PHYSIOLOGICAL REVIEWS
Vol. 81, No. 1, January 2001
Printed in U.S.A.

Sodium-Potassium-Adenosinetriphosphatase-Dependent Sodium Transport in the Kidney: Hormonal Control

ERIC FÉRAILLE AND ALAIN DOUCET

Division of Nephrology, Geneva University Hospital, Geneva, Switzerland; and Centre National de la Recherche Scientifique Unité de Recherche Associée 1859, Service de Biologie Cellulaire, Centre d'Etudes de Saclay, Gif sur Yvette, France

Féraille, Eric, and Alain Doucet. Sodium-Potassium-Adenosinetriphosphatase-Dependent Sodium Transport in the Kidney: Hormonal Control. *Physiol Rev* 81: 345–418, 2001.—Tubular reabsorption of filtered sodium is quantitatively the main contribution of kidneys to salt and water homeostasis. The transcellular reabsorption of sodium proceeds by a two-step mechanism: Na^+-K^+-ATPase-energized basolateral active extrusion of sodium permits passive apical entry through various sodium transport systems. In the past 15 years, most of the renal sodium transport systems (Na^+-K^+-ATPase, channels, cotransporters, and exchangers) have been characterized at a molecular level. Coupled to the methods developed during the 1965–1985 decades to circumvent kidney heterogeneity and analyze sodium transport at the level of single nephron segments, cloning of the transporters allowed us to move our understanding of hormone regulation of sodium transport from a cellular to a molecular level. The main purpose of this review is to analyze how molecular events at the transporter level account for the physiological changes in tubular handling of sodium promoted by hormones. In recent years, it also became obvious that intracellular signaling pathways interacted with each other, leading to synergisms or antagonisms. A second aim of this review is therefore to analyze the integrated network of signaling pathways underlying hormone action. Given

the central role of Na^+-K^+-ATPase in sodium reabsorption, the first part of this review focuses on its structural and functional properties, with a special mention of the specificity of Na^+-K^+-ATPase expressed in renal tubule. In a second part, the general mechanisms of hormone signaling are briefly introduced before a more detailed discussion of the nephron segment-specific expression of hormone receptors and signaling pathways. The three following parts integrate the molecular and physiological aspects of the hormonal regulation of sodium transport processes in three nephron segments: the proximal tubule, the thick ascending limb of Henle's loop, and the collecting duct.

I. INTRODUCTION

Mammalian kidneys play a major role in the homeostasis of extracellular compartment. Despite large qualitative and quantitative variations in dietary intake of solutes and water, the kidneys are able to maintain the composition and the volume of the extracellular compartment within very narrow margins. This homeostatic function of kidneys requires the presence of numbers of specific carriers able to transport a large variety of substrates and their fine control by specific factors and hormones.

Since the onset of modern renal physiology, tremendous efforts have been made to describe the transport properties of the kidney tubule and to analyze their regulatory factors. This led, in the mid 1980s, to an almost coherent cellular description of the transport properties of the successive segments constituting the nephron, as well as to the localization and characterization of hormonal regulation of these processes (566).

During the past 10–15 years, most efforts have permitted the evolution from this cellular level of understanding to a molecular one. This evolution mainly results from *1*) the molecular cloning of membrane transporters and hormone receptors involved in solute and water transport and its regulation, *2*) the characterization of new extracellular regulatory factors and deciphering of new intracellular signaling pathways, *3*) the acknowledgement that intracellular signaling pathways should not be considered as linear and parallel chains of interactions, but as intricated and interactive networks. Such combinatorial organization markedly increases the diversity of signaling. *4*) Lastly, the idea has slowly emerged that the cornerstone of kidney transport machinery, Na^+-K^+-ATPase, is not a house-keeping protein that does not participate actively to rapid adaptations of kidney function but is an important molecular target of hormonal regulation. For this last reason, we have chosen to take Na^+-K^+-ATPase as a leading thread in the analysis of the hormonal control of sodium transport in the kidney.

This review has been focused on the regulatory pathways of sodium transport that share the following criteria: *1*) mechanisms are deciphered, at least partially, at the molecular level; *2*) transport is dependent on Na^+-K^+-ATPase; and *3*) transport mechanism has a functional relevance with sodium homeostasis.

In the two first sections of this review, we summarize the general properties and the renal specificities of Na^+-K^+-ATPase and of intracellular signaling pathways. The three following parts are devoted to the hormonal regulation of cation transport in proximal tubule, thick ascending limb of Henle's loop, and collecting duct. For each structure, the cellular and molecular mechanisms of sodium transport are analyzed before discussing the actions of the main hormones. For each hormone, the general physiological response is first described, and then the regulation of Na^+-K^+-ATPase and other transporters is analyzed in terms of intrinsic changes in properties and of signaling mechanisms.

II. SODIUM-POTASSIUM-ADENOSINETRIPHOSPHATASE IN SODIUM TRANSPORT ALONG THE RENAL TUBULE

Epithelial cell layers separate compartments of distinct compositions and ensure transfer of water and solutes between them. The serosal compartment, in equilibrium with blood plasma, is characterized by the constancy of its composition. In contrast, the composition of the mucosal compartment varies greatly from one epithelium to another and with time. Epithelial cells are characterized by their functional polarization, since their apical membrane facing the mucosal compartment has receptors as well as transport and permeability properties distinct from their basolateral membrane bathed by the serosal compartment. This polarity is maintained by targeting to and/or withdrawal of newly synthesized proteins from a specific cell pole, and by prevention of planar diffusion of membrane constituents between the apical and basolateral domains by specific proteins located at the intercellular junctional complexes (115, 131).

In renal tubular cells, as in all sodium-reabsorbing epithelia, Na^+-K^+-ATPase is exclusively located in the basolateral membrane (723), the infoldings of which are closely surrounded by mitochondria. In contrast, the sodium gradient generated by Na^+-K^+-ATPase between intra- and extracellular compartments is mainly dissipated across the apical membrane. A net transfer of sodium from mucosal toward serosal compartment results from this architectural organization. Quantitatively, net sodium reabsorption is the major function of renal Na^+-K^+-ATPase, and a close relationship exists between the abundance of Na^+-K^+-ATPase and the sodium reabsorption capacity of the different segments of nephron (323). In humans, kidneys reabsorb over 600 g sodium/day and

utilize over 2 kg of ATP for this process. Accordingly, kidney cells are rich sources of Na^+-K^+-ATPase; they contain up to 50 million pumps per cell (248) compared with a few hundred to a few thousand pumps in nonpolarized cells.

Renal Na^+-K^+-ATPase energizes not only sodium reabsorption, but also the secondary active transport (reabsorption or secretion) of large amounts of a wide variety of substances, including other ions and uncharged solutes. Indeed, passive entry of sodium into the cell is often coupled to the transport of other solute(s) at the level of symport or antiport systems. In turn, transport of charged solutes generates transmembrane voltage and concentration gradients that serve as driving force for passive electrolyte movements through ion channels. Transcellular movements of solutes may also generate a transepithelial potential difference that drives ion movements along the paracellular pathway, especially in leaky epithelia.

In summary, Na^+-K^+-ATPase can be considered as an energetic transducer that converts metabolic energy into rapidly mobilizable ionic solute gradients. Despite its ubiquitousness and its quantitative prevalence in kidney epithelial cells, it is worth recalling that Na^+-K^+-ATPase and sodium and potassium gradients are not a universal source of energy. This function may also be achieved by H^+-ATPase and proton gradient in most lower eukaryotes, but also in some cells of higher eukaryotes, such as the intercalated cells of the collecting duct.

A. General Properties of Na^+-K^+-ATPase

The main function of Na^+-K^+-ATPase is to pump intracellular sodium ions out of the cells and extracellular potassium ions within the cells, at the expense of ATP hydrolysis. Although it can be considered either as an ion transporter (the sodium pump) or as an enzyme (Na^+-K^+-ATPase), it is essential to remind that these are two aspects of a same function achieved by a single protein complex.

1. Enzymatic properties

Some 40 years ago, 1997 Nobel Prize winner J. C. Skou (748) first reported that microsomal membrane fractions from crab nerve contain an ATP-hydrolyzing activity stimulated by concentrations of sodium and potassium usually found in intracellular and extracellular fluids, respectively (747). This requirement for both sodium and potassium ions remains the fundamental characteristic of Na^+-K^+-ATPase.

Na^+-K^+-ATPase activity is stimulated by sodium (acting at the cytosolic face of the membrane) with an apparent mean affinity constant ($K_{0.5}$) in the 5–15 mM range in the presence of 5–10 mM K^+, and under these conditions the maximum velocity (V_{max}) is achieved with 60–100 mM of sodium. Because intracellular sodium concentration is in the 5–20 mM range, Na^+-K^+-ATPase works well below its V_{max} in intact cells. Thus any increase in intracellular sodium concentration stimulates Na^+-K^+-ATPase activity which, in turn, pumps more sodium out of the cell and thereby contributes to restore the initial intracellular sodium concentration. Conversely, any decrease in intracellular sodium concentration slows down the pump and participates in maintaining cellular homeostasis. This autoregulatory process is highly efficient because sodium activation of Na^+-K^+-ATPase displays a marked positive cooperativity; thus small variations of sodium concentration around the $K_{0.5}$ induce large variations of Na^+-K^+-ATPase activity. In addition, any regulatory process that alters the sodium affinity of Na^+-K^+-ATPase also alters the pump activity. From the extracellular side, Na^+-K^+-ATPase is stimulated by potassium with an apparent Michaelis constant (K_m) in the millimolar range (0.5–1.5 mM). Thus extracellular potassium is not rate limiting for ATPase activity, except in the case of severe hypokalemia.

The requirement for intracellular sodium is almost absolute except for lithium, which is transported (although at a slower rate than sodium) by human erythrocytes and kidney cells. This has no physiological relevance because lithium concentration in body fluids is very low. The selectivity for potassium is less strict since it can be replaced by rubidium and ammonium with almost similar affinities and efficiencies. Na^+-K^+-ATPase-mediated transport of ammonium instead of potassium into the cells has a physiological relevance in the kidney medulla since it participates to the recycling of ammonium. Transport of rubidium by Na^+-K^+-ATPase has no physiological significance, but use of rubidium as a potassium surrogate proved to be a precious tool for studying the sodium pump. Indeed, the radioactive isotope $^{86}Rb^+$ is much easier to handle in the laboratory than $^{42}K^+$ because it has a much longer radioactive half-life ($\approx$18 days vs. 12 h).

The energy necessary to move sodium and potassium against their transmembrane electrochemical gradients is provided by the hydrolysis of the "energy-rich" ATP molecule, as other nucleotides triphosphate are hydrolyzed at much slower rates. The true substrate of Na^+-K^+-ATPase is the ATP-Mg complex, but the dependency on magnesium is not absolute because other divalent cations (manganese, cobalt) can substitute for magnesium. However, most divalent cations, in particular calcium, inhibit ATPase activity.

For each ATP molecule hydrolyzed, Na^+-K^+-ATPase moves two potassium ions into the cell and three sodium ions out of the cell. An important consequence of this $3Na^+$:$2K^+$ stoichiometry is the electrogenicity of Na^+-K^+-ATPase and therefore its dependence on membrane potential (see below).

As with all P-type ATPases, Na^+-K^+-ATPase is transiently phosphorylated during its activation. P-type ATPases are also called E_1-E_2 ATPases because they exhibit two main conformation states that can be either unphosphorylated (E_1 and E_2) or phosphorylated (E_1-P and E_2-P). The two conformation states of Na^+-K^+-ATPase are characterized by their respective affinities for sodium, potassium, and ATP and by the accessibility of the cationic sites at the intracellular or extracellular sides of the membrane: E_1 conformation confers a high affinity for ATP and sodium and a low affinity for potassium, both cation sites being accessible from the intracellular side, whereas under the E_2 conformation, the cation sites are accessible from the outside and display low affinity for sodium and high affinity for potassium. Na^+-K^+-ATPase cycles through these different conformations according to the so-called Albers-Post model (Fig. 1). ATP, magnesium, and sodium bind to E_1 on the intracellular side of the pump, allowing phosphorylation of E_1 (E_1-P) and "occlusion" of sodium ions that are no longer accessible from either side of the membrane. After release of ADP, the exergonic transconformation of E_1-P to E_2-P occurs and promotes the extracellular delivery of sodium and the binding of extracellular potassium. This latter process induces dephosphorylation of E_2-P and potassium occlusion. Spontaneous reversion to E_1 releases potassium inside the cell, completing the reaction cycle (reviewed in Ref. 449).

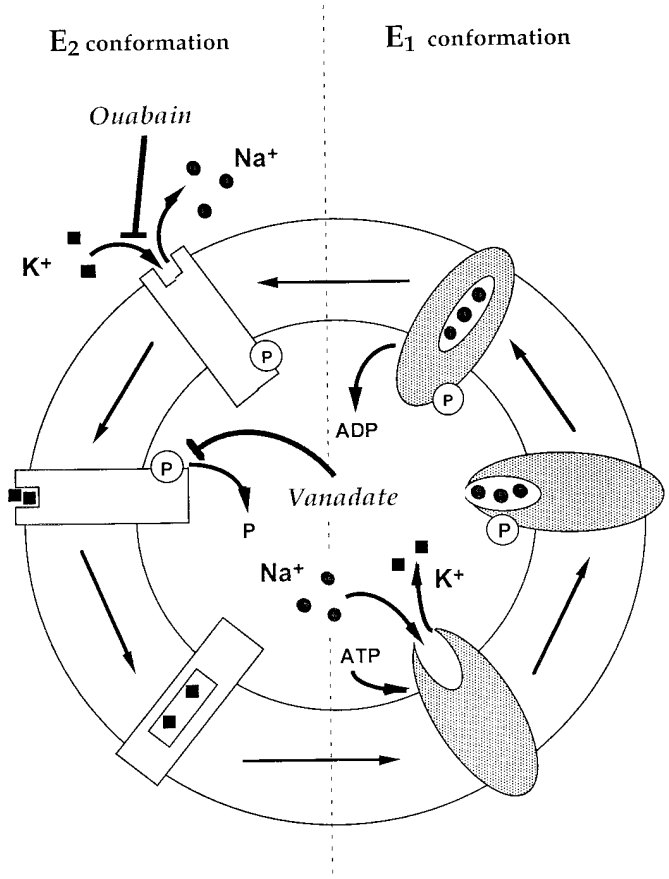

FIG. 1. Catalytic cycle of Na^+-K^+-ATPase. This models shows the transition between the main conformational forms of Na^+-K^+-ATPase catalytic subunit (E_1, E_1-P, E_2-P, and E_2) with either accessible or occluded cation binding sites. It also indicates the sites of action of Na^+-K^+-ATPase inhibitors vanadate and ouabain.

2. Pharmacology and toxicology

A) VANADATE. Vanadate acts as a structure analog of phosphate to inhibit all P-type ATPases through binding to their phosphorylation site and blockade under their E_2 configuration (Fig. 1). Although vanadate was initially considered as a putative physiological modulator of Na^+-K^+-ATPase (130), this now appears unlikely because the redox state prevailing within cells reduces vanadate to inactive vanadyl.

B) DIGITALIS GLYCOSIDES. Digitalis glycosides are natural and potent inhibitors of Na^+-K^+-ATPase (710) used in therapy as well as in the laboratory. Ouabain (G-strophantin) is generaly used in vitro because of its better, although limited, water solubility, whereas digoxin is the most widely used digitalic in therapy. Ouabain binds to an extracellular domain of Na^+-K^+-ATPase under its E_2 conformation and decreases its affinity for potassium, and vice versa, as a competitive inhibitor (Fig. 1). As a clinical counterpart, digitalic poisoning is more severe in hypokalemic patients. Through impediment of potassium binding, ouabain prevents the dephosphorylation of the enzyme and the associated transconformation from E_2 to E_1. The affinity of Na^+-K^+-ATPase for ouabain varies within a wide range of concentrations (from nM to mM) between species (rats, mice, and *Bufo marinus* are rather resistant to ouabain) and between organs or cells from a given species (kidneys being less sensitive to ouabain than brain and heart). These differences are accounted for in part by the molecular heterogeneity of Na^+-K^+-ATPase (see below).

For over 40 years, ouabain has been considered as a highly specific inhibitor of Na^+-K^+-ATPase. In particular, despite its functional and structural similarities with Na^+-K^+-ATPase, gastric H^+-K^+-ATPase is not sensitive to ouabain. However, it is now established that ouabain also inhibits several nongastric forms of H^+-K^+-ATPase (431).

C) PALYTOXIN. Palytoxin, a nonprotein toxin produced by a marine coelonterate, not only inhibits Na^+-K^+-ATPase but transforms it into a sodium channel (358). The tumor-promoting activity of palytoxin is related to its ability to increase intracellular sodium by this mechanism (482).

3. Structure and structure-function relationship

Purification (438) and molecular cloning (456, 615, 730, 734, 735, 736) have shown that Na^+-K^+-ATPase consists of two main subunits (α and β) that are associated in a 1:1 molar ratio.

The α-subunit (~1,000 amino acid residues and 110 kDa) displays all the functional properties described above (binding sites for sodium and ATP and phosphorylation site on the cytoplasmic domain, and binding sites for potassium and ouabain on the extracellular domain) and is therefore considered as the catalytic subunit. It consists of 10 membrane-spanning domains (M_1-M_{10}) with intracellular NH_2- and COOH-terminal domains and a long M_4-M_5 intracytoplasmic loop (Fig. 2).

Site-directed mutagenesis of amino acid residues and covalent chemical modifications of the α-subunit have permitted the identification of functionally important amino acids and domains (reviewed in Ref. 396). The ATP binding domain and the phosphorylation site are located into the long M_4-M_5 cytoplasmic loop; their highly conserved sequence is a molecular signature of P-ATPases. The M_4, M_5, and M_6 transmembrane domains likely constitute the cation occlusion site and the ion pore. The intracellular NH_2-terminal domain might play a role in cation gating. M_1-M_2 as well as part of the M_3-M_4 ectodomains are involved in ouabain binding. The M_7-M_8 ectodomain is the main site of interaction with the β-subunit. Finally, several intracellular amino acid residues are phosphorylation sites for protein kinases (see below).

The β-subunit is smaller (~300 amino acids) and displays a single membrane-spanning domain and a large extracellular domain with several *N*-linked glycosylation sites. This ectodomain is responsible for the interaction with α-subunit. Although the β-subunit has no enzymatic or transport activity, its association with an α-subunit is an absolute requirement for ATPase and pump activities: it allows the folding of newly synthetized α-subunits and their targeting from the endoplasmic reticulum to the plasma membrane, as well as the stabilization of α-subunits within the membrane (328, 330, 828).

Na^+-K^+-ATPase may also contain a γ-subunit (53 amino acids, ~10 kDa), which was first recognized as copurifying with α- and β-subunits (296). More recently, this γ-subunit has been cloned (554); its mRNA is abundantly expressed in kidney and at lower level in other epithelia but is absent in other tissues. Thus, conversely to the α- and β-subunits, the γ-subunit is not an absolute requirement for functional Na^+-K^+-ATPase although, when present, it is an integral part of Na^+-K^+-ATPase. The γ-subunit contains a single transmembrane domain with an extracellular NH_2 terminus. Expression in *Xenopus* oocyte indicates that the γ-subunit reaches the cell membrane only when associated with the $\alpha\beta$-complex (in a 1α:1β:1γ stoichiometry) (69). Functionally, coexpression of γ-subunit with α- and β-subunits was described as modifying the voltage sensitivity of potassium activation (69), decreasing the affinity of the pump for ATP (805, 806) as well as for sodium and potassium (28).

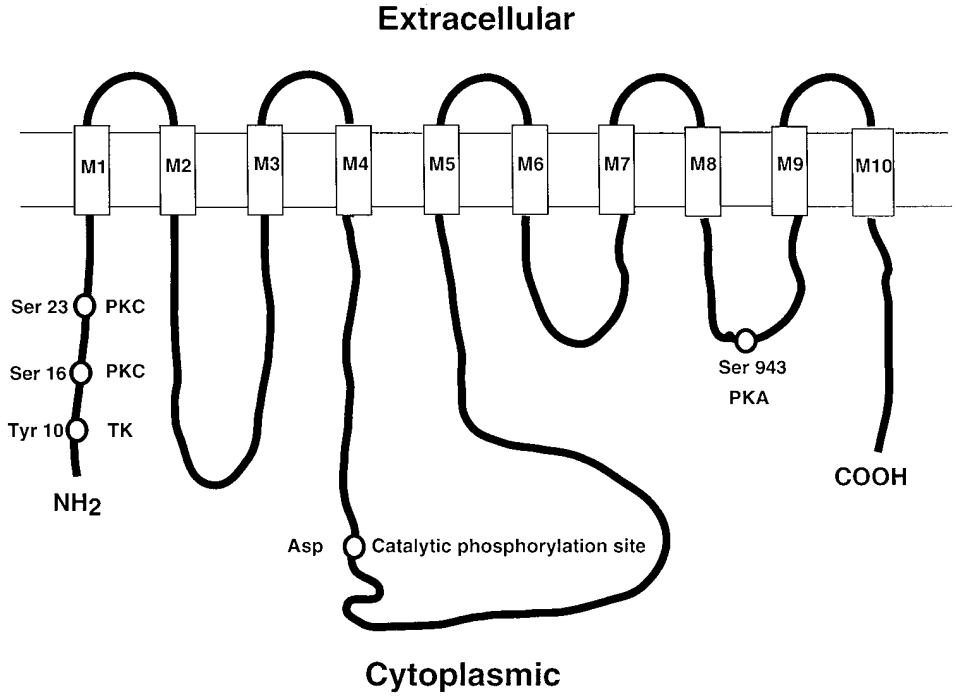

FIG. 2. Topology of the Na^+-K^+-ATPase α-subunit and localization of the phosphorylated amino acids. The Na^+-K^+-ATPase α-subunit displays 10 membrane-spaning domains with intracytoplasmic NH_2 and COOH termini. The currently identified amino acids phosphorylated by protein kinase C (PKC) and tyrosine kinases (TK) are located in the extreme NH_2 terminus of the α-subunit. Both Tyr-10 and Ser-16 are conserved in all cloned α_1-subunits, whereas Ser-23 is specific of the rat α_1-subunit. The protein kinase A (PKA) phosphorylation site is located at Ser-943 into the M_8-M_9 intracellular loop. Also indicated is the Asp residue within the M_4-M_6 large intracellular loop that is phosphorylated during the catalytic cycle of the pump.

4. *Molecular and functional heterogeneity of Na^+-K^+-ATPases*

Despite canonical characteristics, the Na^+-K^+-ATPase is functionally highly heterogeneous. Molecular cloning and expression of several isoforms of Na^+-K^+-ATPase catalytic subunits provided some molecular basis for this heterogeneity. As yet, four genes encoding Na^+-K^+-ATPase α-subunits and three genes encoding β-subunits have been cloned from mammals (reviewed in Ref. 396). In addition, two splice variants of the γ-subunit (γ_1 and γ_2) have been recently characterized in rat kidney (483). The γ_1-subunit has the amino acid sequence predicted by the published cDNA sequence, whereas the γ_2-subunit contains a different sequence of its 7 NH_2-terminal amino acids and is acetylated on its first methionine. Experiments in which specific α- and β-isoforms were coexpressed in heterologous cellular systems indicate that all types of $\alpha\beta$-dimers tested are functional. However, it remains to be demonstrated that all the combinations between different α- and β-isoforms are functionally expressed in normal cells. The following discussion on the properties of these different isoforms is focused around α-subunits because they condition the main properties of the holoenzyme.

Sequence comparison between the different α-isoforms in a given species reveals a very high degree of conservation, suggesting the existence of a common ancestor gene. The four isoforms of Na^+-K^+-ATPase α-subunit (α_1-α_4) are differentially expressed among tissues: the α_1-isoform is ubiquitous and is the most abundant, if not the only, form in the kidney; the α_2-isoform is predominantly expressed in heart, skeletal muscle, and brain; the α_3 is expressed in neural tissue and ovary; whereas expression of the α_4-isoform is restricted to testis (786, 895).

In rat, it is well established that α-subunit isoforms endow different affinities for cardiac glycosides: α_3 [dissociation constant (K_D) $\approx$2 nM] > α_2 ($K_D\approx$100 nM) > α_4 ($K_D\approx$300 nM) > α_1 ($K_D\approx$1 mM) (602, 648, 895). The very low affinity of rat α_1-isoform for ouabain mainly results from the presence of a positively charged arginine residue and a negatively charged aspartate residue at the two ends of the first (M_1-M_2) ectodomain of the α-subunit (649). Such differences in ouabain sensitivities are not found in all species; in humans, for example, the α_1-α_3 isoforms display similar affinities for ouabain (190).

Isoforms of Na^+-K^+-ATPase α-subunits may also differ by their voltage dependence. The equilibrium potential of Na^+-K^+-ATPase (i.e., the membrane potential for which the energy necessary to move three sodium and two potassium ions across the cell membrane equals the free energy of hydrolysis of one molecule of ATP) is around −280 mV. From that membrane potential, at which the pump cannot work, the pumping rate of Na^+-K^+-ATPase should theoretically increase as the membrane depolarizes to 0 mV and to positive potentials. Such relationships between membrane potential and Na^+-K^+-ATPase pumping rate (as evaluated by the pump current) were experimentally observed within a wide range of membrane potentials (from −100 to + 50 mV) in excitable cells (which mainly contain the α_1- and α_3-isoforms) such as axons (660) and myocytes (318). However, in renal epithelial cells (which mostly contain the α_1-isoform), the pump current does increase with membrane potential within the −175 to −75 mV range, but reaches a plateau at higher physiological potentials (−75 to −25 mV) (397). Whether these distinct behaviors are intrinsic properties of the distinct isoforms originating in these two types of tissues or result from the specific cellular environment remains unknown. The marked voltage dependency of Na^+-K^+-ATPase in excitable cells is teleologically sound, since stimulation of the pump during membrane depolarization facilitates the recovery of intracellular sodium concentration during action potential. Also physiologically sound is the absence of such regulation in epithelial cells since their membrane potential does not vary much.

Thus the expression of functionally distinct isoforms of the Na^+-K^+-ATPase α-subunit in specific tissues may have physiological consequences. In addition, these properties might be modulated by the association with different isoforms of β-subunit and/or the absence or presence of the different splice variants of the γ-subunit.

5. *Regulation of Na^+-K^+-ATPase through phosphorylation of α-subunit*

An emerging but important regulatory mechanism for Na^+-K^+-ATPase activity in intact cells is phosphorylation by protein kinases. Phosphorylation of the Na^+-K^+-ATPase α-subunit has been first recognized using purified preparations of Na^+-K^+-ATPase incubated in the presence of protein kinase C (PKC) or protein kinase A (PKA) (83, 283, 522). PKA- and PKC-mediated phosphorylation of Na^+-K^+-ATPase α-subunit has been subsequently revealed in tissue homogenates (167) and in intact cells (66, 67, 86, 105, 134, 135, 295, 496, 529, 555, 616).

A single PKA phosphorylation site has been mapped to Ser-943 (Fig. 2) located in a typical PKA consensus site conserved in all cloned Na^+-K^+-ATPase α-subunits (66, 283, 294). In addition, two PKC phosphorylation sites are currently identified (Fig. 2). The first one, located at Ser-16, is conserved among all cloned α_1-subunits and lies within an unusual PKC phosphorylation motif (66, 68). The second one, located at Ser-23, is found only in the rat α_1-subunit and is comprised of a typical PKC consensus site within the lysine-rich cluster of the NH_2-terminal domain of the α_1-subunit (68, 73, 284). Finally, phosphorylation of the Na^+-K^+-ATPase α_1-subunit at Tyr-10 (Fig. 2) has been recently identified (276), similarly to the closely

related gastric H^+-K^+-ATPase α-subunit (809). It should be mentioned that phosphorylation of the identified sites does not account for the whole basal phosphorylation of the Na^+-K^+-ATPase α_1-subunit (66, 68). These findings, together with the identification of threonine phosphorylation of rat α_1-subunit in intact cells (276), indicate that an additional phosphorylation site(s) remains to be identified.

The functional effects of serine phosphorylation of the α-subunit are still highly debated. Results obtained in transfected COS-7 cells have suggested that PKA phosphorylation of the α-subunit has an inhibitory effect on Na^+-K^+-ATPase activity (294). However, in vitro PKA phosphorylation of shark rectal gland Na^+-K^+-ATPase stimulates its activity, whereas the activity of the pig kidney enzyme is unchanged under similar conditions (186). It should be mentioned that in native rat kidney epithelial cells, PKA phosphorylation of the Na^+-K^+-ATPase is associated with stimulation of its activity (135, 467).

Similarly, in response to PKC phosphorylation of its α-subunit, Na^+-K^+-ATPase activity was either stimulated (134, 628, 835), inhibited (73, 166, 168, 835), or unchanged (77, 285). These discrepancies observed in intact cells may be accounted for in part by the presence of both indirect effects of PKC phosphorylation such as internalization of active Na^+-K^+-ATPase units (165) and direct effects of phosphorylation such as an increase in apparent affinity for sodium (276). This later effect of phosphorylation of Na^+-K^+-ATPase α-subunit was recently demonstrated using COS-7 cells stably transfected with either wild-type or Ser-16 (the ubiquitous PKC phosphorylation site) mutant Na^+-K^+-ATPase α_1-subunits: *1*) phorbol esters increased the apparent sodium affinity of wild-type Na^+-K^+-ATPase; *2*) when nonspecific increase in fluid-phase endocytosis was prevented, mutation of Ser-16 prevented the stimulatory effect of phorbol esters on the transport activity of Na^+-K^+-ATPase; and *3*) mutant α_1-subunits in which Ser-16 was substituted by an acidic residue (Asp or Glu) mimicking constitutive phosphorylation exhibited an increased apparent sodium affinity (276). This effect of Ser-16 phosphorylation on the apparent sodium affinity of Na^+-K^+-ATPase is in agreement with the results of Logvinenko et al. (515), who showed that in vitro phosphorylation of purified Na^+-K^+-ATPase by PKC shifts the conformational equilibrium of the Na^+-K^+-ATPase toward the E_1 conformation, i.e., the conformation displaying high affinity for sodium (see sect. II*A1*). These observations are also consistent with earlier studies showing that the α_1-subunit NH_2-terminal domain is involved in conformational changes of the enzyme. Indeed, tryptic cleavage of the α_1-subunit occurring between Lys-30 and Glu-31 (440), or truncation of the NH_2 terminus by site-directed mutagenesis (854, 880), displaces the E_1-E_2 conformational equilibrium toward the E_1 conformation through stimulation of potassium deocclusion (880), and thereby may account for the increased apparent sodium affinity of Na^+-K^+-ATPase (440). This recent report (276) agrees with a growing number of studies documenting a stimulation of Na^+-K^+-ATPase activity in response to PKC activation (277, 356, 393, 530, 628), but contrasts with a few others (73, 285). It should be stressed, however, that these last two studies (73, 285) focused on the role of the additional rat-specific Ser-23 phosphorylation site and not on that of the ubiquitous Ser-16 phosphorylation site. These two phosphorylation sites might be targets for different PKC isozymes and/or produce different physiological effects. Indeed, phosphorylation of Ser-23 per se does not alter Na^+-K^+-ATPase activity (165, 285) but promotes endocytosis of the Na^+-K^+ pump in proximal tubule and in opossum kidney (OK) cells (165, 166). This hypothesis is supported by recent data indicating that *1*) in OK cells, the opposite effects of phorbol esters and dopamine on the transport activity of Na^+-K^+-ATPase rely on classical PKC-β and atypical PKC-ζ activation, respectively (79); and *2*) the transport activity of rat α_1-β complexes expressed in *Xenopus* oocytes is inhibited while that of the endogenous *Xenopus*α_1-β complexes, which were previously shown to be exclusively phosphorylated on Ser-16 (68), are stimulated by injection of purified rat PKC (835). In addition, secondary regulatory mechanisms that may be cell specific and/or brought about by experimental conditions, such as oxygen (81, 277) or calcium (161) availability, may also explain some discrepancies.

In response to the activation of receptor tyrosine kinases, i.e., insulin, insulin-like growth factor I (IGF-I), or epidermal growth factor (EGF) receptors, stimulation of Na^+-K^+-ATPase activity is almost always reported (178, 209, 266, 279, 280, 531, 544, 766). These observations are consistent with the recent identification of Tyr-10-dependent stimulation of Na^+-K^+-ATPase activity in native and cultured renal proximal tubule epithelial cells (278).

B. Na^+-K^+-ATPase Along the Nephron

1. Anatomic and topographic segmentation of the nephron

Figure 3 schematically depicts the topographical organization and the axial segmentation of the rat nephron and lists the abbreviations used in this review for the different nephron segments.

The proximal tubules extend from the glomeruli down to the thin segments, at the junction between the outer and inner stripes of the kidney outer medulla. Its apical cell border is characterized by a well-developed brush border made of densely packed microvilli. The basolateral plasma membrane forms deep infoldings that are in close contact with mitochondria. Both apical microvilli and basolateral membrane infoldings consider-

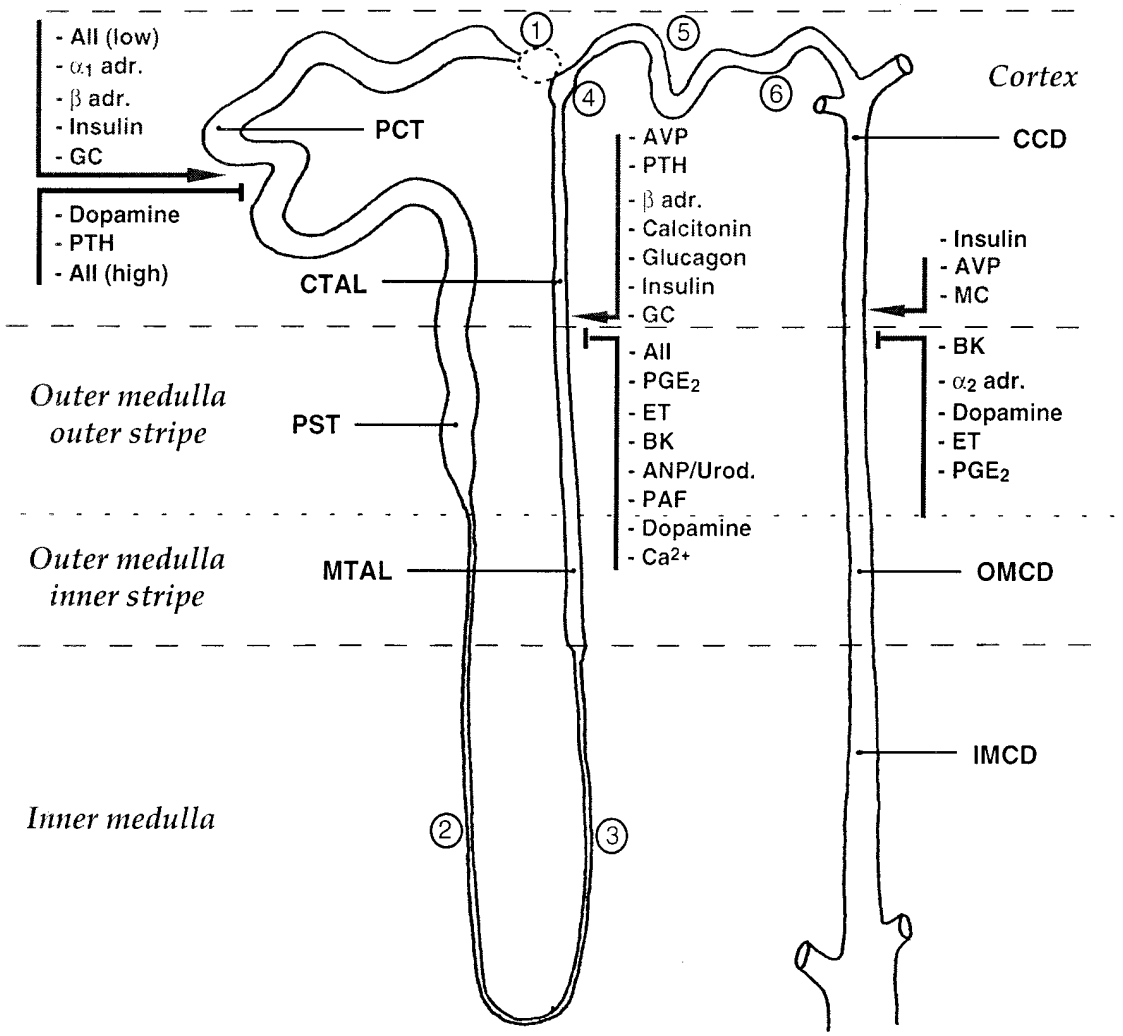

FIG. 3. Topology of the main nephron segments and sites of action of hormones controling sodium transport. Schematical representation of a nephron with its successive portions: *1*) glomerulus; PCT, proximal convoluted tubule; PST, proximal straight tubule; *2*) thin descending limb of Henle's loop; *3*) thin ascending limb; MTAL, medullary thick ascending limb of Henle's loop; CTAL, cortical thick ascending limb; *4*) macula densa; *5*) distal convoluted tubule; *6*) connecting duct; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct. For the three structures analyzed in this review (proximal tubule, thick ascending limb of Henle's loop, and collecting duct), we mentioned the main hormones or factors that stimulate (→) and inhibit (⊢) sodium reabsorption: ANG II, angiotensin II (low and high referring to pico- and micromolar concentrations); adr, adrenergic agonists; AVP, arginine vasopressin; PTH, parathyroid hormone; GC, glucocorticoids; MC, mineralocorticoids; PGE_2, prostaglandin E_2; ET, endothelin; ANP/Urod, atrial natriuretic peptide and urodilatin; PAF, platelet-activating factor; BK, bradykinin.

ably increase the membrane surface area available for transport. Intercellular cell junctions are shallow, and the epithelium is leaky. The proximal tubule is usually subdivided in three successive portions on a morphological basis: S_1 includes the initial and mid proximal convoluted tubule (PCT), S_2 includes the late PCT and the cortical portion of the proximal straight tubule (PST), and S_3 consists of the outer medullary PST. In most species but rat, the cell size, the density of apical microvilli, and the height of brush border and basolateral infoldings decrease from S_1 to S_3, and along with these morphological changes, the transport capacity of the proximal tubule also decreases from S_1 to S_3.

The thin segments of the loop of Henle extend from the end of PST up to the junction with thick ascending limbs. Despite their importance for urine concentration by a countercurrent mechanism, they are exclusively the site of passive solute and fluid exchanges. Accordingly, their Na^+-K^+-ATPase activity is very low (see below), and therefore, they are not considered in the following sections.

The thick ascending limb of Henle's loop (TAL) ex-

tends from the junction between the inner and outer medulla (where the thin segments end) up to the macula densa, or a few micrometers beyond, in the superficial cortex. It therefore includes a medullary and a cortical portion (MTAL and CTAL, respectively). On the basis of morphological criteria, TAL appears as made of a single type of cell that displays deep basolateral membrane infoldings surrounding numerous mitochondria. Apical membrane forms only few and short microvilli, and the junctional complexes are numerous and of the shallow type. Functionally these intercellular junctions are permeable to solutes but highly impermeable to water.

The distal convoluted and connecting tubules (DCT and CNT, respectively) complex, which extends from the macula densa to the first branching with another tubule, represents an heterogeneous portion from morphological, functional, and biochemical points of view. It consists of three cell types (DCT cells, connecting cells, and intercalated cells), the distribution of which along the DCT and CNT varies with species. In most species, however, the three cell types are present along most of the length of this nephron portion. Despite the functional and pharmacological importance of the DCT/CNT segments, in particular for calcium reabsorption and as the site of action of thiazide diuretics, their shortness and their cellular heterogeneity have precluded the determination of the molecular mechanism underlying the regulation of their transport properties. Therefore, this regulation is not discussed in the following sections.

Collecting ducts constitute the last segment of the nephron. They extend throughout the kidney, from the outermost cortex to the tip of the papilla, and therefore, they encounter surroundings of different compositions. On the basis of topographical criteria, the collecting duct is usually subdivided into three successive portions: the cortical collecting duct (CCD), the outer medullary collecting duct (OMCD), which is itself subdivided into outer stripe and inner stripe subsegments ($OMCD_o$ and $OMCD_i$, respectively), and the inner medullary collecting duct (IMCD) also subdivided into three subsegments of equal length ($IMCD_1$, $IMCD_2$, and $IMCD_3$). The CCD and OMCD are made of two distinct cell types, namely principal, or light, cells (accounting for 60–65% of whole cells) and intercalated, or dark, cells (accounting for the remaining 35–40% cells). Intercalated cells are further subdivided into two subtypes called type A and type B intercalated cells (or α- and β-cells). Principal cells are characterized by a deeply invaginated basolateral membrane, the infoldings of which are closely associated with mitochondria, and a rather smooth apical membrane with few blunt microvilli and a single central cilium. Intercalated cells are characterized by a great number of mitochondria. Type A cells display extensive apical microplicae, numerous subapical tubulovesicular structures, and a rather nonextensive basolateral membrane. In contrast, type B cells display fewer apical microplicae, the tubulovesicular structures are scattered throughout the cell, and the basolateral membrane is extensive. In fact, these are archetypal descriptions of types A and B intercalated cells, as one can distinguish intermediate subtypes of intercalated cells featuring only a few of those structural properties: intercalated cells can evolve from one phenotype to the other in response to different stimuli, and intermediate subtypes may correspond to evolving cells (8). Under normal conditions, there is an axial gradient in the relative proportion of the two subtypes of intercalated cells along the CCD and OMCD: type B cells are preponderant in the most cortical portion of the collecting duct (25–28% B cells vs. 10–12% A cells), whereas they almost disappear at the transition between $OMCD_o$ and $OMCD_i$.

The $OMCD_i$ and $IMCD_1$ consist of principal cells and type A intercalated cells. The proportions of intercalated cells decrease from 35–40% at the transition between $OMCD_o$ and $OMCD_i$ to 10% at the $IMCD_1$-$IMCD_2$ transition. The morphology of principal cells also varies from the most cortical regions of CCD down to the innermost region of $IMCD_1$. The most prominent aspect of axial changes is the decrease in size of the basolateral membrane and in the density of intracellular organelles, which correlates with a decrease in transport capacity.

$IMCD_2$ and $IMCD_3$ are made of IMCD cells that appear structurally homogeneous, eventhough there might be several functionally distinct subtypes of IMCD cells.

2. Methods used to study Na^+-K^+-ATPase in kidney tubules

Given this axial heterogeneity of nephrons, understanding the precise contribution of Na^+-K^+-ATPase to tubular cation transport has required the development of techniques allowing its study at the level of well-characterized nephron segments. Because this approach has obvious limitations, we thought it of interest to briefly discuss the advantages and limits of the methods presently available.

Although early approaches were based on isolation of well-defined portions of renal tubule from freeze-dried kidney sections (718), all the techniques presently used apply to nephron segments microdissected from fresh tissue. Moderate hydrolysis of kidney interstitium with collagenase allows the isolation of large numbers of samples from all the nephron subsegments. The main limitation of this approach results from the small size of microdissected samples: a 1-mm-long nephron segment accounts for ~300–400 cells, 100–250 ng of proteins, 3–5 ng RNAs, and 60–100 pg poly(A) RNAs. Nonetheless, techniques are now available for quantifying the enzymatic and transport activity of Na^+-K^+-ATPase, the number of catalytic units, as well as the amount of protein and

of mRNA coding its different subunits at the level of single or homogeneous populations of nephron segments.

The enzymatic (ATP-hydrolytic) activity of Na^+-K^+-ATPase can be measured at the level of single nephron segments by monitoring the rate of hydrolysis of exogenous ATP. The amplification factor required by the small size of the sample is provided either by using radioactive ATP (224) or by enzymatically coupling the production of ADP to the generation of a fluorescent metabolite (323, 633). Whatever the method used, the assay needs to be carried out on broken or permeabilized cells to permit the access of exogenous ATP to intracellular catalytic sites. The Na^+-K^+-ATPase activity should be discriminated from other ATPase activities present in the nephron segments on the basis of its sodium and potassium dependence rather than its sensitivity to ouabain, since this drug may inhibit other ATPases (nongastric H^+-K^+-ATPase) potentially present in the tubular sample. Alternatively, it is possible to use specific inhibitors of these ouabain-sensitive H^+-K^+-ATPases (such as Sch 28080) to circumvent this contamination. Measurement of Na^+-K^+-ATPase activity allows us to define and control the concentration of substrates during the assay, and thereby to determine the kinetical parameters of the enzyme, in particular its V_{max} and $K_{0.5}$ for cations. As a counterpart, permeabilization of cell membranes entails the loss of regulatory parameters such as the transmembrane voltage, the membrane limitation to potassium recycling, or intracellular diffusible regulatory molecules. Also, Na^+-K^+-ATPase activity does not inform about the in vivo pumping activity of Na^+-K^+-ATPase, as the latter is highly dependent on intracellular sodium concentration.

In nonpermeabilized nephron segments, the activity of Na^+-K^+-ATPase can be determined through its pumping capacity by measuring ouabain-sensitive rubidium uptake under initial rate conditions (163). Although this method is widely used in a great variety of tissues, it has two main pitfalls. The first one, which results from the nonspecificity of ouabain (see above), can be easily circumvented by using Sch 28080 in the assay to abolish the activity of ouabain-sensitive H^+-K^+-ATPases. More importantly, ouabain-sensitive rubidium uptake is calculated as the difference between the rates of intracellular accumulation of rubidium measured in the absence and presence of saturating concentrations of ouabain, respectively. Such a calculation implies that Na^+-K^+-ATPase-independent rubidium intake is similar in the absence and presence of ouabain. This is very unlikely, since inhibition of Na^+-K^+-ATPase by ouabain abolishes the concentration gradient of rubidium across cell membranes and may thus increase the driving force for passive, Na^+-K^+-ATPase-independent rubidium movements. Thus ouabain-insensitive rubidium uptake probably overestimates Na^+-K^+-ATPase-independent rubidium uptake, and therefore, ouabain-sensitive rubidium uptake underestimates Na^+-K^+-ATPase activity. However, because ouabain-insensitive rubidium uptake in renal tubule cells represents a minute fraction of the total rubidium uptake (163), this underestimation might be limited. An additional technical difficulty of this measurement in isolated nephron segments results from their huge Na^+-K^+-ATPase activity: the kinetics of rubidium uptake is fast, and measurement under initial rate conditions must be performed within 0.5–1 min. Changes in rubidium uptake reflect alterations of the V_{max} of the pump and/or changes of its efficiency brought about by changes in intracellular concentration of sodium and/or in affinity for sodium.

Despite its importance, the regulation of the pump affinity for intracellular sodium concentration remains very difficult to evaluate in intact nephron segments, since this requires the precise clamping and monitoring of intracellular sodium concentration. An elegant method has been proposed (97) in which the rate of sodium efflux is monitored as a function of time in nephron segments initially loaded with $^{22}Na^+$ by cold exposure in a potassium-free medium. Because the apical entry of sodium is blocked during the efflux study, i.e., the specific radioactivity of $^{22}Na^+$ remains constant, at any time one can calculate the sodium pumping rate of Na^+-K^+-ATPase from the rate of appearance of $^{22}Na^+$ in the superfusate and the intracellular concentration of sodium from the remaining quantity of $^{22}Na^+$. This allows the correlation of Na^+-K^+-ATPase functional activity in intact cells to intracellular sodium concentration. Unfortunately, this method has not been given the large use it deserves.

To elucidate whether changes in Na^+-K^+-ATPase activity are due to activation of preexisting units or induction of new ones, it is possible to quantify these units as well as the mRNAs encoding them. Quantification of Na^+-K^+-ATPase units is feasible on single nephron segments by measuring the specific binding of [^{3}H]ouabain under saturating conditions (248). Contamination by ouabain-sensitive H^+-K^+-ATPase can be prevented by Sch 28080, which is a competitor of ouabain (119). Unfortunately, this method is hardly applicable to the rat because its kidney Na^+-K^+-ATPase displays a low affinity for ouabain. Alternatively, relative quantification can be made by Western analysis with specific antibodies against the different subunits of Na^+-K^+-ATPase (547). Although it requires pooling several tens of nephron segments in each sample, this approach is now routinely used. It is even possible to discriminate between plasma membrane pump and intracellular pools by biotinylation of membrane proteins and streptavidin precipitation before Western blotting analysis (136).

Finally, the transcripts of Na^+-K^+-ATPase subunits can be quantitated by quantitative RT-PCR (118, 818). Given the sensitivity of PCR and the abundance of renal Na^+-K^+-ATPase, this is feasible on very short portions of nephron (0.1 mm).

3. Distribution and properties of Na$^+$-K$^+$-ATPase along the nephron

Measurements of Na$^+$-K$^+$-ATPase activity in microdissected segments of nephrons from different mammalian species have revealed the heterogeneity of distribution of this pump along the nephron (323, 454). In all species studied as yet, Na$^+$-K$^+$-ATPase activity is high in the TAL and DCT, intermediate in the proximal tubule (PCT and PST), relatively low in the collecting duct, and vanishingly low in the thin segments of Henle's loop. This distribution profile is paralleled by the number of Na$^+$-K$^+$-ATPase units determined either by [^{3}H]ouabain binding ($\alpha\beta$-complexes) (248) or by Western blotting (α- and β-subunits) (547).

From the number of specific [^{3}H]ouabain binding sites and the V_{max} of Na$^+$-K$^+$-ATPase catalytic activity, one can calculate a molecular activity of ~2,000 cycles · ouabain binding sites^{-1} · min^{-1} in all the nephron segments (248). This molecular activity is much lower than the 10,000 cycles · ouabain binding sites^{-1} · min^{-1} reported for the Na$^+$-K$^+$-ATPase purified from kidney (439), suggesting the presence of cellular components that downregulate the enzyme activity in the cell.

With the assumption of a 1 ATP:2 K$^+$ stoichiometry (see sect. II*A1*), comparison of Na$^+$-K$^+$-ATPase activity and ouabain-sensitive rubidium uptake indicates that in intact tubular cells the pump is working at 20–30% of its maximum rate, which is consistent with measurements of intracellular sodium concentration and sodium affinity (163).

Despite technique availability, only one report, on rat OMCD, provides absolute quantification of Na$^+$-K$^+$-ATPase subunits mRNA expression (118). In this study, mRNAs for the β_1-subunit were greater than threefold more abundant than those coding for α_1-subunit, suggesting that the transcription of α_1-subunit mRNAs is the rate-limiting step in the regulation of Na$^+$-K$^+$-ATPase expression. Absolute quantification of mRNAs and of [^{3}H]ouabain binding sites indicated that there are 24,000 Na$^+$-K$^+$-ATPase units per α_1-subunit mRNA, which suggests either a high efficiency of translation or a slow turnover rate of the protein.

The functional properties of Na$^+$-K$^+$-ATPase vary along the rabbit nephron: compared with proximal tubules and TAL, the collecting ducts display a higher affinity for ouabain (220) and for sodium (48). In addition, Na$^+$-K$^+$-ATPase activity is inhibited by a specific anti-α_3-isoform monoclonal antibody in collecting ducts and by an anti-α_1-isoform monoclonal antibody in proximal tubules and thick ascending limbs (46). In the rat nephron, two functional forms of Na$^+$-K$^+$-ATPase displaying different sensitivities to ouabain and to the anti-α_1- and anti-α_3-antibodies are coexpressed in each nephron segment (275).

However, despite some controversy (6, 176), most studies (162, 821) failed to demonstrate the presence of α_2- or α_3-isoforms in the rat nephron (α_4 has not been looked but seems restricted to testis). The $\alpha_1\beta_1$ heterodimer is likely the exclusive Na$^+$-K$^+$-ATPase complex expressed in kidney tubules, and the functional axial heterogeneity of kidney Na$^+$-K$^+$-ATPase might result from cell-specific regulation. An interesting possibility would be that coexpression of the γ-subunits might be responsible for the observed differences (at least for sodium affinity).

III. HORMONE SIGNALING ALONG THE NEPHRON

A. General Mechanisms of Hormone Signaling

1. Receptors coupled to G proteins and adenylyl cyclase

Many peptide hormones, catecholamines, eicosanoids, nucleotides, and calcium ions act through binding to plasma membrane receptors coupled with specific multimeric G proteins. These G proteins are constituted by the association of a Gα-subunit with a Gβ- and a Gγ-subunit. Binding of a ligand to its cognate receptor induces a conformational change that is transmitted to the G protein, causing GDP release and GTP binding by the Gα-subunit, and promoting its dissociation from G$\beta\gamma$-subunit complex. The free Gα-subunit and G$\beta\gamma$-heterodimer each activate target effectors. The reaction is turned off by GTP hydrolysis and reassociation of the G protein subunits. Among the multiple targets of G protein subunits, adenylyl cyclase and phospholipase C-β couple the binding of an agonist to its receptor with the modulation of PKA and PKC activity, respectively.

In the kidney, peptide hormones, e.g., parathyroid hormone and vasopressin, and catecholamines, e.g., epinephrine and dopamine, bind to G protein-coupled receptors and lead to the activation of adenylyl cyclases. Adenylyl cyclases are stimulated by Gα_s (795) but are either further stimulated (322, 793) or inhibited by G$\beta\gamma$ (453, 753, 796) according to their subtypes (Fig. 4). Other mediators such as angiotensin II or norepinephrine can decrease the generation of cellular cAMP through an inhibition of adenylyl cyclases by Gα_i subunits (Fig. 4).

Stimulation of adenylyl cyclase increases the intracellular concentration of cAMP leading to the activation of cAMP-dependent protein kinase (PKA) (Fig. 4). PKA is a heterotetramer consisting of a dimer of regulatory (R) subunits that maintains two catalytic (C) subunits in an inactive state. Upon binding of cAMP to the R subunits, active C subunits are released and can phosphorylate substrates. PKA phosphorylates serine or threonine resi-

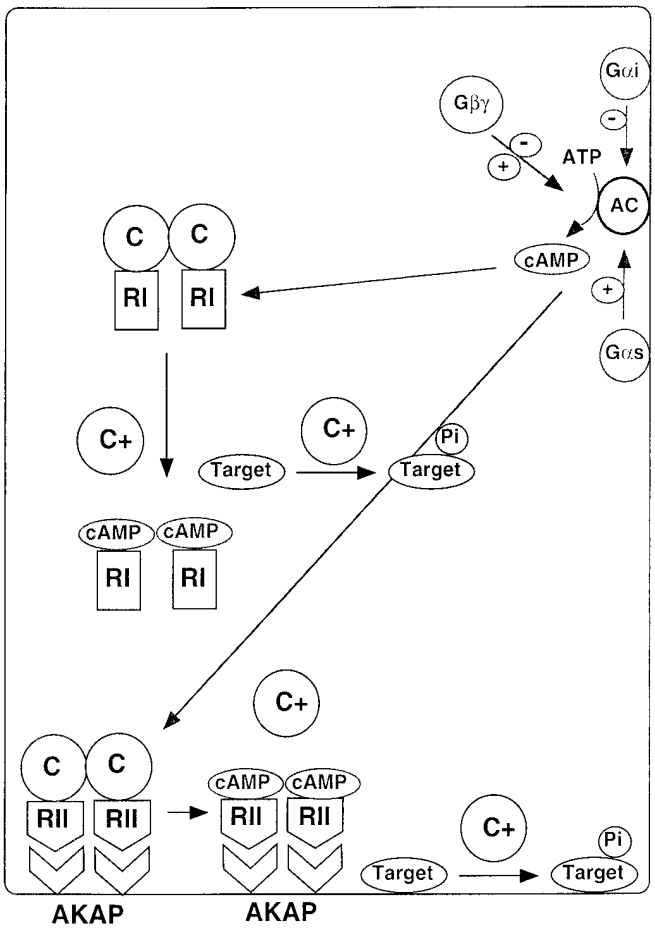

FIG. 4. Schematic overview of the cAMP-PKA signaling pathway. Activation of cell-surface serpentine receptors (not shown) induces the dissociation of heterotrimeric G proteins into free α-subunits and $\beta\gamma$-subunits that may either activate or inhibit adenylyl cyclases (AC). The cAMP generated by adenylyl cyclases binds to the regulatory subunits (RI or RII) of PKA, allowing the dissociation of the catalytic subunits (C). The free catalytic subunits (C+) phosphorylate target membrane or cytosolic proteins. Subcellular targeting of PKA is mediated by interactions between A-kinase anchoring proteins (AKAPs) and the RII isoform of the regulatory subunit. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets.

dues located in consensus sites exhibiting the Arg-Arg-Xaa-Ser/Thr or Lys-Arg-Xaa-Xaa-Ser/Thr motifs. To date, three isoforms of C subunit (α, β, and γ) and two isoforms of R subunits (RI and RII) have been identified in mammals. Some level of specificity of the PKA signal relies on the different affinities for substrates of C subunit isoforms as well as their binding properties to regulatory subunits (320). In addition, the RI and RII subunits exhibit different cAMP binding affinities and differential subcellular localization (203, 797). The type I PKA holoenzyme (containing RI) is predominantly cytoplasmic, whereas the type II PKA holoenzyme (containing RII) is mostly targeted to several subcellular compartments through binding to A-kinase anchoring proteins (AKAP) (203), conferring a further level of specificity. It is interesting to mention that some AKAP, e.g., AKAP79, can act as scaffolding proteins and also bind PKC isozymes (469) and protein phosphatase 2B (179).

Recent pieces of evidence suggest the presence of alternate PKA-independent cAMP signaling pathways. Two intracellular cAMP-binding proteins were cloned and shown to act as guanine nucleotide exchange factors for the small G protein Rap-1 (207, 457). Thus cAMP can activate the Rap-1 pathway independently of PKA activation. Interestingly, one of these two cAMP binding proteins (Epac or cAMP-GEF-I) is expressed at very high levels in the kidney (207, 457). Further studies are needed to identify the functional targets of the cAMP/Epac/Rap-1 pathway, in particular in kidney.

Termination of the signal is accounted for by activation of compartimentalized phosphodiesterases (169, 729) and desensitization of adenylyl cyclases and receptors (225, 354, 434, 516, 863).

2. Receptors coupled to G proteins and phospholipase C

In addition to the modulation of adenylyl cyclase activity, peptide hormones, e.g., parathyroid hormone and angiotensin II, and catecholamines, e.g., norepinephrine and dopamine, may trigger G protein-mediated activation of phospholipase C (PLC)-β and subsequent PKC activation.

PKCs constitute a superfamily of protein kinases comprising 11 isozymes. These isozymes are currently grouped into three families (Fig. 5) according to their sensitivities to physiological and pharmacological activators (511, 553). The most studied group is the conventional PKCs, which includes the α-, βI-, βII-, and γ-isoforms. PKC-βI and -βII isoforms are generated by alternative splicing of the same gene. Conventional PKCs are activated by phosphatidylserine (PS) in a diacylglycerol (DAG)/phorbol esters and calcium-dependent manner. DAG and phorbol esters decrease the calcium concentration required for activation and dramatically increase PKC sensitivity to PS. PKCs δ, ϵ, η, and θ are members of the novel PKC family. These isozymes are calcium insensitive but remain activated by DAG and phorbol esters in the presence of PS. Finally, the members of the atypical PKC family, PKC-λ and PKC-ζ, are insensitive to calcium, DAG, and phorbol esters, and their mode of activation is not clearly established. PKCs phosphorylate serine or threonine residues located in consensus sites exhibiting the Ser/Thr-Xaa-Lys/Arg motif. A first level of specificity is conferred by the cell-specific expression of PKC isozymes. For instance, the kidney expresses high levels of the classical PKC-α but, in contrast to brain, β- and γ-isozymes are undetectable (451, 612). The novel PKC-δ and -ϵ as well as the atypical PKC-ζ are also ex-

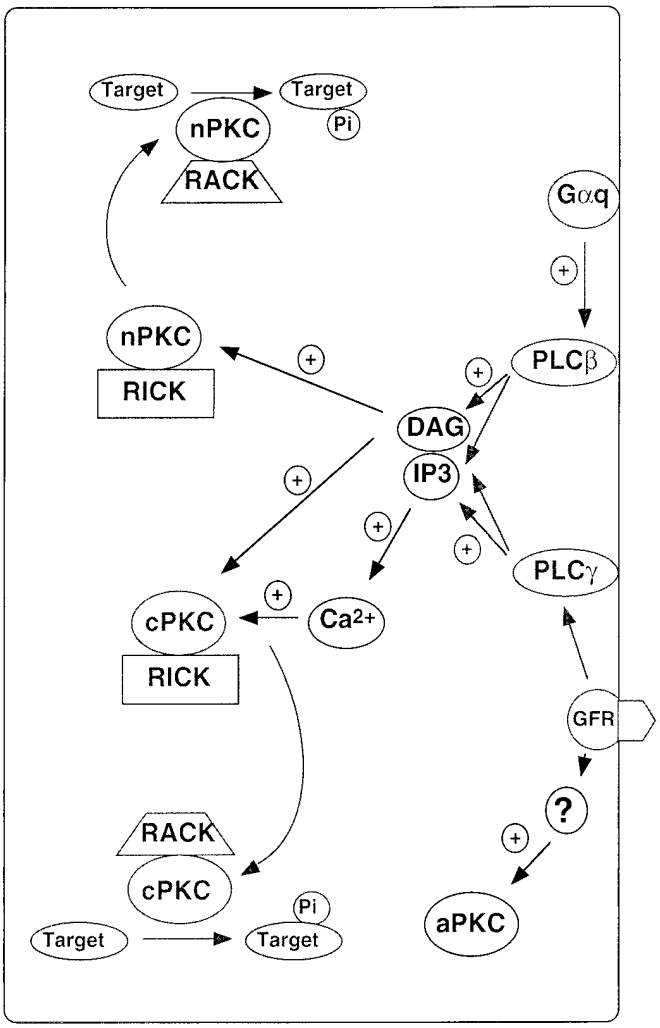

FIG. 5. Schematic overview of the PKC signaling pathway. Activation of cell-surface serpentine receptors (not shown) induces the dissociation of heterotrimeric G proteins into free α-subunits and $\beta\gamma$-subunits that may activate phospholipase C-β (PLC-β). Alternatively, growth factor receptors (GFRs) may be coupled to the PLC-γ isoform. PLCs generate diacylgylcerols (DAG) and inositol trisphosphate (IP_3) that increases cytosolic calcium. The classical isoforms of PKC (cPKC) are activated by calcium and DAG, the novel isoforms of PKC (nPKC) are calcium insensitive but DAG activated, whereas the atypical PKC (aPKC) are both calcium and DAG insensitive. The current model for PKC targeting to specific subcellular compartments assumes that once activated, PKC isozymes translocate from receptors for inactive C-kinase (RICK) to receptors for activated C-kinase (RACK). Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+)effect on their targets.

pressed in the kidney cortex and medulla (451, 612). A second level of specificity is conferred by the differential pattern of PKC isoform activation in response to an agonist. In the kidney PCT, PKC-α, -δ, and -ϵ are activated in response to phorbol esters, whereas only PKC-α and -ϵ are activated in response to angiotensin II (451). The identification of PKC isozyme-specific anchoring proteins provides further specificity to the signal by targeting either active or inactive PKC to various subcellular compartments (505, 558).

3. *Tyrosine kinase receptors*

Insulin and many growth factors, e.g., IGF-I, EGF, and platelet-derived growth factor (PDGF), share the property to bind to receptors exhibiting an intrinsic tyrosine kinase activity (Fig. 6). Binding of the ligand to the receptor activates its tyrosine kinase activity and induces receptor autophosphorylation as well as phosphorylation of substrate proteins on tyrosine residues (458). It is generally admitted that growth factor receptors form homodimers either in the absence of ligand, e.g., PDGF

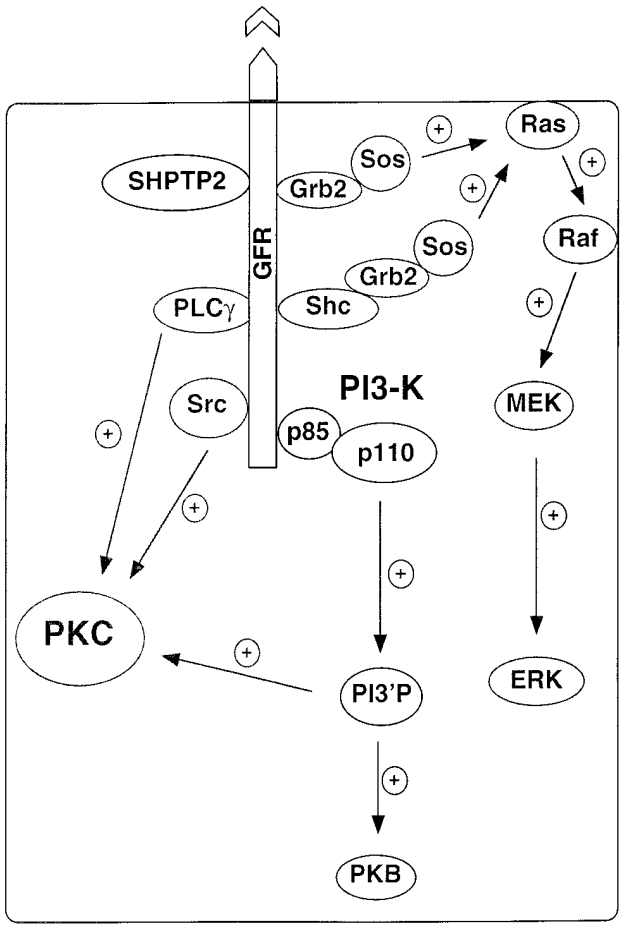

FIG. 6. Schematic overview of growth factor receptor signaling. Once activated by ligand binding, GFRs dimerize and undergo autophosphorylation on specific tyrosine residues. Phosphotyrosines generate binding sites for SH2 and PTB domains. For instance, phosphorylated receptors can bind PLC-γ, the nonreceptor tyrosine kinase Src, the tyrosine phosphatase SHPTP2, the p85 subunit of the phosphatidylinositol 3-kinase (PI 3-K), and the adaptor proteins Grb2 and Shc. PLC-γ, Src, and PI 3-K link GFR to the PKC pathway, PI 3-K links GFR to the protein kinase B (PKB) pathway, and Grb2 links GFR, directly or through Shc binding, to Sos which activates Ras leading to Raf, MEK, and ERK activation. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) effect on their targets.

receptor, or after ligand binding, e.g., EGF receptor, and that autophosphorylation results from the phosphorylation of one receptor monomer by the other (382). Because it is well established for EGF and PDGF receptors, tyrosine phosphorylation of the receptor creates specific binding sites for SH2 or PTB domains contained in many proteins involved in the subsequent steps of the signaling cascade (180, 455, 458, 499). These interactions, together with growth factor receptor aggregation, induce the focal formation of signaling complexes. In the insulin signaling pathway, tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) instead of the insulin receptor itself mediates the binding of signaling intermediates (875, 876). Receptor tyrosine kinases and IRS-1 can be associated with nonreceptor tyrosine kinases of the Src family (783, 824), phospholipase C-γ (474, 913), the regulatory p85 subunit of phosphatidylinositol 3-kinase (PIK) (35, 258, 452, 474), the adaptor proteins Grb2 (582, 857) or Shc (93, 357), and the tyrosine phosphatase SHPTP-2 (74, 488, 905). These protein-protein interactions may link receptor protein kinases to *1*) a second round of tyrosine phosphorylation of specific target proteins by nonreceptor tyrosine kinases, e.g., c-Src (458, 614, 761); *2*) PKC activation through activation of PLC-γ and PIK (205, 458, 568), as well as isoform-specific tyrosine phosphorylation, e.g., PKC-δ (205, 477) and PKC-θ (677); *3*) PIK-dependent generation of phosphatidylinositol 3-products (132, 661) and activation of protein kinase B (297); and *4*) Ras-dependent activation of extracellular regulated kinases (ERK) secondary to the binding of Sos (a guanine nucleotide exchange factor for Ras) to Grb2 and Shc (521, 650, 666) and activation of Raf-1 kinase by active Ras (877).

Several mechanisms participate in the termination of the signal: dephosphorylation of receptors and signaling complexes by tyrosine phosphatases (174, 263, 777, 858), serine-threonine phosphorylation of receptors (265, 569, 807) and docking proteins, e.g., IRS-1 (626, 794), and internalization of activated receptors through clathrin-coated pits (133, 437, 754, 763, 883).

4. Nuclear receptors

Traditionally, steroids, thyroid hormone, vitamins A- and D-derived hormones, and some fatty acids are thought to primarily alter the transcription of specific mRNAs and protein synthesis. This mechanism of action was initially proposed on the basis of the sensitivity of the functional actions of these agents to inhibitors of transcription and translation such as actinomycin D and cycloheximide (644). It also accounts for the latency and slow development of the cells responses to these agents. Later, this hypothesis was confirmed by cloning the cDNAs encoding intracellular receptors for these factors (61, 256, 312). Indeed, steroids and related agents bind to a superfamily of intracellular receptors that interact with regulatory elements of DNA, and thereby control the transcription of specific genes.

Steroid-thyroid-retinoid receptors consist of three major distinct domains: an immunogenic domain, a DNA binding domain, and a hormone binding domain. The DNA binding domain, which is the best conserved among different steroid receptors, consists of ~70 amino acids and contains two zinc finger structures (in which cysteines are coordinated by zinc), each followed by an α-helix domain. These structures are important for recognition and binding to DNA and for dimerization of the receptor (256). The DNA binding domain recognizes specific target sequences on the DNA, called hormone response elements (HREs), which are very similar for the different receptors. For example, glucocorticoid, progesterone, androgen, and mineralocorticoid receptors recognize the same glucocorticoid response elements (GREs) with the consensus sequence 5′-NGGTACANNNTGTTCTN-3′. The hormone binding domain, a well-conserved region among distinct steroid receptors, consists of 250 amino acids forming an hydrophobic pocket that participates not only to hormone binding but also to the dimerization of the receptor, its nuclear translocation, and the resulting activation of the transcription (264). This region contains also the binding site for the 90-kDa heat shock protein (HSP90), a chaperone associated with the unbound receptor that facilitates the hormone response (636). The NH_2-terminal domain is highly variable between steroids and species. Its varies from 25 to 602 amino acids for the vitamin D and the mineralocorticoid receptors, respectively. This hypervariable region contains the epitopes of most antireceptor antibodies and is therefore called the immunogenic domain. Along with the hormone binding domain, it displays transactivation activity.

Circulating steroids are supposed to reach their intracellular receptors by passive diffusion across the cell membrane, although active or facilitated transport mechanisms have been proposed (12, 106, 827). Hormone binding activates the receptor and confers it the ability to interact with DNA. In case of the mineralocorticoid receptor, activation is associated with the release of HSP90 (658). Finally, receptor/DNA interaction induces the activation/repression of several genes whose protein products modify the functional properties of target cells. From a kinetic point of view, functional alterations are usually classified as early and late responses, but the overall response should better be considered as a continuous cascade of induction/repression of several genes encoding proteins, with various maturation and life span, acting either as transcription factors or as peripheral effectors accounting for the phenotypic changes.

In addition to this traditional mode of action of steroids, several other mechanisms may be involved. First, genomic effect via a direct interaction between the ste-

roids and nuclear DNA has been described (385, 825). Second, several pieces of evidence suggest nongenomic response via transduction through membrane receptors (reviewed in Ref. 862). This would account, for example, for very rapid responses such as the quasi-immediate inhibition of corticotropin release induced by abrupt increase in the blood concentration of cortisol. This mechanism is supported by ligand-binding experiments that revealed high-affinity membrane binding sites for several steroids (610, 861). However, the molecular characterization of these receptors is not yet available. Finally, steroid receptors may trigger cell response by protein-protein interaction (548), without binding to DNA. For example, glucocorticoid receptors have been shown to modulate AP-1- and NFκB-induced transcription by direct protein-protein interaction (313, 634). In support of this mechanism, it was recently shown that transgenesis expression of a mutant glucocorticoid receptor unable to bind DNA allows survival of the mice, whereas the deletion of glucocorticoid gene is lethal (665).

B. Hormone Receptors and Signaling Pathways Along the Nephron

The following discussion on hormone receptors and cognate signaling pathways is restricted to the three renal target selected, i.e., the proximal tubule, TAL, and collecting duct principal cell.

1. Parathyroid hormone

In the late 1960s, the pioneering work of Chase and Aurbach showed that in vivo infusion of parathyroid hormone (PTH) increases urinary excretion of cAMP (154) and that in vitro addition of PTH stimulates adenylyl cyclase activity in kidney cortex homogenates (155). A few years later, the precise mapping of PTH-responsive nephron segments was obtained by measuring the in vitro effect of PTH on adenylyl cyclase activity in microdissected nephron segments (416). PTH-sensitive adenylyl cyclase was evidenced in PCT, PST, and CTAL from rat, rabbit, and human kidney (142, 143, 566) and in MTAL from some but not all species (143).

Although PTH receptors may be coupled to transducers other than adenylyl cyclase (see below), further attempts to localize renal PTH receptors that were not based on stimulation of adenylyl cyclase confirmed this distribution profile. The presence of PTH receptors in proximal tubules and CTAL was found by ligand binding (311, 682) and by localization of the mRNAs encoding the cloned PTH/PTH-related peptide (PTHrP) receptor (490, 674, 914). In contrast, the PTH-2 receptor cloned from brain is not expressed in the kidney (70, 833).

In rat proximal tubules, PTH receptors are expressed at both the apical and basolateral plasma membrane (311, 388) (Fig. 7). However, adenylyl cyclase is much better stimulated by basolateral than apical PTH (311), in agreement with in situ microperfusion study of the rat PCT showing a small effect of luminal with respect to basolateral PTH (59). Poorly efficient coupling between apical PTH binding and adenylyl cyclase is consistent with the recent demonstration of specific binding of full-length PTH and its biologically active NH_2-terminal fragments to megalin (388). Megalin is a proximal tubule multifunctional endocytic receptor (172) that likely accounts for the largest part of specific PTH binding to apical membranes of proximal tubule cells (388). Thus biological effects of PTH on the proximal tubule are essentially mediated by basolateral PTH/PTHrP receptors (59), whereas apical megalin (Fig. 7) may account for the cel-

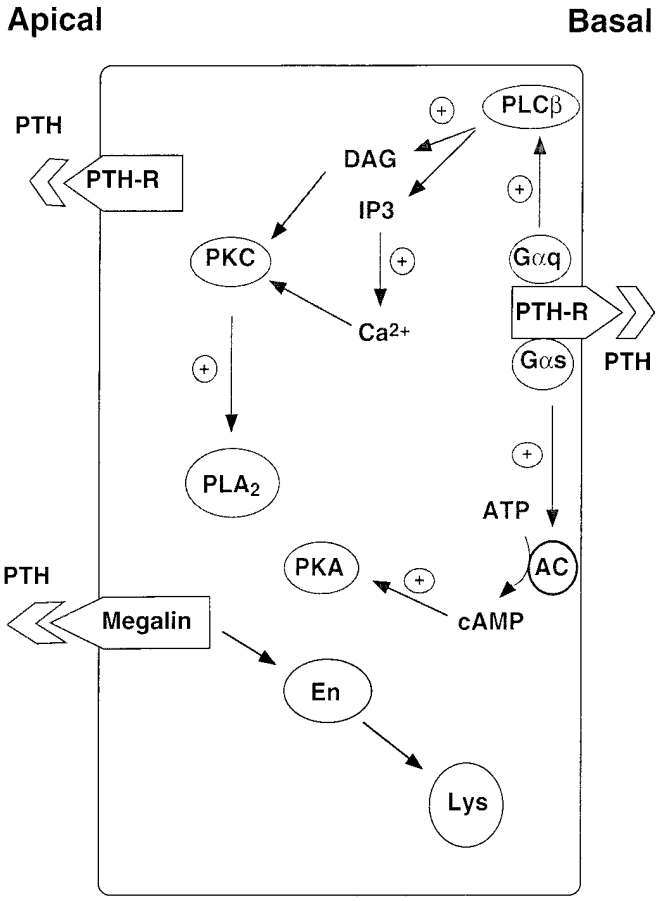

FIG. 7. PTH signaling in proximal tubule cells. Binding of PTH to basolateral PTH receptor (PTH-R) activates $G\alpha_s$ and $G\alpha_q$ that couple to adenylyl cyclase (AC) and PLC-β, respectively. Activated PLC generates DAG and IP_3, which increases cytosolic calcium. Calcium and/or DAG activate PKC isozymes which in turn may activate phospholipase A_2 (PLA_2). AC generates cAMP, which activates PKA. On the apical side, PTH may also bind PTH-R but might preferentially bind megalin, which undergoes endocytosis (En) leading to PTH degradation in lysosomes (Ly). Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) effect on their targets.

lular uptake and subsequent lysosomal degradation of filtered PTH (388).

In proximal tubules, PTH not only activates adenylyl cyclase but also PLC and PKC (229) (Fig. 7). Indeed, PTH dose-dependently stimulated inositol trisphosphate (IP_3) and DAG production and/or increased cytosolic calcium in rat PCT suspensions (288, 642, 792) as well as in primary cultures of dog proximal tubules and OK cells (400, 401).

The coupling of the PTH/PTHrP receptor to both adenylyl cyclase and PLC is further attested by its link with both $G\alpha_s$ (that activates adenylyl cyclase) and $G\alpha_{q/11}$ (that activates PLC) in osteoblast-like cells and transfected human embryonic kidney cells (724). This dual coupling of PTH/PTHrP receptor is dependent on different structural determinants of PTH, since various NH_2-terminal fragments of PTH activate PKC either alone or together with PKA in transfected AP-1 cells (34).

In addition to PKA and PKC, PTH also activates phospholipase A_2 (PLA_2) in rat PCT (208, 608, 672). The presence of a PTH-mediated control of mitogen-activated protein (MAP) kinases remains controversial (837, 838).

2. Dopamine

Dopamine binds to two pharmacologically distinct groups of receptors (556): D_1-like receptors (formerly central D_1 and peripheral DA_1 receptors) and D_2-like receptors (formerly central D_2 and peripheral DA_2 receptors). Each of these groups consists of molecularly distinct isoforms: two forms of D_1-like receptors (called D_1 and D_5 receptors, or D_{1A} and D_{1B} receptors in rodents) and three forms of D_2-like receptors (D_2, D_3, and D_4 receptors) have been cloned. Both D_1-like and D_2-like receptors are expressed in kidneys.

Expression of D_1-like receptors was first demonstrated in PCT, PST, MTAL, CTAL, and CCD by autoradiography using specific ligands (271, 406, 462, 675, 790). Expression of the D_{1A}-D_1 receptor isoform was confirmed at mRNA and protein levels in rat proximal tubules (603, 604, 912), as well as in OK and LLC-PK_1 cells (448, 589), and in human CCD (603, 604, 617), whereas it was hardly detected in human and rat medulla (603, 604, 617). Expression of D_{1B}-D_5 receptor isoform remains to be demonstrated. D_1-like receptors are expressed on both apical and basolateral membranes (271, 604) (Fig. 8).

D_2-like binding sites (Fig. 8) were demonstrated in proximal tubules (271) and attributed to the D_2 and D_3 receptor isoforms (321, 605, 760). D_2 and D_3 receptor mRNAs were also detected in the rat outer medulla (321), but the D_3 receptor protein was not detected in the rat TAL (605). D_3 and D_4 receptors were also evidenced both at mRNA and protein levels in rat CCD (605, 782). In summary, the renal expression level of dopamine receptors is high in proximal tubules, intermediate in CCD, and low in TAL.

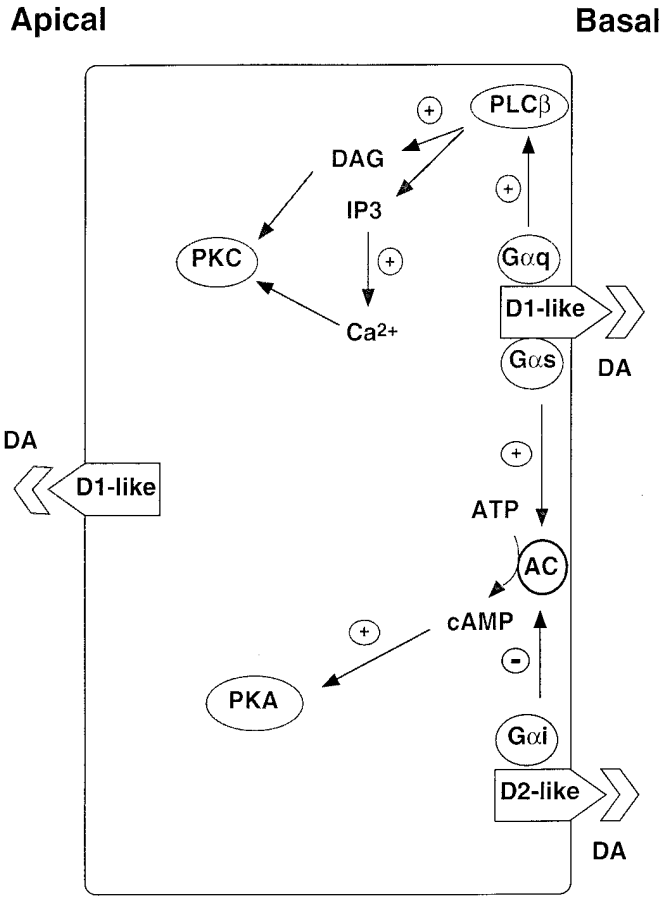

FIG. 8. Dopamine (DA) signaling in proximal tubule cells. Binding of DA to D_1-like receptors expressed in both apical and basolateral membrane domains activates $G\alpha_s$ and $G\alpha_q$ that couple to AC and PLC-β, respectively. Activated PLC generates DAG and IP_3, which increases cytosolic calcium. Calcium and/or DAG activate PKC isozymes. AC generates cAMP which activates protein kinase A (PKA). Binding of DA to D_2-like receptors activates $G\alpha_i$ which inhibits AC. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets.

It is generally assumed that dopamine D_1-like and D_2-like receptors are coupled to adenylyl cyclase activation through $G\alpha_s$ and inhibition through $G\alpha_i$, respectively (409, 441, 556). However, the D_1 receptor was shown to coprecipitate with $G\alpha_q$ (271), and its dual coupling to both $G\alpha_s$ and $G\alpha_{q/11}$ was demonstrated in basolateral membranes from rat proximal tubules (408). In this preparation, the dopamine D_1 receptor agonist fenoldopam induced a dose- and time-dependent binding of equivalent amounts of [^{35}S]GTPγS to $G\alpha_s$ and to $G\alpha_{q/11}$. Coupling of D_1-like receptors to $G\alpha_q$ may account for the reported activations of PLC-β, phosphoinositide breakdown, and PKC activation (448, 846, 921, 922) in suspensions of rat proximal tubules (Fig. 8). Activation of PLC and PKC likely accounts for dopamine-induced generation of ara-

chidonic acid reported in isolated rat PCTs (608). Fenoldopam also reduced the proportion of membrane-associated growth factor-activated PLC-γ in rat kidney cortex, but not in kidney medulla (921), suggesting that activation of D_1-like receptors reduced PLC-γ activity.

The membrane recruitment of various PKC subtypes in response to D_1-like agonists was studied in suspensions of rat proximal tubules obtained after in vivo infusion of drugs into the renal artery (915). Under these conditions, D_1-like agonists did not alter the cellular distribution of the classical PKC-α and atypical PKC-λ, whereas they increased the membrane abundance of the new PKC-θ and decreased that of the new PKC-δ and atypical PKC-ζ. In contrast to this latter finding, stimulation of PKC-ζ was suggested on the basis of its requirement for dopamine effect on sodium transport in OK cells (236). This discrepancy may rely on that dopamine binds to both D_1-like and D_2-like receptors and that activation of PKC-ζ might be dependent on the activation of both receptors subtypes, as shown previously for the effect of dopamine on Na^+-K^+-ATPase activity in guinea pig neostriatal neurons (84) and rat PCT (82).

Coupling between MAP kinases and dopamine receptors has been described in nonrenal tissues (525, 874, 930). However, this coupling remains to be established in renal epithelial cells.

3. Adrenergic receptors

Epinephrine, mainly synthesized by adrenal glands, reaches its renal targets through the blood circulation, whereas norepinephrine is mainly released locally by renal nerve endings. Epinephrine and norepinephrine bind to several classes of receptors that differ by their pharmacological and biological properties. These receptors, members of the G protein-coupled receptor superfamily, are classified as α_1-, α_2-, and β-receptors.

A) α_1-ADRENERGIC RECEPTORS. Based on pharmacological properties, α_1-adrenergic receptors were subdivided into α_{1A}- and α_{1B}-subtypes. Later, three molecular species of α_1-adrenergic receptors were cloned (α_{1A}, α_{1B}, and α_{1D}) (216).

The presence of α_1-adrenergic receptors in rat and human kidney cortex and outer medulla as well as in proximal tubules (Fig. 9) and MTALs was first evidenced by ligand binding studies (273, 274, 435, 545, 574, 831). Although the three α_1-isoforms are expressed in proximal tubules (273, 506a, 830), the α_{1A} predominates (65%) in rat PCT (273), whereas in TAL, only the α_{1B}- and α_{1D}-isoforms are expressed (273, 830). The presence of α_1-adrenergic receptors in collecting duct is more controversial: they were not detected in an initial study (830), whereas all three isoforms (α_{1A}, α_{1B}, and α_{1D}) were later found in rat CCDs (881).

α_1-Adrenergic receptors couple predominantly to pertussis toxin-insensitive $G\alpha_{q/11}$ proteins (903) that, in association with Gβγ (904), activate PLC-β (749, 921) and trigger the IP_3/DAG/PKC cascade (42, 907) (Fig. 9). In nonrenal cells, α_1-adrenergic receptors can be coupled to *1*) phospholipase D and phosphatidylcholine hydrolysis (42, 750), *2*) PLA_2 activation and arachidonate generation (629, 873, 907), and *3*) ERK activation (191, 204, 528), but there is currently no evidence for such coupling in renal epithelial cells.

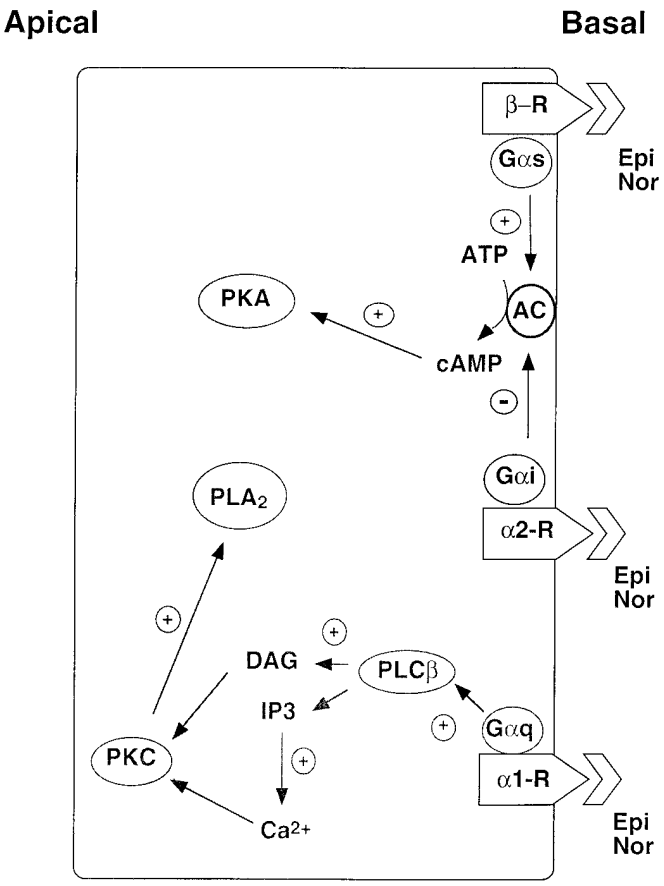

FIG. 9. Adrenergic agonists signaling in proximal tubule cells. Binding of norepinephrine (Nor) or epinephrine (Epi) to α_1-adrenergic receptors (α_1-R) leads to the activation of $G\alpha_q$ that couples PLC-β. Activated PLC generates DAG and IP_3, which increases cytosolic calcium. Calcium and/or DAG activate PKC isozymes, which in turn may activate PLA_2. α_2-Adrenergic receptors (α_2-R) are coupled to $G\alpha_i$, which inhibits AC. β-Adrenergic receptors (β-R) are coupled to $G\alpha_s$, which activates AC. The generation of cAMP by AC activates PKA. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets.

B) α_2-ADRENERGIC RECEPTORS. At least three isoforms of α_2-adrenergic receptors (α_{2A}, α_{2B}, and α_{2C}) account for the pharmacologically defined α_2-adrenergic receptors (216). The presence of α_2-adrenergic receptor was first suggested in kidney cortex and PCT on the basis of the ability of epinephrine and of specific α_2-receptor agonists to inhibit PTH-stimulated adenylyl cyclase in vitro and in vivo (829, 897, 898). Ligand binding studies confirmed this hypothesis in rat, guinea pig, and human kidney cortex

(575, 831, 919) and in rat proximal tubules (435, 473, 545, 784) (Fig. 9) and further demonstrated expression of α_2-adrenergic receptors in rat and human kidney medulla (575, 831) as well as in rabbit MTAL (561). In proximal tubules, α_2-adrenergic receptors are twice more abundant than α_1-adrenergic receptors (435, 831) and are mainly accounted for by the α_{2B}-isoform (473, 545, 550) present at the basolateral cell border (403). In contrast, the $\alpha_{2A/C}$-receptor isoform accounts for α_2-adrenergic receptors expressed in OMCD (550) where the α_{2B} is not detected (403, 550).

The negative coupling of α_2-adrenergic receptors to adenylyl cyclase via pertussis toxin-sensitive $G\alpha_i$ protein was recognized first (518, 897, 898) (Fig. 9). In nonrenal cells, α_2-adrenergic receptors coupling to $G\alpha_q$ and PLC-β activation (147, 332) and to activation of ERK pathway (10, 204) has been described.

c) β-ADRENERGIC RECEPTORS. The β-adrenergic receptors are subdivided into three molecular subtypes called β_1, β_2, and β_3 (214, 301, 581). β-Adrenergic receptors were first revealed by radioligand binding studies in rat proximal tubules (Fig. 9), although at a much lesser density than α-adrenergic receptors (784). Further functional characterization of β-adrenergic receptors, based on increased cAMP production in response to the preferential β_2-agonist isoproterenol, indicated marked species differences in the proximal tubule expression of β-adrenergic receptors; in rat they are expressed in native and cultured proximal tubules (365, 429); in mouse, guinea pig, and dog kidney they are only found in PST but not in PCT (39, 494, 577), whereas they are not detected in either PCT or PST from rabbit kidney (39, 244) although functional effects were described (71). In primary cultures of rat proximal tubule cells grown on semi-permeable filters, β-adrenergic receptors are found on both apical and basolateral membrane domains (365). However, such a distribution needs to be confirmed in native tissue, since cell dedifferentiation, with loss of membrane polarity, occurs very rapidly in primary cultures of renal epithelial cells.

β-Adrenergic receptors are also found in mouse MTAL (39) and in rat outer medulla (550). Both β_1- and β_2-adrenergic receptors are expressed in the rat CCD and OMCD (535), most likely in intercalated cells (374), and therefore do not participate to the control of sodium transport.

The classical positive coupling of β_2-adrenergic receptors to the $G\alpha_s$/adenylyl cyclase/PKA cascade is also found in proximal tubule cells (365, 429, 577) (Fig. 9). In nonrenal cells, β_2-adrenergic receptors also activate MAP kinases of the ERK family through $\beta\gamma$-subunits of pertussis toxin-sensitive G protein and activation of Ras (7, 193, 204, 526, 527).

4. Angiotensin II

Two pharmacologically distinct subtypes of angiotensin II (ANG II) receptors have been cloned: AT_1 receptors (580) mediate hemodynamic (426, 607, 778, 817) and sodium-retaining effects (139, 442) of ANG II, whereas AT_2 receptors (446, 573) appear to counteract the effects of AT_1 receptors on hemodynamics and sodium balance (381, 746). Conversely to humans who exhibit a single AT_1 gene, rodents display duplicated AT_1 receptor genes encoding for AT_{1A} and AT_{1B} subtypes (692, 916).

Binding studies demonstrated the presence of ANG II receptors in rat PCT and to a lesser extent in PST (572), and this was later confirmed in humans (911). AT_1 receptors were demonstrated in rat and rabbit proximal tubules at mRNA and/or protein levels (108, 123, 368, 557, 625, 802). Both AT_{1A} and AT_{1B} isoforms of ANG II receptors are expressed in rat proximal tubules (although the AT_{1A} isoform predominates) (108) and are functionally undistinghishable (108, 160, 426, 692, 817). AT_1 receptors are expressed at the apical and basolateral poles of proximal tubule (58, 368, 498, 564, 653, 655). Expression of AT_1 receptor mRNAs (predominantly AT_{1A}) was also found in rat TAL and collecting duct (108) and was functionally confirmed in rat TAL (108) and CCD (108, 715). Functional (513, 745) and biochemical (175) evidence indicate that AT_2 receptors are expressed in the kidney, and a recent study has demonstrated the presence of AT_2 receptor mRNA and protein in proximal tubules (557) (Fig. 10).

As in other cell types (711), proximal tubule AT_1 receptors are coupled to activation of PLC-β through $G\alpha_q$ protein (712), IP_3 generation, increased intracellular calcium concentration (108, 218, 443, 641), and activation of the classical PKC-α and novel PKC-ϵ (451). In proximal tubule cells, ANG II is also linked to the classical $G\alpha_i$-mediated inhibition of adenylyl cyclase (509, 641, 804, 896) (Fig. 10). ANG II-induced inhibition of cAMP production in PCT may also result from increased intracellular calcium concentration because calcium-inhibited type 6 adenylyl cyclase is expressed in this tissue (141).

In addition to positive coupling with PLC and negative coupling with adenylyl cyclase, increasing pieces of evidence indicate that angiotensin receptors may trigger PLA_2 and MAP kinases. In primary cultures of rabbit proximal tubule cells, ANG II dose-dependently stimulated an apical membrane-associated PLA_2 activity (64, 370, 428) and thereby increased arachidonic acid release (428). ANG II-induced generation of arachidonic acid was also observed in LLC-PK_1 cells (64). Stimulation of PLA_2 by ANG II is mediated by AT_2 receptors (Fig. 10), since it is prevented by the specific AT_2 antagonist PD123319, whereas the specific AT_1 antagonist losartan is ineffective (228).

Another potentially important signaling pathway triggered by ANG II, in particular in proximal tubule cells (228, 801), is the ERK pathway (562, 719, 920). MAP kinases ERK1–2 (877) are activated by ANG II through at least two independent pathways: a PKC-dependent and

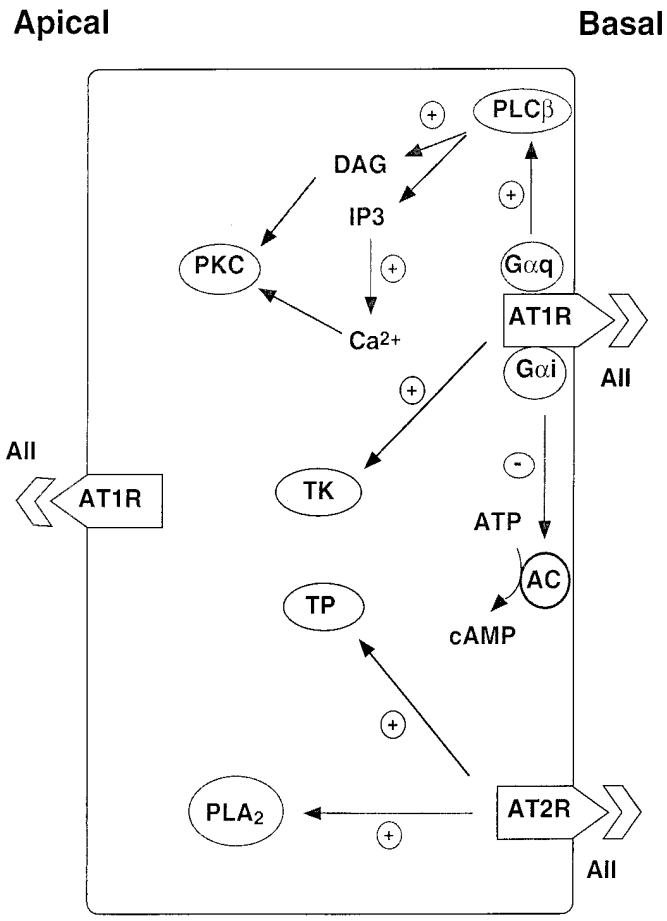

FIG. 10. Angiotensin II (ANG II) signaling in proximal tubule cells. Binding of ANG II to AT_1 receptors (AT_1R) expressed at both apical and basolateral membrane domains activates $G\alpha_i$ and $G\alpha_q$ that couple negatively to AC and positively to PLC-β, respectively. Activated PLC generates DAG and IP_3, which increases cytosolic calcium. Calcium and/or DAG activate PKC isozymes. AT_1 receptors may also couple to several tyrosine kinases (TK). Binding of ANG II to AT_2 receptors (AT_2R) activates PLA_2. In addition, AT_2 receptors might be coupled to the activation of tyrosine phosphatases (TP). Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets.

Ras-independent pathway (501, 787, 933), and a tyrosine-kinase and Ras-dependent pathway (238, 421, 691, 713). The latter pathway is likely mediated through Src-dependent (421, 713) transactivation of EGF receptors (237, 239, 576). Intriguingly, activation of AT_2 receptors leads to ERK1–2 inhibition in neurons (402), whereas ERKs are activated through an AT_2 receptor-mediated activation of PLA_2 and subsequent generation of arachidonic acid in cultured renal proximal tubule cells (228).

In nonrenal cells, ANG II activates several tyrosine kinases (Fig. 10) through AT_1 receptors (689), including *1*) Pyk-2, a calcium-regulated tyrosine kinase (114, 237, 689); *2*) the Janus kinase Jak-2, which phosphorylates transcription factors of the STAT family (500, 536, 836); *3*) c-Src (237, 500, 689, 705); and *4*) the EGF receptor, which is transactivated by c-Src (239, 576). Activation of AT_2 receptors exert opposite actions through the stimulation of tyrosine phosphatase(s) (120, 227, 584). The AT_1-mediated activation of tyrosine kinases remains to be demonstrated in renal epithelial cells.

5. Vasopressin

Two pharmacologically defined subtypes of vasopressin receptors found in the kidney have been cloned: the V_2 receptor (517) classically coupled with the activation of adenylyl cyclase and the V_{1a} receptor (565) coupled with the activation of PLC and the release of intracellular calcium stores (Fig. 11).

The pioneering work of Chase and Aurbach (155) showed that in vitro addition of vasopressin stimulated adenylyl cyclase activity in kidney medulla homogenates. Several years later, the precise mapping along the nephron of vasopressin response was determined by measuring the in vitro effect of vasopressin on adenylyl cyclase activity in microdissected nephron segments (417). Vasopressin-sensi-

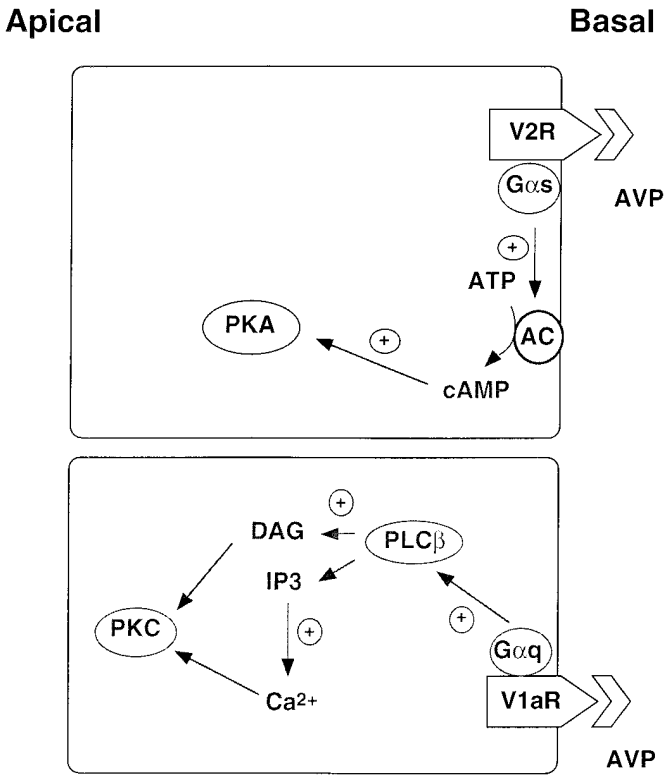

FIG. 11. Vasopressin (AVP) signaling in principal cells of the collecting duct. Binding of AVP to V_2 receptors (V_2R) activates $G\alpha_s$ that couples to AC. The generation of cAMP by AC activates PKA. Conversely, binding of AVP to V_{1a} receptors ($V_{1a}R$) coupled to PLC-β generates diacylglycerols (DAG) and IP_3, which increases cytosolic calcium. Calcium and/or DAG activate PKC isozymes. V_2R and $V_{1a}R$ might be expressed either by the same cell type or by different cell types. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets.

tive adenylyl cyclase was evidenced in CTAL and MTAL from rat and rabbit (417, 418, 815), but not human kidney (142), whereas CCD and OMCD from all three species responded to vasopressin (142, 417, 418). These results are consistent with the expression of the cloned V_2 receptor (517) in these nephron segments. The expression of V_2 receptor in TAL and along the collecting duct was confirmed at the mRNA level by RT-PCR on microdissected rat nephron segments (293, 613, 803) and at the protein level by immunohistochemistry on rat kidney sections (597). In addition to the basolateral expression of V_2 receptors in all vasopressin-responsive segments, a significant level of apical expression was observed in the terminal IMCD (597).

The presence of V_{1a} receptors in vasopressin-responsive nephron segments was suspected on the basis of a vasopressin-induced increase in intracellular calcium (194, 414, 422, 769) and phosphoinositide breakdown (171, 798). Pharmacological studies with specific inhibitors of either V1 or V_2 receptors have indicated that in rat and rabbit CCD and OMCD, the vasopressin-induced increase in intracellular calcium relies on V_{1a} receptors (17, 122, 918), whereas in rat IMCD the transient increase in intracellular calcium is mediated by V_2 receptor (150, 231, 737) and/or oxytocin receptors (532). In addition, the presence of functional V_{1a} receptors has been demonstrated in rabbit CTAL (595). The presence of V_{1a} receptor in rat CCD and OMCD was subsequently confirmed by ligand binding (16), detection of mRNAs in microdissected nephron segments (293, 803), and by immunolocalization (339). In agreement with pharmacological studies, V_{1a} receptor expression was not detected in the rat terminal IMCD (339, 293).

In addition to its classical positive coupling to adenylyl cyclase, activation of the V_2 receptor (517) also induces transient increases in intracellular calcium (150, 231) independently of cAMP production (737). The cloned V_{1a} receptor (565) is classically linked to activation of PLC, generation of IP_3, increase in intracellular calcium, and activation of PKC. In nonrenal cells, V_{1a} receptors are also linked to tyrosine kinase (927) and Ras-dependent activation of MAP kinases (5, 376, 894).

6. Corticosteroids

Early [^{3}H]aldosterone binding experiments in kidney preparations revealed two high-affinity receptors, called type I (higher affinity, 1–2 nM) and type II (lower affinity, 80 nM), which were later characterized as the physiological mineralocorticoid and glucocorticoid receptors (MR and GR), respectively (272, 314, 541). Both MR and GR were cloned in the mid 1980s (27, 390).

GRs are ubiquitous, and accordingly, they were found along the whole nephron by ligand binding experiments (102, 491) and localization of mRNAs (254, 808). However, immunolocalization experiments failed to detect GR in proximal tubule (261). This intriguing result together with the absence of nuclear ligand binding in proximal tubule (102) may suggest that proximal tubules express a specific form of GR. Conversely, MRs are specifically expressed in tight epithelia. In the nephron, they were initially localized by binding experiments in the distal segments of the nephron, from DCT to IMCD, with a maximal binding capacity in CCD and OMCD (223, 262). This localization was confirmed at the mRNA level (254, 808) and by immunolocalization (479, 519, 688). In the rabbit CCD, mineralocorticoid staining is absent in 15–20% of the cells, suggesting that intercalated cells are devoid of MR, or at least that MRs are expressed at a much lower level in intercalated cells than in principal cells (519).

The colocalization of MR and GR in the distal segments of the nephron raises the problem of the specificity of corticosteroid action (259). Specifically, the question of how aldosterone may exert specific actions in the distal nephron is set by two types of observations: *1*) the MR displays a similar intrinsic affinity for physiological mineralo- and glucocorticoids, but blood glucocorticoid concentration are ~100-fold higher than aldosterone concentration so that MRs should be permanently saturated by glucocorticoids in vivo. *2*) If MRs and GRs recognize the same responsive elements (GREs) at the nuclear level, they should promote the same physiological responses.

There is no specific renal answer to the second point, but it is generally admitted that hormone-receptor complexes can interact with DNA sequences other than archetypal responsive elements, and therefore, MR and GR may have both common and different nuclear targets (313). For example, MRs and GRs were shown to have the same efficiency in GREs but to induce distinct actions on plfG, a low-affinity responsive element that binds the AP-1 transcription factor. GR repressed AP-1-induced transcription, whereas MR was inactive (627). In addition, some effects of steroids may not be mediated by receptor/DNA interaction (see sect. III*A4*).

With regard to the first point, there is clear evidence that renal mineralocorticoid target cells degrade glucocorticoids into compounds of low affinity for MRs, and thereby prevent continuous MR saturation by heterologous ligand. 11β-Hydroxysteroid dehydrogenase (11β-OHSD) catalyzes the oxidation of 11-hydroxysteroids into 11-ketosteroids, e.g., cortisol and corticosterone into cortisone and 11β-dehydrocorticosterone, respectively, two compounds displaying low affinity for the MR (315). Aldosterone target tissues only contain the type 2 11β-OHSD which, in contrast to the type 1 11β-OHSD, displays a high affinity for 11-hydroxysteroids, and is therefore the most likely candidate to endow specificity on the MR (478). Type 2 11β-OHSD activity is high in rabbit CCD and OMCD and low in aldosterone-insensitive segments (103). After cloning of type 2 11β-OHSD (9), its expression in

aldosterone-sensitive nephron segments was confirmed at the mRNA and protein levels (585).

IV. HORMONAL CONTROL OF SODIUM TRANSPORT ALONG THE PROXIMAL TUBULE

A. General Transport and Regulatory Properties of Proximal Tubules

1. Transport properties

The proximal tubule reabsorbs over 70% of the filtered sodium, potassium, chloride, bicarbonate, phosphate, and water and virtually all the filtered glucose and amino acids. The rates of reabsorption of water and of many solutes decrease from S_1 to S_3, but the cellular and molecular mechanisms underlying these transports remain mostly similar in the successive subsegments of the proximal tubule.

Luminal sodium-dependent cotransport systems (nomenclature given in parentheses) account for the active uptake of glucose (SGLT), phosphate (NaPi-2, NPT-1, and Npt-1 in rabbit, human, and mouse, respectively), sulfate (NaSi-1), amino acids, and several organic acids in the proximal tubule (Fig. 12*A*). However, most luminal sodium entry within proximal tubule cells (>80%) is directly coupled to active proton extrusion, and secondarily to bicarbonate or chloride reabsorption. Thus regulation of sodium transport in the proximal tubule is discussed only with regard to bicarbonate and chloride reabsorption processes, although other transports may be important, especially for glucose and phosphate balance.

Apical proton extrusion is mediated by Na^+/H^+ exchange (Fig. 12*A*), and to a much lesser extent by V-type H^+-ATPase. Na^+/H^+ exchangers constitute a family of

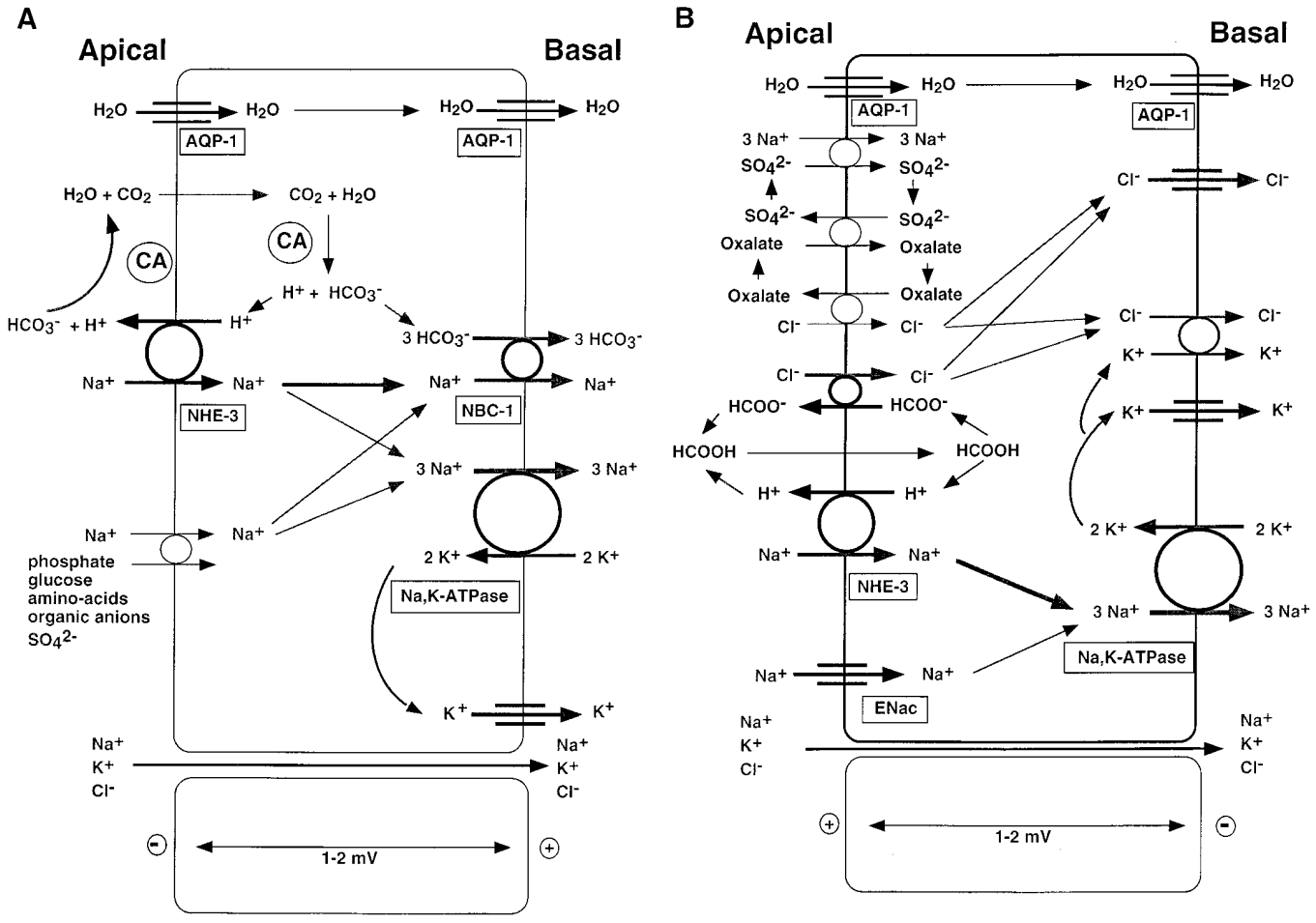

FIG. 12. Cellular mechanism of sodium, potassium, anion, and water transport in proximal convoluted tubules. The situations prevailing in early and late proximal tubule are depicted in *A* and *B*, respectively. Arrows indicate net fluxes of water and solutes. The names of the currently cloned transporters are mentioned into rectangular boxes. CA, carbonic anhydrase; AQP-1, aquaporin-1.

electroneutral antiporters that physiologically couple the downhill entry of sodium into the cell to the active extrusion of proton. Four isoforms of Na^+/H^+ exchangers (NHE-1 to -4) have been cloned and found in the kidney. Functionally, these isoforms mainly differ by their affinity for sodium (K_m from 4–18 mM for rat NHE-1 and NHE-3 to 40 mM for rat NHE-2), their sensitivity to amiloride and its derivative ethylisopropylamiloride (EIPA) (NHE-3 is less sensitive to amiloride and EIPA than other isoforms), their tissue distribution (NHE-1 is ubiquitous), and their polarity in epithelial cells (NHE-1 and NHE-4 are basolateral, whereas NHE-2 and NHE-3 are apical) (596, 620, 926). In the proximal tubule, apical proton extrusion is mainly mediated by NHE-3 (902), although the NHE-2 isoform might be also present (392).

The sequence of events ultimately resulting in net bicarbonate reabsorption is summarized by Figure 12*A*. Despite the impermeability of the luminal membrane of proximal cells to bicarbonate, proton secretion results in an equivalent bicarbonate reabsorption: the brush border contains an ecto carbonic anhydrase that allows the rapid dehydration of the carbonic acid formed from filtered bicarbonate and secreted proton. Carbon dioxide diffuses into the cellular compartment by nonionic diffusion, or possibly through the water channel aquaporin 1 (AQP-1) (182). In the intracellular compartment, where the pH is higher than in the lumen, bicarbonate is regenerated by the reverse sequence of reactions, catalyzed by intracellular carbonic anhydrase. Bicarbonate regenerated in the cells crosses passively the basolateral membrane through an electrogenic Na^+-HCO_3^- cotransport system (NBC-1) (676). This electrogenic Na^+-HCO_3^- cotransport system, which is inhibited by the stilbene derivative DIDS, couples the downhill extrusion of three bicarbonate ions to the active extrusion of one sodium ion. This improves the overall energetic efficiency of the system. For nine bicarbonate ions reabsorbed, nine sodium ions enter the cell via the apical Na^+/H^+ exchanger, six of which leave the cell through Na^+-K^+-ATPase (at the expense of the hydrolysis of 2 molecules of ATP), whereas the remaining three are transported along the Na^+-HCO_3^- transport system. Thus 4.5 sodium ions, instead of 3, are reabsorbed through the transcellular pathway for each ATP molecule hydrolyzed.

As tubular fluid flows along the proximal tubule, its pH and bicarbonate concentration decrease, whereas chloride concentration increases, because this mechanism of proton secretion favors the reabsorption of $NaHCO_3$ over that of NaCl. Luminal fluid acidification reduces the efficiency of the Na^+/H^+ exchanger by increasing the proton gradient. In the mid and terminal proximal tubule, this is palliated in part by indirect coupling of proton secretion to chloride reabsorption rather than to bicarbonate reabsorption. Two mechanisms account for this coupling (Fig. 12*B*): *1*) apical chloride/formate exchange with recycling of formate by nonionic cellular difusion of the formic acid generated by the protonation of formate in the lumen, and *2*) apical chloride/oxalate exchange with recycling of luminal oxalate through oxalate/sulfate exchange coupled to Na^+-sulfate cotransport (25, 26). Given the presence of basolateral exit pathways for chloride (both chloride channels and K^+-Cl^- cotransporters), sodium reabsorption drives chloride reabsorption. In addition, increased luminal concentration of chloride above that in the interstitium generates a driving force for its passive reabsorption via the intercellular route. In turn, this generates a lumen-positive voltage in the mid-terminal proximal tubule, which serves as driving force for intercellular cation reabsorption (Fig. 12*B*). Because the ionic conductance of the shunt pathway is high for both sodium and chloride, paracellular reabsorption fluxes of both ions are high, whereas the transepithelial voltage remains in the millivolt range.

In addition to apical sodium-coupled transporters, a recent study (885) suggested the participation of the epithelial sodium channels (ENaC) (see sect. VI*A* for properties of ENaC) in apical sodium entry in rat PCT (Fig. 12*B*). This conclusion is based on the following: *1*) micromolar concentrations of luminal amiloride (that inhibit ENaC but not NHE-3) hyperpolarized the apical membrane of in vitro microperfused rat PCT, and *2*) mRNAs encoding the three subunits of ENaC were detected by RT-PCR in rat PCT.

Because luminal and basolateral membranes of proximal tubules cells are made freely permeable to water through the expression of AQP-1 (594), net water reabsorption is passively driven by the overall osmotic balance of solute fluxes.

2. Regulatory properties

Sodium and fluid reabsorption by the proximal tubule is controlled by many hormones and neurotransmitters including PTH, dopamine, epinephrine and norepinephrine, angiotensin II, insulin, and glucocorticoids. PTH is the main regulator of phosphate transport and an important modulator of bicarbonate reabsorption in proximal tubule. However, PTH most likely plays an accessory role in the overall sodium and fluid balance. The bulk of sodium and water reabsorption by the proximal tubule is controlled positively mainly by ANG II and epinephrine/norepinephrine and negatively by dopamine. Insulin may play a physiological role in postprandial periods. Glucocorticoids play a role in the day-to-day control of the reabsortion process through transcriptional regulation of sodium transporters. In addition to this stricto sensu hormonal control, local factors, including endothelins and nitric oxide, may modulate the proximal tubule reabsorption process, and the reader may refer to recent reviews on that topic (476, 537).

Because hormones trigger different signaling pathways in a same cell (see sect. III*B*), we first present data obtained through unique activation of either cAMP/PKA pathway by forskolin and/or permeant analogs of cAMP, or of phorbol ester-sensitive PKC pathway.

B. cAMP/PKA Signaling Pathway

Both in vivo and in vitro microperfusion studies demonstrated that luminal cAMP and cell-permeant cAMP analogs inhibit the proximal tubule reabsorption of fluid, sodium, and bicarbonate (59, 549, 725). This inhibitory effect was observed in tubules microperfused with an ultrafiltrate-like solution containing ~25 mM bicarbonate. In contrast, when the perfusate contained 5 mM bicarbonate, which mimicks the situation prevailing in the late proximal tubule, cAMP analogs and forskolin stimulated fluid and NaCl reabsorption (848). This stimulation was dependent on transcellular chloride transport through apical chloride/anion exchangers and basolateral chloride channels and required the presence of a chloride gradient. This suggests that cAMP might both *1*) inhibit bicarbonate transporters, e.g., apical Na^+/H^+ exchanger and/or basolateral Na^+-HCO_3^- cotransporter, and consequently inhibit the fraction of sodium and fluid transport coupled to bicarbonate reabsorption, and *2*) stimulate sodium and chloride transporters not coupled to bicarbonate transport, e.g., apical sodium channels and basolateral chloride channels and K^+-Cl^- cotransporter.

1. Stimulation of Na^+-K^+-ATPase

A large body of evidence indicates that cAMP-PKA modulates the activity of Na^+-K^+-ATPase in proximal tubules. Most studies concluded to a stimulatory effect of cAMP on proximal tubule Na^+-K^+-ATPase measured either in intact cells by *1*) ouabain-sensitive oxygen consumption (63), *2*) Na^+-K^+-ATPase-dependent basolateral membrane potential variation (110), and *3*) ouabain-sensitive rubidium uptake (135, 136), or in permeabilized isolated PCTs (80, 136) and kidney cortex homogenate (337) by the Na^+-K^+-ATPase hydrolytic activity. In one study, inhibition of ouabain-sensitive oxygen consumption was reported in response to a cAMP analog (673). However, this inhibitory effect likely resulted from insufficient supply of aerobic metabolic substrates leading to the activation of cell protective mechanisms. Such mechanisms include PLA_2 activation (121, 170, 645) and generation of inhibitors of Na^+-K^+-ATPase through cytochrome *P*-450-monoxygenase arachidonate metabolism (11) (this inhibitory pathway has been well established in TAL and is further discussed in sect. v*B1*). Indeed, incubation solution used in this study did not contain lactate, a major metabolic substrate in PCT cells (444, 826), which is mandatory to support active sodium transport (163). Finally, in two other reports, incubation of isolated rat PCTs at room temperature with exogenous or endogenous cAMP did not alter Na^+-K^+-ATPase activity, revealing the temperature dependence of the stimulatory effect of cAMP (82, 702). This latter observation is consistent with membrane redistribution events. Indeed, the stimulation of Na^+-K^+-ATPase activity by cAMP analogs was observed at V_{max} and was associated with increased number of pump units at the plasma membrane at the expense of early endosomes (136). This suggests that latent intracellular Na^+-K^+-ATPase units located in an early endosomal compartment can be translocated to the basolateral membrane in response to PKA activation in PCT cells, although a PKA-induced inhibition of Na^+-K^+-ATPase endocytosis cannot be excluded (Fig. 13).

Because the effects of cAMP are mainly mediated by PKA, phosphorylation of the Na^+-K^+-ATPase α-subunit might be implicated in the redistribution of Na^+-K^+-

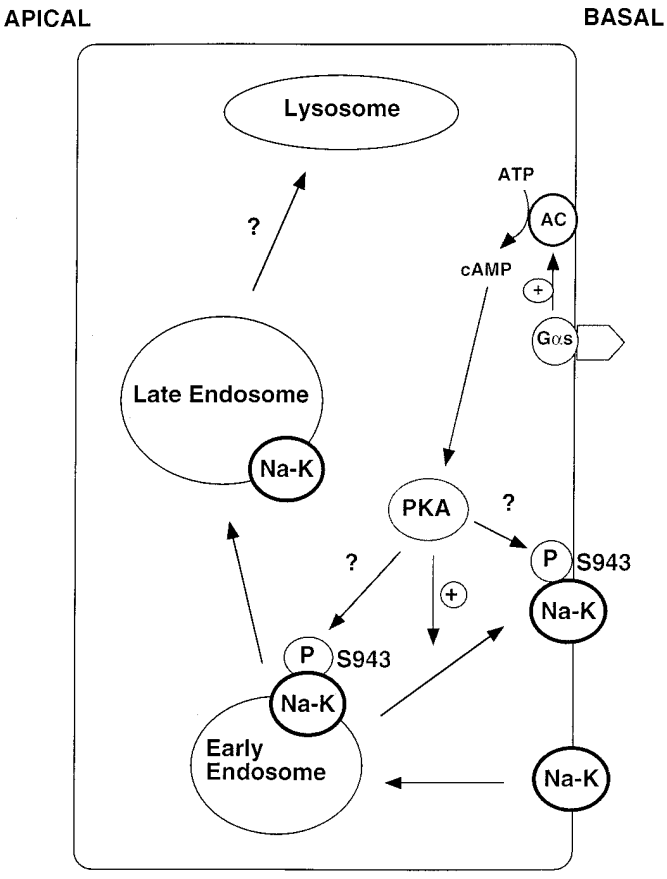

FIG. 13. Mechanism of stimulation of Na^+-K^+-ATPase by cAMP in proximal convoluted tubule. Activation of AC through $G\alpha_s$ increases cellular cAMP levels and activates PKA. Activation of PKA induces translocation of Na^+-K^+-ATPase units from early endosomes to the basolateral plasma membrane domain. Whether PKA phosphorylates (P S943) Na^+-K^+-ATPase units located at the plasma membrane or in early endosomes remains to be determined. Arrows indicate the direction of the signaling cascade.

ATPase observed in proximal tubule. Phosphorylation of Na^+-K^+-ATPase α-subunit by PKA was evidenced in vitro (83, 167) and in intact cells (66, 135, 294). The PKA phosphorylation site was mapped at the well-conserved Ser-943 of the α_1-subunit (66, 283, 294). cAMP-mediated phosphorylation of Na^+-K^+-ATPase and stimulation of ouabain-sensitive rubidium uptake occurred with the same time course in intact cells of rat kidney cortex (135), suggesting that both events are linked. However, the definite relationship between cAMP-stimulated phosphorylation and stimulation of Na^+-K^+-ATPase activity remains to be established.

2. Inhibition of Na^+/H^+ exchanger and Na^+-HCO_3^- cotransporter

The inhibitory effect of cAMP on sodium and bicarbonate reabsorption is mediated at least in part by coordinate inhibition of the apical Na^+/H^+ exchange (869) and of the basolateral Na^+-HCO_3^- cotransport (685). cAMP-induced inhibition of Na^+/H^+ exchange is also observed in OK cells (128, 384, 643). Reconstitution studies in proteoliposomes demonstrated that cAMP-induced inhibition of the brush-border Na^+/H^+ exchanger NHE-3 was dependent on active PKA (866, 870) and required a dissociable phosphoprotein cofactor (870, 871). This cofactor, called NHE-RF, was recently cloned from a rabbit renal cDNA library (872), and soon after, an analogous cofactor (E3KARP) was cloned from a human fibroblast cDNA library (924). Both NHE-RF and E3KARP directly bind to the cytoplasmic tail of NHE-3 (924) through their PDZ domains (923). Phosphorylation of NHE-3 by PKA was also demonstrated in transfected AP-1 fibroblasts (559, 929, 932) and in OK cells (929). The cAMP-induced inhibition of NHE-3 requires its PKA phosphorylation, since a dominant-negative PKA-regulatory subunit, as well as mutation of the two serine residues (Ser-552 and Ser-605) of the rat NHE-3 which are phosphorylated in response to PKA activation in intact cells, abolished the effect of cAMP on NHE-3 activity (929). Conversely, phosphorylation of NHE-RF and E3KARP is not necessary for the inhibition of NHE-3 by cAMP (484, 932). A direct interaction between E3KARP and the cytoskeletal protein ezrin has been recently demonstrated (484, 923), and because ezrin plays the anchoring role of AKAP for type II PKA (484), the following model has been proposed. The ezrin-E3KARP/NHE-RF complex would bring in close vicinity type II PKA and NHE-3 so as to facilitate the preferential PKA phosphorylation of NHE-3 in response to cAMP binding. This phosphorylation would reduce the intracellular pH dependence of the Na^+/H^+ exchanger (484). It is interesting to mention that NHE-RF also interacts with the proximal tubule basolateral Na^+-HCO_3^- cotransporter, and reconstitution assays have shown that NHE-RF is required for its PKA-dependent inhibition (76). These observations illustrate the complexity of the regulation of ion transporters through a network of interactions with cytoskeleton, adaptor proteins, and protein kinases (Fig. 14).

3. Stimulation of bicarbonate-independent transporters

cAMP-induced stimulation of sodium chloride and fluid reabsorption observed in the absence of luminal bicarbonate likely results from the coordinated regulation of several transporters at the apical and basolateral pole of the cell. At the basolateral border, stimulation of Na^+-K^+-ATPase activity *1*) increases basolateral sodium exit; *2*) hyperpolarizes the basolateral membrane (110), and thereby increases chloride exit via basolateral chloride channels (785); and *3*) increases the driving force for paracellular chloride reabsorption. At the apical border, epithelial sodium channels (885) are good condidates, since they might be activated when sodium entry through apical Na^+/H^+ exchanger is decreased. Indeed, ENaC activity is closely related to the sodium availability and is upregulated by a decreased rate of sodium entry (307, 885). Furthermore, cAMP increases ENaC activity in the collecting duct (305), therefore inferring that similar regulation may occur in PCT.

In summary, cAMP promotes a shift from sodium-bicarbonate toward sodium-chloride reabsorption in PCT through inhibition of apical Na^+/H^+ exchanger and basolateral Na^+-HCO_3^- cotransporter. In this context, the stim-

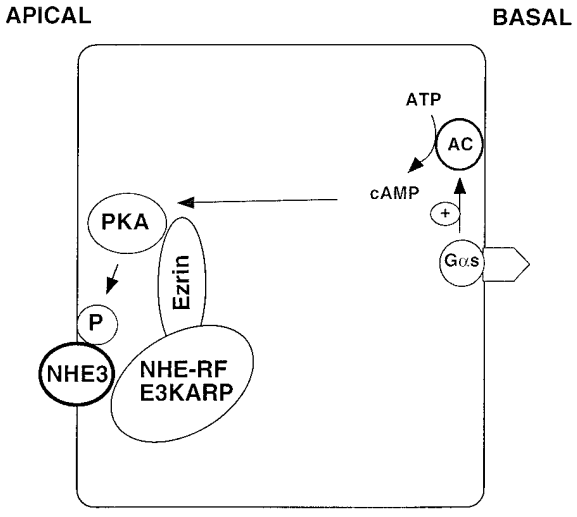

FIG. 14. Mechanism of inhibition of the apical Na^+/H^+ exchanger NHE3 by cAMP in proximal convoluted tubule. Activation of AC through $G\alpha_s$ increases cellular cAMP level and activates PKA which phosphorylates NHE3. Ezrin, a cytoskeletal protein, binds both the regulatory (RII) subunit of PKA and E3KARP-NHE-RF. E3KARP and NHE-RF bind to the cytoplasmic tail of NHE3. The E3KARP-ezrin complex brings type II PKA and NHE3 in close vicinity, thus favoring the phosphorylation of NHE3. Arrows indicate the direction of the signaling cascade.

ulation of Na^+-K^+-ATPase by cAMP could play a key role in the enhanced sodium chloride reabsorption by maintaining or increasing the electrochemical gradients necessary for sodium and chloride reabsorption independently of the apical Na^+/H^+ exchanger and basolateral Na^+-HCO_3^- cotransporter activities.

C. PKC Signaling Pathway

In the in situ microperfused rat PCT, phorbol esters and dioctanoylglycerol stimulated water and solute reabsorption, whereas inhibition of PKC decreased them (510). In an other study, in contrast, an inhibitory effect of phorbol esters and dioctanoylglycerol was reported in the in vitro microperfused rabbit PCT (54). However, this inhibition likely resulted from the lack of pyruvate and lactate in bathing solution which, as discussed above, may lead to the activation of cell protective mechanisms that downregulate active sodium transport (for further discussion see below and sect. v*B1*). Finally, a third study in the in situ microperfused rat PCT reported an initial stimulation of fluid reabsorption by phorbol esters lasting 10 min, followed by an inhibitory effect (847). This finding may result from *1*) sequential activation of different isoforms of PKC by phorbol esters, *2*) downregulation of PKCs after prolonged exposure to phorbol esters, or *3*) activation of the above-mentioned cell protective mechanisms in response to toxic effects of phorbol esters.

1. Stimulation of Na^+-K^+-ATPase

Primary modulation of Na^+-K^+-ATPase activity most likely plays a key role in the effect of phorbol esters on sodium transport in the proximal tubule. The studies of Bertorello et al. (79, 81) and Satoh et al. (702) concluded that direct in vitro activation of PKCs by phorbol 12,13-dibutyrate (PDBu) decreased the V_{max} of Na^+-K^+-ATPase activity through an activation of PLA_2 and of arachidonic acid metabolism (608, 702), but this effect was no longer observed in well-oxygenated isolated rat PCTs incubated in the presence of oxidative metabolic substrates (277). Thus phorbol esters inhibit proximal tubule Na^+-K^+-ATPase and sodium transport as a result of poor metabolic status that triggers cell protective mechanisms, whereas under adequate metabolic conditions, phorbol esters stimulate both sodium transport and Na^+-K^+-ATPase activity.

In well-oxygenated rat PCTs, PDBu did not alter the V_{max} of Na^+-K^+-ATPase activity but increased ouabain-sensitive rubidium uptake, as a result of increased Na^+-K^+-ATPase apparent affinity for sodium (277). In suspensions of rat proximal tubules, the stimulatory effect of phorbol ester on the transport activity of Na^+-K^+-ATPase was correlated and cosaturated with an increase in the phosphorylation level of Na^+-K^+-ATPase α-subunit (134). This suggested a causal relationship between PKC-dependent phosphorylation and increased sodium affinity of Na^+-K^+-ATPase (277). This hypothesis was recently confirmed by the demonstration that phosphorylation of the Na^+-K^+-ATPase α_1-subunit at Ser-16 increases the apparent sodium affinity of the enzyme in COS-7 cells (276) (see sect. II*A5*).

2. Stimulation of Na^+/H^+ exchanger and Na^+-HCO_3^- cotransporter

In addition to modulating Na^+-K^+-ATPase activity, phorbol esters may also alter sodium-dependent transporters in proximal tubule. Stimulation of the proximal tubule apical Na^+/H^+ exchanger by purified phorbol ester-sensitive PKC has been documented in isolated rabbit brush-border membranes (868). Similar findings were obtained with brush-border Na^+/H^+ exchangers incorporated into artificial proteoliposomes (865), suggesting that PKC-dependent phosphorylation of the Na^+/H^+ exchanger mediates the observed stimulation of Na^+/H^+ exchange. This stimulatory effect of PKC-dependent phosphorylation was questioned by several studies on fibroblast-like cells and in epithelial-like Caco-2 cells transfected with NHE-3, which reported a phosphorylation-associated inhibition of Na^+/H^+ exchange activity by phorbol esters (493, 816, 917, 925). In addition, Na^+/H^+ exchange was acutely inhibited by phorbol esters in OK cells (384), which express exclusively the NHE-3 isoform (33). However, a recent study has demonstrated that individual clones of Na^+/H^+ exchanger-deficient AP-1 cells stably transfected with the rat NHE-3 may exhibit opposite reponses to phorbol esters (879). Indeed, clones with stimulatory, inhibitory, or no response were observed despite a similar pattern of phorbol ester-induced phosphorylation analyzed by phosphopeptide mapping. Therefore, the effect of PKC-dependent phosphorylation of NHE-3 is indirect and largely dependent on cell-specific factors that may determine the stimulatory effect observed in proximal tubule brush-border membranes.

The activity of the basolateral Na^+-HCO_3^- cotransporter is also increased in response to phorbol esters in isolated rabbit proximal tubule cells (910). In addition, the activity of the cotransporter is increased by phosphorylation by purified PKC of basolateral membranes isolated from rabbit proximal tubules (685). Altogether, these effects are consistent with the phorbol ester-induced alkalinization of dog proximal tubules cells (552).

In summary, activation of phorbol ester-sensitive PKCs stimulates the activity of sodium-coupled acid-base transporters in PCT which, together with the stimulation of Na^+-K^+-ATPase, may increase bicarbonate reabsorption in this segment. The pleiotropic coordi-

nated stimulation by phorbol esters of both apical sodium entry and basolateral sodium exit allows the increase of net transepithelial reabsorption of sodium without altering its intracellular concentration. This whole process most likely requires phosphorylation of the Na^+-K^+-ATPase α-subunit, of NHE-3, and of the Na^+-HCO_3^- cotransporter.

D. ANG II

1. ANG II is locally produced

Several studies indicated that luminal proximal tubule concentrations of ANG II are 1,000-fold (nM range) (109, 591, 931) to 100-fold (0.1 nM range) (99, 590) higher than plasma concentrations (pM range), indicating that PCTs secrete either circulating or locally generated ANG II. Several lines of evidence suggest that the major part of luminal ANG II derives from local synthesis: *1*) proximal tubule cells express angiotensinogen (218, 420), renin (560), and angiotensin I converting enzyme (116, 137, 911); *2*) both angiotensin I (591) and angiotensin I converting enzyme (137) are secreted into the PCT lumen; and *3*) circulating and proximal tubule luminal ANG II concentrations may vary independently (99). Only a very small fraction of luminal ANG II is excreted in the urine, since it is almost entirely reabsorbed and degraded by proximal tubules (631, 651). In contrast to PTH, the role of the multifunctional endocytic receptor megalin (172) has not been evaluated in the clearance of ANG II from the proximal tubule luminal fluid.

2. Solute and fluid transport

A biphasic effect of ANG II on sodium reabsorption by the PCT has been recognized in the late 1970s. By in vivo microperfusion of rat PCT and peritubular capillaries, Harris and Young (366, 367) demonstrated that low concentrations of ANG II (10^{-12} to 10^{-10} M) stimulated sodium and fluid reabsorption, whereas high concentrations of ANG II (3×10^{-7} to 3×10^{-6} M) inhibited sodium and water transport. This early observation was subsequently confirmed using either the same technique in rat (156, 507) or the in vitro microperfusion of rabbit PCT (218, 720). In rats, the stimulatory effect of low concentrations of ANG II was observed along the whole proximal tubule (326, 507), but ANG II was less potent in PST than in PCT (507). ANG II exerted its dose-dependent effects on PCT fluid and solute reabsorption at both the basolateral (367, 508, 720) and the apical side of the tubule (58, 498, 508), but it was more potent when applied to the lumen of rabbit PCT (498). In addition, the action of luminal ANG II can be modulated by basolateral ANG II (498). Indeed, the concomitant application of a high concentration of luminal ANG II (inhibitory) and a low concentration of basolateral ANG II (stimulatory) stimulated fluid reabsorption. It is important to mention, however, that the micromolar concentrations of ANG II that inhibit fluid reabsorption in proximal tubule are never observed under physiological conditions, even at the luminal border of PCTs.

In tubules perfused with an ultrafiltrate-like solution (containing more than 20 mM bicarbonate), the stimulatory effect of ANG II on sodium and fluid reabsorption was associated with increased bicarbonate reabsorption (507). However, in contrast to the inhibitory effect of PTH (206, 549), ANG II increased sodium chloride reabsorption even when tubules were perfused with bicarbonate-free solutions (890).

Micropuncture and clearance studies have shown that under basal conditions, ANG II may exert a tonic control on sodium transport by PCT (931). This was confirmed using the in vitro microperfused rabbit PCT in which perfusion with an inhibitor of the angiotensin converting enzyme or an AT_1 receptor antagonist decreased fluid reabsorption (653). Similar observations were obtained by in vivo microperfusion of rat PCT (752, 906). In addition, regulation of proximal tubule ANG II secretion by extracellular volume may account in part for the homeostasic adaptation of proximal tubule fluid reabsorption to volume changes (654). Indeed, in volume-depleted rats, in vivo luminal perfusion of ANG II had no effect on fluid reabsorption, whereas administration of an AT_1 receptor antagonist inhibited it, suggesting a high basal concentration of luminal ANG II sufficient for maximal activation of fluid transport. Conversely, in volume-expanded rats, the AT_1 receptor antagonist was ineffective, whereas the fluid reabsorption was stimulated by luminal exogenous ANG II, suggesting a basal luminal ANG II concentration below the stimulation threshold.

On the basis of pharmacological studies using nonpeptidic antagonists, the stimulatory and inhibitory effects of ANG II on sodium and fluid transport were attributed to AT_1 receptors (654, 893).

3. Sodium-dependent transporters

Consistent with its effects on sodium, bicarbonate, and fluid reabsorption, ANG II modulates Na^+-K^+-ATPase activity in a biphasic manner (88). At low concentrations, the stimulatory action of ANG II on Na^+-K^+-ATPase results from either a V_{max} effect (327) or from increased apparent affinity for sodium (22). The precise mechanism of control of Na^+-K^+-ATPase activity by ANG II as well as the signaling pathway mediating these effects remain to be investigated.

The effect of ANG II on the apical Na^+/H^+ exchange has been investigated more extensively. Stimulation of the apical Na^+/H^+ exchange in response to low concentrations of ANG II was first suggested on the basis of the

abolition by amiloride of the increase in sodium and bicarbonate transport in rat PCT microperfused in situ (508). Stimulation of the apical Na^+/H^+ exchanger was directly demonstrated in the in vitro microperfused rabbit PCT where low concentration of ANG II increased the rate of EIPA-sensitive intracellular alkalinization (331). This observation was confirmed by measurement of the activity of the Na^+/H^+ exchanger taken as *1*) the initial rate of amiloride-sensitive sodium uptake in freshly isolated rabbit (690) and rat proximal tubule cells (335) or *2*) the initial rate of sodium-dependent intracellular pH recovery after acid loading in suspensions of rat PCT (398). Similarly, in brush-border membrane vesicles (BBMVs) isolated after exposure of rabbit proximal tubule cells to low concentration of ANG II, the activity of the Na^+/H^+ exchanger was stimulated (95, 690). Conversely, the activity of the Na^+/H^+ exchanger, measured as the initial rate of sodium-dependent intracellular pH recovery after an acid load, was decreased by high concentrations of ANG II in suspensions of rat PCT (398). The precise mechanism of Na^+/H^+ exchanger stimulation by ANG II remains to be established. In some studies, ANG II was reported to increase the V_{max} of proton efflux (95, 690), raising the possibility that ANG II might increase the expression of NHE-3 at the apical membrane.

Studies in rat proximal tubule suggested that the stimulatory effect of low concentration of ANG II relies in part on decreased cAMP cell content (398, 509). In the in situ microperfused rat PCT, ANG II decreased cAMP content in luminal fluid, and its effect on fluid transport was partially prevented through inhibition of $G\alpha_i$ by pertussis toxin and was abolished by a cell-permeant cAMP analog (509). These results suggest that ANG II may act in part through the $G\alpha_i$-mediated inhibition of adenylyl cyclase, and thus by reversing the inhibitory effect of cAMP on NHE-3. Alternately, studies with PKC inhibitors (398) or activators (510) suggested that activation of the phorbol ester-sensitive PKC-α and PKC-ϵ by low concentration of ANG II (451) participates to the stimulation of Na^+/H^+ exchange. It is most likely that the stimulation of the apical Na^+/H^+ exchanger by low concentration of ANG II results from complex interactions between multiple signaling pathways, since PLA_2 (498, 564) and c-Src (819) may also participate to this effect. The inhibitory effect of high concentration of ANG II on Na^+/H^+ activity likely relies on PLA_2-mediated generation of arachidonic acid and subsequent synthesis of active metabolites through the cytochrome *P*-450-monooxygenase pathway (398, 498).

Stimulation of the basolateral Na^+-HCO_3^- cotransport also participates to increase bicarbonate reabsorption in response to low concentration of ANG II. Using the in vitro microperfused rabbit PCT, Giebel et al. (331) have shown that ANG II stimulated the rate of basolateral Na^+-HCO_3^- cotransport, measured as the rate of DIDS-sensitive sodium-dependent intracellular pH recovery in tubules loaded with the fluorescent probe 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF). This was confirmed by electrophysiological measurement of the Na^+-HCO_3^- cotransport rate in the same preparation (183) and by measurement of the bicarbonate-sensitive sodium uptake in basolateral membrane vesicles from rabbit kidney cortex (240, 686). This stimulatory effect of low concentration of ANG II is likely mediated by an activation of PKC and by a decrease in intracellular cAMP, since it was prevented by inactivation of $G\alpha_i$ by pertussis toxin and by inhibition of PKC by calphostin C (686). In contrast, high concentrations of ANG II inhibited the Na^+-HCO_3^- cotransport measured in the in vitro microperfused rabbit PCT by bicarbonate-dependent changes in basolateral membrane potential (184).

In conclusion, ANG II controls sodium and fluid reabsorption by the proximal tubule through a coordinated modulation of the sodium transporters present at apical and basolateral membrane domains. However, ANG II appears to alter apical and basolateral sodium transporters within a different time frame, since it exerts a transient biphasic effect on intracellular sodium concentration (669, 892). The apical sodium transport step, mediated by Na^+/H^+ exchange, might be altered first since the intracellular sodium concentration initially increased or decreased in response to low or high concentrations of ANG II, respectively.

E. Epinephrine and Norepinephrine

1. Stimulatory effect of α-adrenergic agonists

The direct α-adrenergic control of fluid and solute reabsorption in the PCT has been demonstrated in the 1980s. In the in situ perfused rat kidney, infusion of norepinephrine or epinephrine increased fluid reabsorption (152, 867), and this effect was reversed by the α-adrenergic antagonists phenoxybenzamine (152) and phentolamine (867). In agreement with the lack of luminal binding sites, luminal norepinephrine had no effect on fluid reabsorption by PCT (152). At the level of isolated PCTs, norepinephrine stimulated *1*) sodium reabsorption in *Ambystoma* (3); *2*) sodium, chloride, and bicarbonate reabsorption and ouabain-sensitive oxygen consumption in rabbit (60); and *3*) ouabain-sensitive rubidium uptake in rat (39). The stimulatory effect of norepinephrine on proximal tubule reabsorption is mediated at least in part by α_1-adrenergic receptors, since prazozin, a specific α_1-antagonist, decreased chloride reabsorption by the in situ microperfused rat PCT (891) and antagonized the decrease in bicarbonate excretion induced by renal nerve stimulation in anesthezized dogs (611). In contrast to prazozin, specific α_2-antagonists did not alter ion transport in these settings (611, 891). Moreover, an inhibition of fluid reabsorption has been reported in response to the

specific activation of α_2-adrenergic receptors by clonidine in the isolated perfused rabbit PCT (683).

Stimulation of proximal tubule sodium transport in response to norepinephrine is largely mediated by a primary increase in Na^+-K^+-ATPase activity as shown by the decrease in intracellular sodium concentration in *Ambystoma* proximal tubule (3) and by direct measurement of the hydrolytic activity of Na^+-K^+-ATPase in basolateral membranes isolated from norepinephrine-treated rabbit PCTs (60). These results were confirmed in isolated rat PCTs by measurements of the hydrolytic activity of Na^+-K^+-ATPase in response to the α-adrenergic agonist oxymetazoline (23). Attempts to delineate the α-adrenergic receptor subtype involved in the stimulation of proximal tubule Na^+-K^+-ATPase indicated that the specific α_1-antagonist prazosin prevented the stimulation of Na^+-K^+-ATPase in response to α-agonists (23, 340). Surprisingly, however, the stimulatory effect of the nonspecific α-agonist oxymetazoline was fully prevented by the α_2-antagonist yohimbine (23). Further studies, using more specific tools, are therefore required to reach a definitive conclusion about the respective roles of α_1- and α_2-adrenergic receptors in this effect. The signaling pathway leading to the stimulation of Na^+-K^+-ATPase activity in response to α-adrenergic agonists is far from being fully elucidated. It appears to be dependent on a diacylglycerol-insensitive and staurosporine-sensitive protein kinase (39, 340) and on the calcium/calmodulin-dependent protein phosphatase 2B (calcineurin) (23).

In addition to Na^+-K^+-ATPase, norepinephrine also modulates, directly or indirectly, sodium-coupled acid-base transporters in proximal tubule, since α-adrenergic agonists increased intracellular pH (334). In BCECF-loaded suspensions of rat proximal tubules, both α_1- and α_2-agonists increased intracellular pH through stimulation of Na^+/H^+ exchange, since this effect was dependent on extracellular sodium and fully prevented by EIPA. Stimulation of Na^+/H^+ exchange by both α_1-adrenergic(333, 506a) and α_2-adrenergic (177, 333, 599) agonists has been confirmed in isolated rat (333) and rabbit proximal tubules (599), as well as in primary and immortalized cultures of mouse proximal tubules (506a) and OK cells (177). Studies with antisense oligonucleotides and subtype-specific antagonists demonstrated that the stimulation of Na^+/H^+ exchange in mouse proximal tubule cells is mediated for half by α_{1A}-receptors and for the remaining half by α_{1B}-receptors, whereas α_{1D}-receptors are not involved in this regulatory pathway (506a). Because α-adrenergic control of the proximal tubule Na^+/H^+ exchange has been investigated only in intact cells, it is not possible to discriminate between a primary stimulation of Na^+/H^+ exchangers and a secondary activation driven by decreased intracellular sodium concentration brought about by increased Na^+-K^+-ATPase activity. The signaling pathway responsible for Na^+/H^+ exchange stimulation remains to be determined. Specific agonists of α_1- and α_2-adrenergic receptors had synergistic effects in rat PCT (333), suggesting either that the number of receptors might be rate limiting or, more likely, that the two subtypes of receptors trigger different signaling pathways. In OK cells, the α_2-adrenergic stimulation of Na^+/H^+ exchange is independent of the decrease in cellular cAMP concentration (177).

In conclusion, the antinatriuretic effect of α_1-adrenergic agonists (40) is at least in part explained by an increase in sodium reabsorption in the proximal tubule mediated by stimulation of basolateral Na^+-K^+-ATPase and apical Na^+/H^+ exchange, while the effect of α_2-adrenergic agonists remains controversial.

2. Stimulatory effect of β-adrenergic agonists

Activation of the β-adrenergic receptors with isoproterenol increased sodium reabsorption in the in vitro microperfused rabbit PCT (71), and blockade of β-adrenergic receptors by propranolol inhibited fluid reabsorption in the in situ microperfused rat PCT (867). The mechanism of action of β-adrenergic agonists on sodium transport has been investigated in primary cultures of rat proximal tubule cells that maintained, at least in part, their functional polarity (743, 744). In this experimental system, β-adrenergic agonists stimulated the apical sodium transport, estimated by sodium uptake, and Na^+-K^+-ATPase-mediated transport measured by ouabain-sensitive rubidium uptake. Whether β-agonists primarily stimulated the apical Na^+/H^+ exchanger and/or the basolateral Na^+-K^+-ATPase remains to be determined.

A ligand-induced association of the COOH-terminal tail of the β_2-adrenergic receptor with NHE-RF, the protein cofactor required for the cAMP-mediated inhibition of NHE-3 (924), has been recently demonstrated (361). In this study, Chinese hamster ovary cells expressing NHE-RF were stably transfected with both NHE-3 and wild-type or mutant β_2-adrenergic receptors. Isoproterenol did not alter the activity of NHE-3 in the cells expressing the wild-type β_2-receptor, whereas an inhibitory effect was observed in the cells expressing a mutant β_2-receptor that did not bind NHE-RF. In addition, the inhibitory effect of recombinant NHE-RF on NHE-3 activity measured in the presence of active PKA and ATP in rabbit proximal tubule brush-border membranes was prevented by incubation with a GST fusion protein containing the COOH-terminal end of the β_2-receptor. Altogether, these findings indicate that sequestration of NHE-RF by activated β_2-adrenergic receptors may prevent the phosphorylation on NHE-3 by PKA and thereby impede its inhibition. This mechanism could antagonize the effect of a basal level of PKA activity and thereby could explain in part the stimulatory effect of

β-adrenergic agonists on sodium and fluid reabsorption by the proximal tubule.

F. Dopamine

1. Local production of dopamine and effect on sodium transport

Both neuronal and extraneuronal dopamine production occur in the kidney. The local synthesis of dopamine by proximal tubules is attested by *1*) the presence of intracellular dopamine immunoreactivity (359), *2*) the inhibition of sodium transport by precursors of dopamine such as L-dopa or glu-dopa and by inhibitors of dopamine degradation by COMT (241, 727), and *3*) the release of dopamine by proximal tubule cells incubated with L-dopa (38, 855). Dopamine synthesis also takes place in OK cells and LLC-PK_1 cells (345, 758, 759). In kidney cortex (38, 489) as well as in LLC-PK_1 cells (759), uptake of L-dopa occurs at both apical and basolateral sides. In human and rat kidney cortex slices, dopamine synthesis from L-dopa is dependent on the presence of extracellular sodium and on Na^+-K^+-ATPase activity, suggesting a sodium-coupled mechanism of L-dopa uptake (756, 757). Locally synthesized dopamine is preferentially released into the tubular lumen (855, 856). The proximal tubule synthesis of dopamine is increased by high sodium intake and may therefore participate to the excretion of the sodium load (85, 856).

Indeed, dopamine is an important local regulator of sodium and fluid reabsorption by the proximal tubule. Dopamine decreases fluid and sodium reabsorption in the in vitro microperfused rabbit PCT (57) and PST (72). In isolated PCT, the effect of dopamine is detected only when sodium and fluid reabsorption is stimulated by norepinephrine (57). In this setting, dopamine acts from the luminal side but not from the basolateral side, in agreement with a physiological role of the luminal secretion of locally synthesized dopamine.

2. Inhibition of Na^+-K^+-ATPase

Effect of dopamine on proximal tubule Na^+-K^+-ATPase has been extensively studied. Inhibition of the hydrolytic activity of Na^+-K^+-ATPase by exogenously added (20, 82) and locally synthesized dopamine (20, 85) was evidenced in isolated rat PCTs in the late 1980s. These results were confirmed by subsequent studies on intact proximal tubule cells which showed that dopamine also inhibited ouabain-sensitive rubidium uptake (727, 751) and ouabain-sensitive oxygen consumption (727, 728). As in neostriatum neurons (84), inhibition of Na^+-K^+-ATPase activity in rat proximal tubule requires both D_1- and D_2-like receptor occupancy, since D_1- or D_2-like agonists alone had no effect (82). Conversely, other authors using the D_2-like agonist bromocriptine reported pertussis toxin-sensitive stimulation of Na^+-K^+-ATPase activity in suspensions of rat proximal tubules (407).

In proximal tubules, the inhibitory effect of dopamine on Na^+-K^+-ATPase activity does not involve the PKA-dependent phosphorylation of the protein phosphatase 1 regulator DARPP-32 (21). Indeed, DARPP-32 was not detected by immunobloting in rat kidney cortex (310), and protein phosphatase 1 activity was not altered by dopamine in suspensions of rat proximal tubule (751). Rather, dopamine-induced inhibition of Na^+-K^+-ATPase activity in rat PCT is primarily mediated by PKC (164, 168, 637), but also requires PLA_2 activation (168, 703) and the synthesis of arachidonic acid metabolites via cytochrome *P*-450-monooxygenase pathway (608).

A dopamine-induced decrease in Na^+-K^+-ATPase V_{max} has been consistently reported in permeabilized (20, 82, 411) and intact rat proximal tubules (727, 728), as well as in OK cells (165). However, dopamine also alters the apparent affinity of the proximal tubule Na^+-K^+-ATPase for cations; it decreased the apparent affinity for potassium and increased that for sodium (20, 411). Thus dopamine-induced increase in the sodium affinity of Na^+-K^+-ATPase may antagonize functionally the effect of the decrease in V_{max} of the enzyme, since sodium is rate limiting in intact cells. In intact cells, dopamine also inhibited the ouabain-sensitive rubidium uptake and oxygen consumption (727, 751), demonstrating an overall inhibition of Na^+-K^+-ATPase. This inhibition might be accounted for by a decrease in apical sodium entry secondary to the inhibition of the Na^+/H^+ exchanger (270, 335, 430, 732) and/or by a more pronounced effect of dopamine on V_{max} than on sodium affinity, leading to a residual decrease in Na^+-K^+-ATPase activity in the presence of physiological intracellular sodium concentrations.

The mechanism of dopamine-induced decrease in V_{max} of Na^+-K^+-ATPase has been extensively studied during the past few years. In suspensions of rat proximal tubule cells and in OK cells, dopamine induced a redistribution of Na^+-K^+-ATPase units from the basolateral plasma membrane to intracellular compartments. In these preparations, dopamine induced a rapid (within minutes) decrease in the number of Na^+-K^+-ATPase units expressed at basolateral membrane (166) and the sequential increase in Na^+-K^+-ATPase abundance in subcellular fractions corresponding to clathrin-coated pits (1 min), early endosomes (2.5 min), and late endosomes (5 min) (164, 166). This redistribution of Na^+-K^+-ATPase was associated with increased PIK activity and was prevented by wortmannin and LY-294002, two specific inhibitors of this kinase (168). This result further underlines the key role of PIK in vesicle trafficking between different subcellular compartments (117, 173, 480, 680, 820). The stimulation of PIK by dopamine was abolished by pretreatment with PKC or PLA_2 inhibitors, suggesting that both enzymes are

involved in this effect (168). Because Na^+-K^+-ATPase α-subunit can be phosphorylated by PKC in vitro (83, 167, 283) and in intact cells, including rat proximal tubule suspensions (66, 134, 295, 555), Chibalin and co-workers (165, 166) evaluated the role of PKC phosphorylation of the α-subunit in dopamine-induced inhibition of Na^+-K^+-ATPase activity. In suspensions of rat proximal tubules, dopamine induced a PKC-dependent phosphorylation of the α-subunit with the same time course as internalization of Na^+-K^+-ATPase (166). The dopamine-induced internalization of Na^+-K^+-ATPase was abolished in OK cells expressing a truncated rat α_1-subunit lacking the first 31 amino acids, i.e., the two PKC phosphorylation sites (Ser-16 and Ser-23) (166), suggesting that phosphorylation of the α-subunit is required for this process. That phosphorylation of the α-subunit is an integral part of the signal leading to internalization of Na^+-K^+-ATPase is further supported by the abolition of the dopamine-induced redistribution of Na^+-K^+-ATPase units in OK cells stably expressing a mutant rat α_1-subunit in which Ser-23 was substituted by alanine (165). Interestingly, despite its dopamine-induced phosphorylation, Ser-16 does not participate in Na^+-K^+-ATPase internalization. Indeed, the dopamine-induced redistribution of Na^+-K^+-ATPase was not altered in OK cells stably expressing a mutant rat α_1-subunit in which Ser-16 was substituted by alanine (165). After endocytosis, the Na^+-K^+-ATPase α-subunit is dephosphorylated by protein phosphatase(s) in the late endosomal compartment (166). It remains to be determined whether Na^+-K^+-ATPase units present in the late endosomal compartment are degraded in lysosomes or recycle back to the plasma membrane (Fig. 15).

3. Inhibition of Na^+/H^+ exchanger

Dopamine may also decrease proximal tubule sodium reabsorption through inhibition of the apical Na^+/H^+ exchanger. Dopamine-induced inhibition of proximal tubule Na^+/H^+ exchange, measured as the amiloride-sensitive sodium uptake, was observed in suspensions of intact rat proximal tubules (335) as well as in rat and rabbit brush-border membranes prepared after preincubation with dopaminergic agonists (270, 430, 732). The effect of dopamine on rat brush-border Na^+/H^+ exchanger is mediated by D_1-like receptors (268, 270, 430), whereas in rabbit, both D_1-like and D_2-like receptors are involved (732). This latter finding is conflicting with the absence of effect of a specific D_2-like antagonist on the inhibition of fluid reabsortion by the rabbit PCT in response to dopamine (57). The signaling pathway responsible for the inhibition of the brush-border Na^+/H^+ exchanger involves both cAMP- and PKA-mediated effects (270) and cAMP- and PKA-independent G protein-linked effects (268).

The dysfunction of the dopamine control of proximal tubule active sodium reabsorption in the pathophysiology of hypertension is out of the scope of this article. The reader can refer to recent reviews focusing on this topic (409, 441).

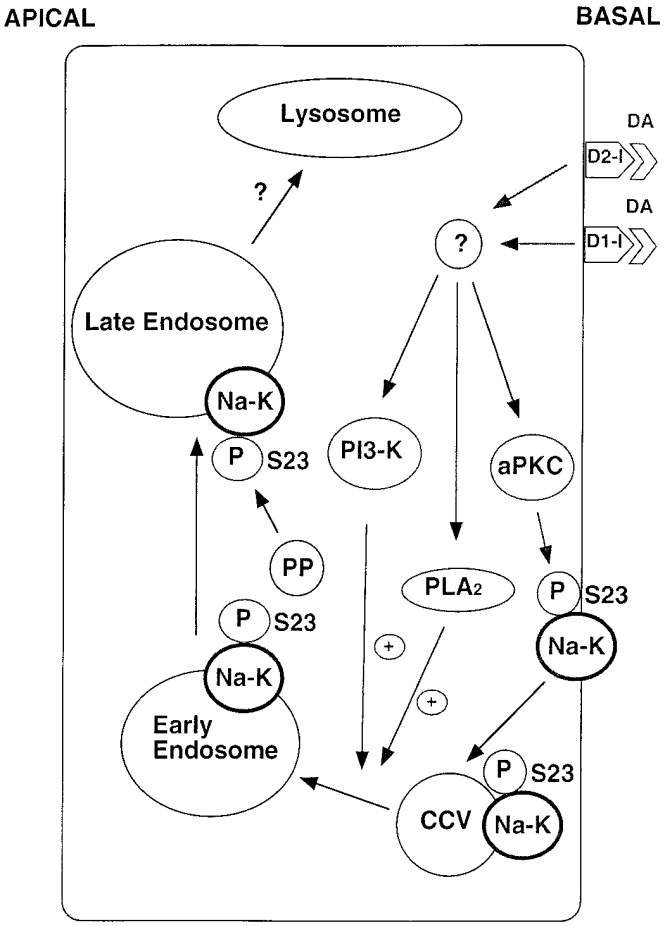

FIG. 15. Regulation of Na^+-K^+-ATPase by dopamine in proximal convoluted tubule. Simultaneous binding of dopamine (DA) to D_1-like (D_1-l) and D_2-like (D_2-l) receptors activates the atypical PKC-ζ (aPKC), PI 3-K, and PLA_2 through yet undetermined signaling intermediate(s). PKC phosphorylates the Na^+-K^+-ATPase α-subunit on serine-23 (P S23), which triggers the endocytosis of Na^+-K^+-ATPase through clathrin-coated pits (CCV) to early and late endosomes. Both PLA_2 and PI 3-K are required for endocytosis of Na^+-K^+-ATPase. The Na^+-K^+-ATPase is then dephosphoryled in the late endosomal compartment by protein phosphatase(s) (PP). Arrows indicate the direction of the signaling cascade.

G. PTH

The recently reviewed control of the sodium-coupled phosphate reabsorption by PTH in the proximal tubule (300, 579) is out of the scope of this article because it does not contribute significantly to sodium homeostasis.

1. Bicarbonate, sodium, and fluid transport

Acute inhibition of proximal tubule bicarbonate and fluid reabsorption by PTH has been demonstrated by in

vivo micropuncture experiments performed in thyroparathyroidectomized dogs (652) and rats (43). These results have been confirmed by in vitro microperfusion of the rabbit PCT (206, 363, 412, 549) and PST (206, 363, 549) and by in situ microperfusion of the rat PCT (59). Inhibition of fluid and sodium reabsorption by PTH was proportional to the decrease in bicarbonate reabsorption (43, 206, 412, 652). In addition, PTH had no effect on fluid and sodium reabsorption when proximal tubules were perfused with low bicarbonate solutions (206, 549). Altogether, these findings indicate that inhibition of fluid and sodium reabsorption by PTH is secondary to its effect on bicarbonate transport. Accordingly, the inhibitory effect of PTH is larger in the early PCT than in the late PCT and PST (206, 363). Inhibition of fluid and bicarbonate reabsorption was observed when PTH was applied to the basolateral but not the apical side of the tubule (59). It was mimicked by luminal cAMP (59) and by basolateral cell-permeant cAMP analogs (549). It should be mentioned that in the whole animal, acute PTH infusion did not decrease significantly acid excretion (43, 652), indicating compensatory mechanism in more distal nephron segments.

2. *Inhibition of Na^+-K^+-ATPase*

The effect of PTH on bicarbonate and fluid reabsorption is dependent on active transport, since it is abolished in tubules pretreated with ouabain (549). Several investigators have studied the effect of PTH on proximal tubule Na^+-K^+-ATPase. In suspensions of rabbit (217) and rat (673) proximal tubules, PTH decreased ouabain-sensitive oxygen consumption. These results were confirmed by the measurement of the hydrolytic activity of Na^+-K^+-ATPase in permeabilized isolated rat PCTs (608) and in basolateral membranes prepared from normal rat kidney cortices taken after acute intravenous infusion of PTH (928). PTH-induced inhibition of Na^+-K^+-ATPase is mediated by a PLC- and PKC-dependent activation of PLA_2 (208) and the subsequent metabolism of arachidonic by the cytochrome *P*-450-monooxygenase pathway (608, 672). Both PTH-(1—34) and PTH-(3—34) inhibited Na^+-K^+-ATPase activity (673, 928), and the effect was not reproduced by cAMP analogs (608). Conversely, PTH effect on Na^+-K^+-ATPase was mimicked by arachidonic acid and by its monooxygenase metabolites (608, 672) and was prevented by inhibitors of PLA_2 (208) and of cytochrome *P*-450 monooxygenase (608, 672).

Inhibition of Na^+-K^+-ATPase activity was observed at V_{max} (608, 673, 928), indicating that PTH decreases either the number of active pumps or the turnover of each pump. PTH effect is unlikely accounted for by endocytosis of active Na^+-K^+-ATPase units, since no redistribution of Na^+-K^+-ATPase was observed by subcellular fractionation after in vivo PTH infusion (928).

However, the physiological relevance of the PTH-induced inhibition of Na^+-K^+-ATPase is not obvious. As already discussed for cAMP (see sect. IV*B1*), the inhibitory effect of PTH on fluid and sodium chloride reabsorption is entirely dependent on the inhibition of bicarbonate reabsorption (206, 549), which is consistent with inhibition of the apical Na^+/H^+ exchange and the basolateral Na^+-HCO_3^- cotransport (see below). In contrast, inhibition of Na^+-K^+-ATPase should also decrease sodium chloride reabsorption in absence of luminal bicarbonate. Because it is mediated by the PLA_2/cytochrome *P*-450 monooxygenase pathway, it remains possible that the inhibitory effect of Na^+-K^+-ATPase observed in vitro is artefactual and results from limited metabolic availability, as the inhibitory effect of cAMP.

3. *Inhibition of Na^+/H^+ exchanger*

PTH controls the apical sodium entry step at the level of apical Na^+/H^+ exchangers. Monitoring intracellular pH with the fluorescent probe BCECF in the in vitro microperfused rabbit PCT (699) and PST (725) have shown that PTH inhibits the apical Na^+/H^+ exchanger. Indeed, in both PCT and PST, PTH reduced the initial rate of intracellular acidification after removal of sodium from bicarbonate-free perfusate, an index of Na^+/H^+ exchanger activity. This inhibitory effect of PTH on the Na^+/H^+ exchange was confirmed by measurement of the EIPA-sensitive sodium uptake in suspensions of rat PCT (335) and in BBMVs from rabbit proximal tubules (445). This inhibitory effect of PTH on the apical Na^+/H^+ exchange was reproduced in OK cells (383, 643) and in a simian virus 40-transformed cell line obtained from rabbit PCT (571). Studies on rat proximal tubules (257, 928), OK cells (33), and transfected nonrenal cells (34) demonstrated that PTH controls the activity of the NHE3 isoform of Na^+/H^+ exchanger.

As mentioned previously, PTH receptors are coupled to both adenylyl cyclase and PLC, leading to the activation of PKA and PKC, respectively. Several lines of evidence indicate that adenylyl cyclase activation is, at least in part, responsible for PTH-induced inhibition of apical Na^+/H^+ exchange in proximal tubule. First, the effect of PTH on the apical Na^+/H^+ exchange was mimicked by either cell-permeant cAMP analogs in suspensions of rabbit proximal tubules (445) and in OK cells (384, 643), or by direct activation of adenylyl cyclases by forskolin in OK cells (383, 384, 643). Second, decreasing cAMP generation by addition of 1,25-dihydroxyvitamin D_3 reduced PTH-induced inhibition of Na^+/H^+ exchange in OK cells (92). Finally, in the OK-P cell subclone, the biologically active NH_2-terminal fragments of PTH [PTH-(1—34)] and PTHrP [PTHrP-(1—34)] that increase cellular cAMP levels in the absence of PLC activation inhibited Na^+/H^+ exchange (533).

However, some studies on cell cultures also suggested a role of PKC in PTH-induced inhibition of proximal tubule Na^+/H^+ exchange. In OK cells, phorbol esters inhibited Na^+/H^+ exchange (383, 384). Biologically active fragments of PTH that activate both PKA and PKC [PTH-(1—34)] or PKC independently of PKA [PTH-(3—34); PTH-(28—42); PTH-(28—48)] inhibited Na^+/H^+ exchange in OK cells (33). Finally, in Na^+/H^+ exchanger-deficient AP-1 cells cotransfected with NHE-3 and PTH/PTHrP receptors, the effect of PTH-(1—34) was altered neither by the specific PKA inhibitor H-89 nor by the specific PKC inhibitor chelerithrine chloride, but it was prevented either by H-89 after downregulation of PKC by overnight incubation with phorbol esters (33) or by H-7 that inhibits both PKA and PKC (34). However, the involvement of PKC in PTH-induced inhibition of Na^+/H^+ exchange appears unlikely in native proximal tubules. Indeed, phorbol esters stimulated apical Na^+/H^+ exchange in suspensions of rabbit proximal tubules (866, 868), as did hormones and neurotransmitters coupled to PKC activation in suspensions of rat proximal tubules (335, 398). Similarly, in AP-1 cells stably transfected with NHE-3, phorbol esters stimulated Na^+/H^+ exchange activity (879). It remains possible that different isozymes of PKC be activated in response to phorbol esters and PTH; these isozymes might be coupled to either stimulatory or inhibitory pathways.

The mechanism of inhibition of proximal tubule Na^+/H^+ exchange by PTH was studied in normal rats (928) and in parathyroidectomized rats (257) given acute intravenous infusion of either PTH or its biologically active fragments. Subcellular fractionation in normal rat kidneys demonstrated a rapid (<1 h) redistribution of NHE-3 after infusion of PTH-(1—34), which activates adenylyl cyclase, PLC, and PLA_2, but not after infusion of PTH-(3—34), which activates PLC and PLA_2, but not adenylyl cyclase (928). These results confirmed the PTH-induced redistribution of Na^+/H^+ exchangers previously reported in suspensions of rat proximal tubules (386). In parathyroidectomized rats, PTH infusion inhibited apical membrane Na^+/H^+ exchange activity and increased NHE-3 phosphorylation after 30 min and decreased the apical membrane NHE-3 immunoreactivity without altering total cellular NHE-3 immunoreactivity after 4 h (257). This suggests that PTH first inhibited apical Na^+/H^+ exchanger through phosphorylation, and subsequently induced its endocytosis. Endocytosis of NHE-3 in response to PTH could explain the redistribution observed in normal rats. However, the decrease in apical membrane NHE-3 content was observed several hours after the redistribution of NHE-3 detected by subcellular fractionation studies. The early mobilization of NHE-3 to a specialized apical membrane subdomain in response to PTH or the coisolation of apical membrane and subapical vesicules containing NHE-3 immunoreactivity (91) could explain this discrepancy.

4. Basolateral bicarbonate transporters

The effect of PTH on the basolateral base transport system is more controversial. Indeed, in the in vitro microperfused rabbit PCT, the activity of the basolateral Na^+-HCO_3^- cotransporter, measured as the initial rate of change in intracellular pH after removal of bath sodium or lowering bath HCO_3 from 25 to 5 mM, was either decreased (624) or unchanged (699) by PTH. In isolated basolateral membranes from rabbit proximal tubule, PTH inhibited in a cAMP- and PLA_2-dependent manner the activity of Na^+-HCO_3^- cotransporter, determined by bicarbonate-dependent sodium uptake (687). In contrast, in the in vitro microperfused rabbit PST, PTH and cAMP stimulated base exit through a Cl^-/HCO_3^- exchanger (725). Taken together with the identification of a cAMP-stimulated basolateral chloride channels in the rabbit PST (726), these observations suggest that PTH promotes the shift from a $NaHCO_3$ to a NaCl reabsorption mode in the PST.

H. Insulin

De Fronzo et al. (198) first demonstrated that insulin exerts an antinatriuretic effect independently of the glycemic status in healthy human. A similar effect was later reported in dog (199) and rat (466). These results indicate that insulin may directly control renal sodium handling. Because insulin was shown to increase fluid and sodium reabsorption in in vitro microperfused rabbit PCT (51), the antinatriuretic effect of insulin is thought to originate in part in proximal tubules.

The stimulatory action of insulin is, at least in part, accounted for by an alteration of Na^+-K^+-ATPase activity. Indeed, in isolated rat PCTs, physiological concentrations of insulin enhance the transport activity of Na^+-K^+-ATPase independently of the apical sodium entry (279, 281). In this setting, insulin did not alter the V_{max} but increased the apparent sodium affinity of Na^+-K^+-ATPase (279). This effect of insulin on rat proximal tubule Na^+-K^+-ATPase activity was independent of PKC; it was prevented by tyrosine kinase inhibition and was mimicked by EGF and orthovanadate, an inhibitor of tyrosine phosphatases (280). These results indicate that insulin modulates Na^+-K^+-ATPase activity through a tyrosine phosphorylation process, raising the possibility of a direct tyrosine phosphorylation of the Na^+-K^+-ATPase.

This latter hypothesis was recently confirmed (278). Indeed, in rat proximal tubules, Na^+-K^+-ATPase α-subunit was phosphorylated at Tyr-10, and insulin increased from 10 to 25% the proportion of tyrosine phosphorylated

Na^+-K^+-ATPase units. Several lines of evidence indicate that the stimulation of Na^+-K^+-ATPase activity by insulin in proximal tubule cells is most likely accounted for by tyrosine phosphorylation of its α-subunit: *1*) substitution of Tyr-10 with Ala (Y10A) abolished the stimulation of ouabain-sensitive rubidium uptake by insulin in OK cells; *2*) the basal exogenous Na^+-K^+-ATPase-mediated rubidium uptake is higher in OK cells expressing the Y10E mutant α_1-subunit, a mutation that mimicks the effect of the negative charge introduced by phosphorylation; and *3*) stimulation of ouabain-sensitive rubidium uptake and phosphorylation of Na^+-K^+-ATPase occurred with the same time course and within the same range of insulin concentrations and cosaturated in rat PCTs.

In addition, insulin may also stimulate directly the apical sodium entry through the Na^+/H^+ exchanger, as shown in suspensions of rat proximal tubule (336).

I. Glucocorticoids

Chronic corticosteroid excess increases whereas chronic corticosteroid deficiency reduces the urine excretion of ammonium, phosphate, and total and titratable acid (226, 404, 405). Effects on phosphate metabolism will not be discussed here because they do not contribute significantly to the sodium balance, and the reader is referred to recent reviews (299, 463). The increased excretion of acid results in part from increased proximal tubule proton secretion and bicarbonate reabsorption (56). Wilcox et al. (882) demonstrated that corticosteroid-induced changes in total and titratable acid urinary output and ammonium excretion are mediated by glucocorticoid receptors, in agreement with the proximal origin of these phenomena.

Glucocorticoids have little or no effect on proximal tubule Na^+-K^+-ATPase. In both rats and rabbits, adrenalectomy did not (or only slightly) reduce(d) Na^+-K^+-ATPase activity (246, 247), and long-term corticosteroid administration did not increase it (245, 323).

In contrast, studies in rat BBMVs demonstrated that glucocorticoid stimulated the apical Na^+/H^+ exchanger (298, 464, 465); although amiloride-sensitive sodium uptake in BBMVs was not altered in vesicles from adrenalectomized (ADX) rats, it was increased in BBMVs from normal or ADX rats treated for 2 days with dexamethasone. This stimulation was accounted for by an increased number of Na^+/H^+ exchangers in BBMV since the exchanger affinities for sodium and proton were not altered by glucocorticoids. Because glucocorticoids stimulate endogenous production of acid (404, 405), and acidosis increases the V_{max} of Na^+/H^+ exchanger in BBMVs (181), the question arose of establishing whether stimulation of Na^+/H^+ exchanger was a primary effect of glucocorticoid or was secondary to intracellular acidification. To answer this question, the effect of glucocorticoids on Na^+/H^+ exchanger activity was determined in BBMV from ADX rats either under normal acid/base balance or made acidotic by addition of NH_4Cl in the drinking solution. Results indicated that dexamethasone was equally efficient in stimulating Na^+/H^+ exchanger in normal and acidotic rats and that acidosis did not increase Na^+/H^+ exchanger activity in the absence of glucocorticoid treatment. This indicated that glucocorticoid effect was independent of acidosis, whereas the effect of acidosis was mediated by glucocorticoids.

Regulation of the number of Na^+/H^+ exchangers in rat brush-border membranes was recently confirmed directly by immunoblot and immunohistochemistry (514). Adrenalectomy did not alter the abundance of the NHE-3 isoform, whereas administration of dexamethasone for 2 days to either normal or ADX rats increased it in proximal tubules. This was a transcriptional effect of glucocorticoids since *1*) the promoter of rat NHE-3 gene contains multiple DNA sequence elements recognized by the GR (127, 447); *2*) in OK and LLC-PK_1 cells transiently transfected with a chimera made of the 5′-regulatory region of NHE-3 gene coupled to the luciferase gene, glucocorticoid treatment induced luciferase activity (447); and *3*) NHE-3 mRNA abundance increased in proximal tubules from rabbits treated for 2 days with dexamethasone (55).

In addition to this long-term (2–3 days) in vivo effect of glucocorticoids, several studies indicate that dexamethasone can exert short-term (few hours) in vitro effects on proximal tubule Na^+/H^+ exchanger. In vitro addition of dexamethasone to microperfused rabbit PCT increased bicarbonate reabsorption (56) as well as Na^+/H^+ exchanger activity within 3 h (55). In rabbit proximal tubule cells, stimulation of Na^+/H^+ exchanger activity was observed as soon as 1 h after dexamethasone addition (90). Short-term stimulation of bicarbonate reabsorption in rabbit PCT resulted from a transcriptional effect of dexamethasone, since it was blocked by actinomycin D and cycloheximide (56). However, no change in NHE-3 mRNA level was observed (55), suggesting that dexamethasone might induce a protein regulating the activity and/or targeting of NHE-3 in brush-border membrane. These findings are at variance with results in OK-P cells in which dexamethasone increased within 4 h both the activity of Na^+/H^+ exchanger (53), the abundance NHE-3 mRNAs, and its transcription rate (52), suggesting a direct transriptional effect of glucocorticoids on NHE-3.

J. Summary

Despite some discrepancies and missing data, a few general conclusions concerning the signaling pathways mediating hormone effects on proximal tubule function can be drawn (Fig. 16). The cAMP/PKA signaling pathway

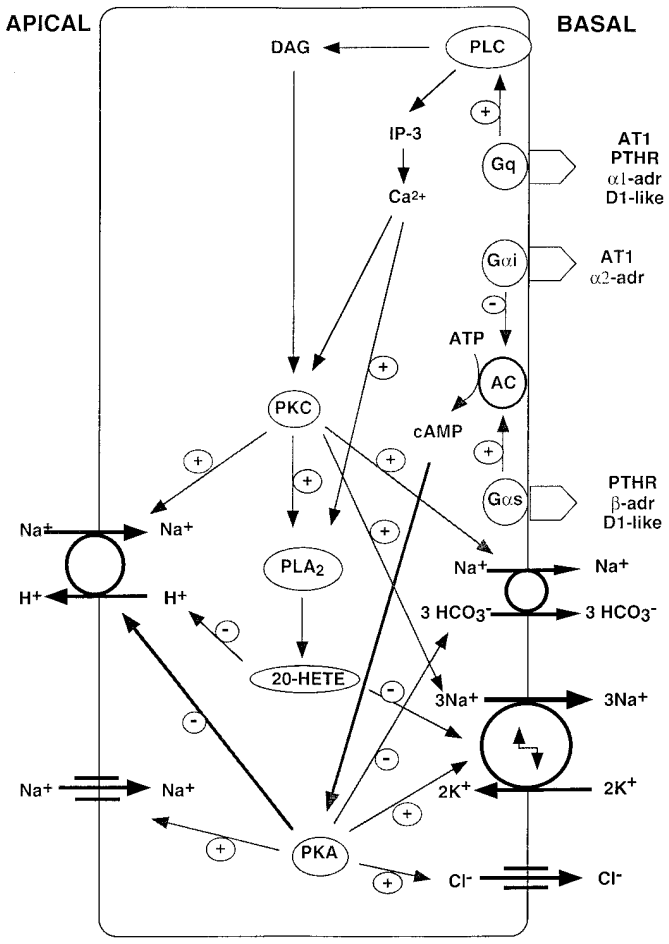

FIG. 16. Overview of the principal signaling pathways controlling sodium transport in proximal tubule cells. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets. AT_1, angiotensin II receptor; PTHR, parathormone receptor; α_1-adr, α_1-adrenergic receptor; α_2-adr, α_2-adrenergic receptor; β-adr, β-adrenergic receptor.

downregulates sodium-bicarbonate reabsorption and upregulates sodium-chloride reabsorption. This shift from a Na^+-HCO_3^- transport mode in proximal tubules to a Na^+-Cl^- one results from the differential regulation of proton/bicarbonate-coupled sodium transporters (the apical NHE-3 Na^+/H^+ exchanger and the basolateral NBC-1 Na^+-HCO_3^- cotransporter) which are inhibited, and of proton/bicarbonate-independent sodium transporters (the basolateral Na^+-K^+-ATPase and possibly the apical epithelial sodium channel ENaC) which are activated. cAMP/PKA-induced stimulation of Na^+-K^+-ATPase involves its phosphorylation and its redistribution toward the basolateral membrane, and therefore increases its V_{max}. cAMP/PKA inhibition of NHE-3 (and probably NBC-1) also involves phosphorylation through a complex network of protein-protein interactions that requires NHE-RF and cytoskeletal components.

Phorbol ester-activated PKCs upregulate both bicarbonate-dependent and -independent transporters. Activation of Na^+-K^+-ATPase results from increased affinity for sodium brought about by its PKC phosphorylation on Ser-16 without change in its V_{max}.

Finally, a PLA_2/arachidonate/cytochrome *P*-450-monooxygenase pathway downregulates the transport properties of proximal tubules, mainly through inhibition of Na^+-K^+-ATPase. This inhibitory pathway may be triggered by some types of PKCs and/or in response to metabolic stress. By inhibiting ATP consumption, it can be considered as a protective mechanism against the deleterious effects of cellular ischemia.

Phorbol ester-sensitive PKCs are the main mediators of the stimulatory effects of low concentrations of ANG II and of α-adrenergic agonists, eventhough decreased cAMP production may also participate to the upregulation of NHE-3. The cAMP/PKA pathway underlies the stimulatory effect of β-adrenergic agonists, with the inhibitory effect of cAMP/PKA on H^+-HCO_3^- transporters being likely prevented through functional inhibition of NHE-RF by direct interaction with activated β-receptors. Finally, the inhibitory effects of dopamine and PTH are mediated in part by the PLA_2/arachidonate/cytochrome *P*-450-monooxygenase pathway (inhibition of Na^+-K^+-ATPase) and in part through cAMP/PKA pathway (inhibition of H^+-HCO_3^- transporters).

In addition, tyrosine kinase phosphorylation of Na^+-K^+-ATPase on Tyr-10 appears as the main mechanism for the stimulatory effect of insulin. In contrast, the stimulatory effect of glucocorticoids mainly results from transcriptional activation of apical transporters.

V. HORMONAL CONTROL OF SODIUM TRANSPORT IN THE THICK ASCENDING LIMB

A. General Transport and Regulatory Properties of the TAL

1. Transport properties

TALs reabsorb ~15% of the filtered sodium and also reabsorb potassium, bicarbonate, and the divalent cations calcium and magnesium. In contrast, there is no net transport of either water, glucose, phosphate, or amino acids. The TAL is called the diluting segment with respect to this ability to reabsorb solutes in excess of water: the fluid delivered by the TAL to the distal convoluted tubule is hypotonic, and its sodium chloride concentration is ~30–40% of that in the glomerular ultrafiltrate. This function is very important for maintaining the water balance of the organism because it allows it to excrete water in excess of solutes in hypotonic states.

In TAL cells (Fig. 17), the sodium gradient generated

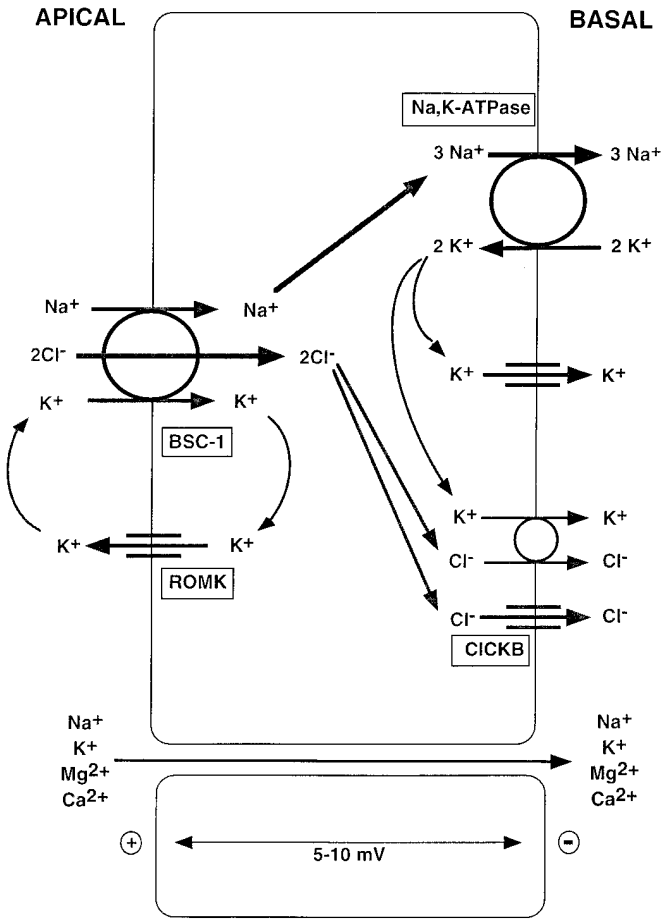

FIG. 17. Cellular mechanism of sodium, potassium, and anion transport in thick ascending limbs of Henle's loop. Arrows indicate net fluxes of solutes. The names of the currently cloned transporters are mentioned into rectangular boxes.

by basolateral Na^+-K^+-ATPase is mainly dissipated by an apical electroneutral Na^+-K^+-$2Cl^-$ cotransport system (343) that couples the downhill entry of sodium to the uphill transport of potassium and chloride. This cotransport system displays a high affinity for both sodium (3–4 mM) and potassium (2 mM) and is specifically inhibited by the loop diuretics furosemide and bumetanide. This Na^+-K^+-$2Cl^-$ cotransporter has been cloned (BSC1 or NKCC2) (319), and the cognate protein was found at the apical pole of TAL cells (450). Potassium ions accumulated by Na^+-K^+-$2Cl^-$ cotransport above Nernst equilibrium are recycled across the apical membrane, allowing adequate potassium availability to the cotransporter, via inwardly rectifying, voltage-insensitive potassium channels (ROMK) (389, 908). Chloride ions leave the cells across the basolateral membrane via chloride channels (ClCK2) (4) and electroneutral K^+-Cl^- cotransport system (KCC1) (338, 344, 502). Conductive diffusion of chloride and potassium depolarizes the basolateral membrane and hyperpolarizes the apical one, respectively. These two diffusion potentials in series combine to generate the lumen-positive transepithelial voltage characterizing the TAL. However, this transepithelial voltage is partly shunted by the electrical conductance of the paracellular pathway; because its conductance is higher for cations than for anions, the shunt current is mainly carried by net sodium reabsorption and to a lesser extent by chloride back flux. The lumen-positive voltage also serves as driving force for calcium and magnesium reabsorption (210, 889). The gene of paracellin-1, a protein accounting for the selective permeability of the shunt pathway to magnesium, was recently cloned, and mutations were shown to be responsible for renal magnesium wasting (742).

With the distal convoluted tubules, TALs display the highest sodium reabsorption capacity and Na^+-K^+-ATPase activity per tubular length unit. Under resting conditions, the quantity of sodium reabsorbed through TAL cells per minute approximately amounts to fourfold the intracellular pool of sodium. Thus maintenance of a constant intracellular sodium concentration requires a tight coordination of the activity of the different transporters involved in apical and basolateral ion movements. And, indeed, dysfunction of any of these transporters leads to dramatic impairment of sodium and water handling. Thus Bartter's syndrome may result from dysfunction of either the apical Na^+-K^+-$2Cl^-$ cotransporter (740), the basolateral chloride channel (738), or the apical potassium channel (741).

TAL also constitutes an important site of fluid acidification and ammonia transport. Despite the presence of an apical V-type H^+-ATPase, bicarbonate transport by TAL is primarily coupled to apical Na^+/H^+ exchanger, both NHE-2 and NHE-3 isoforms being present in apical membranes (149, 780). At the basolateral cell border, bicarbonate leaves the cell via Cl^-/HCO_3^- exchange as well as K^+-HCO_3^- and Na^+-HCO_3^- cotransport (621). Ammonia reabsorption in TAL is active; at the apical cell border, ammonium ions enter mainly by competing with potassium for transport by the apical Na^+-K^+-$2Cl^-$ cotransporter. Intracellularly regenerated ammonia leaves the cell by nonionic diffusion across the basolateral membrane, which displays a much higher permeability to ammonia than the apical membrane (460).

Although these cellular mechanisms of solute reabsorption are essentially similar in MTAL and CTAL, three distinct features characterize the two nephron segments: *1*) compared with kidney cortex, blood supply to the renal medulla is restricted and oxygen can be rate limiting in the MTAL, especially in view of the large ATP requirement necessary for sodium chloride reabsorption in that portion of the nephron. Interestingly, MTALs, but not CTALs, display many feedback regulatory mechanisms that downregulate cation reabsorption and thereby limit anoxia. *2*) In most species except rabbit, reabsorption of divalent cations along the TAL is restricted to the CTAL

(whether in these species paracellin-1 is expressed only in the CTAL remains to be determined). *3*) The largest part of sodium chloride reabsorbed in the MTAL is recycled in the kidney medulla and contributes to generate the gradient of osmotic pressure, whereas salt reabsorbed along the CTAL is rapidly washed out of the kidney by cortical blood flow and therefore contributes to dilute the tubular fluid and to generate free water.

In summary, Na^+-K^+-ATPase not only energizes sodium, potassium, ammonium, calcium, magnesium, chloride, and bicarbonate reabsorption in the TAL, but also serves as the motor for the generation of the corticopapillary osmotic gradient that drives water reabsorption along the collecting duct.

2. Regulatory properties

cAMP is the main stimulus of ion reabsorption in the TAL. Many hormones and mediators, e.g., vasopressin, PTH, glucagon, calcitonin, and β-adrenergic agonists, can be positively coupled to adenylyl cyclase in the TAL and stimulate sodium, potassium, chloride, and divalent cation reabsorption. A peculiarity of the hormonal control of TAL function through cAMP signaling is its redundancy. So many hormones activate a same pool of adenylyl cyclase in the TAL that in vivo the system is always stimulated maximally under normal physiological conditions. In other words, when the concentration of only one of the multiple hormones stimulating adenylyl cyclase in the TAL decreases, the remaining hormones suffice to fully sustain maximal TAL transport functions. This conclusion is based on the following: *1*) characterization of the effects of adenylyl cyclase activating hormones on ion transport by in vivo micropuncture of rat TAL required the development of an hormone-deprived model in which plasma vasopressin, PTH, calcitonin, and glucagon were all four suppressed artificially beforehand (34, 242, 681). *2*) Characterization of hormone action by in vitro microperfusion of TALs requires, before hormone application, a preequilibration period during which nephron segments recover from their in vivo stimulation; during this period, their transepithelial voltage decreases steadily down to a basal level, reflecting destimulation of solute transport. *3*) Although human TALs lack vasopressin-sensitive adenylyl cyclase (142, 684), humans do not display any impairment of their ability to dilute urine, i.e., of their TAL function.

As a counterpart, the major physiological regulation of the cAMP/PKA signaling pathway in TALs may not be through its stimulation but through its inhibition. And, indeed, numerous factors oppose the stimulatory effects of adenylyl cyclase and are functionally important regulatory parameters.

The following discussion mainly focuses on the effects of the cAMP/PKA cascade and on its negative control by ANG II, PGE_2, endothelin, cGMP, and extracellular calcium. The effects of other hormones, including corticosteroids, dopamine, and insulin, are also discussed briefly.

B. cAMP/PKA Signaling Pathway and Related Hormones

As shown by micropuncture and by in vitro microperfusion studies, increased intracellular cAMP level is associated with increased sodium chloride reabsorption in the TAL (192, 211, 212, 243, 788, 887, 888). As expected from the presence of hormone-sensitive adenylyl cyclase in MTAL and/or CTAL of rat, rabbit, and mouse, the stimulatory effect of cAMP was mimicked by basolateral addition of either vasopressin (87, 360, 377, 379, 698, 888, 889), PTH (212, 243, 887), glucagon (211), calcitonin (242), or β-adrenergic agonists (36, 567) to in vitro microperfused CTAL and/or MTAL. The mechanism of the cAMP-dependent increase in sodium chloride transport was further analyzed at the molecular level.

1. Control of Na^+-K^+-ATPase

As already discussed, stimulation of transcellular sodium flux in TAL cells must imply the coordinated stimulation of both apical (Na^+-K^+-$2Cl^-$ cotransporter and potassium channel) and basolateral transporters (Na^+-K^+-ATPase and chloride channel). However, the first study on isolated rat MTAL indicated that forskolin and cAMP analogs reduced Na^+-K^+-ATPase activity (702). Subsequently, this inhibitory effect was shown to result from limited oxygen and metabolic substrate supply that led to cellular ATP depletion and to the triggering of cell protective mechanisms (467). Indeed, in the presence of adequate oxygen supply and oxidative metabolic substrates that increased intracellular ATP level, both forskolin and cAMP analogs stimulated the transport and the hydrolytic activities of Na^+-K^+-ATPase in isolated rat MTALs (467). Vasopressin also increased the V_{max} of Na^+-K^+-ATPase (153, 638). The dependence of the regulation of sodium transport on oxidative metabolism has been observed in other nephron segments (see sect. III*C1* for example), but it is exacerbated in MTAL cells because *1*) cellular ATP is almost exclusively supplied by oxidative metabolism, as the contribution of anaerobic metabolism is very low (826), and *2*) the ATP requirement for Na^+-K^+-ATPase is very high and accounts for as much as 80% of total oxygen consumption (799). Thus oxygen availability may be rate limiting for ATP production and thereby for sodium transport by MTAL, as evidenced both in vitro and in vivo: *1*) in vivo measurements with microelectrodes implanted in the kidney parenchyma indicate that P_{O_2} in kidney medulla was low (32, 492), and it increased markedly in response to transport inhibition by loop diuretics (113); and *2*) in vitro measurements indicated that

basal cellular ATP content was 25% lower in nonoxygenated compared with oxygenated MTALs (467).

Stimulation of Na^+-K^+-ATPase by forskolin and cAMP observed in well-oxygenated MTALs likely resulted from PKA-mediated phosphorylation of its α-subunit. As discussed in section II*A5*, PKA phosphorylates the α-subunit of Na^+-K^+-ATPase on Ser-943 in vitro and in intact cells (66, 83). In suspensions of rat MTALs, cAMP analogs increased the phosphorylation of Na^+-K^+-ATPase α-subunit (467). In addition, the stimulation of Na^+-K^+-ATPase activity was linearly related and cosaturated with the level of phosphorylation of its α-subunit (467), suggesting a causal relationship between the phosphorylation and the stimulation of Na^+-K^+-ATPase.

Inhibition of Na^+-K^+-ATPase activity observed in response to cAMP in hypoxic MTALs resulted from the stimulation of a PLA_2/cytochrome *P*-450-dependent monooxygenase pathway and the synthesis of arachidonic acid metabolites that directly inhibit the pump activity (702). Indeed, inhibition of Na^+-K^+-ATPase was mimicked by arachidonic acid (129, 702) and was prevented by inhibition of either PLA_2 with mepacrine (702) or cytochrome *P*-450-dependent monooxygenase with SKF525A (702). Triggering of this pathway in hypoxic MTALs may not result from a primary effect of cAMP/PKA but rather from decreased Po_2 brought about by an initial stimulation of sodium transport. Thus, whatever the oxygenation status of MTALs, activation of PKA would first stimulate sodium reabsorption, a process that increases oxygen and ATP consumption, as indicated by the decrease in cellular ATP content (467). In the absence of adequate oxygen supply, stimulation of sodium transport would rapidly provoke cellular hypoxia, insufficient ATP synthesis which, in turn, would stimulate the PLA_2/arachidonate/monooxygenase inhibitory pathway. Conversely, when oxygen supply is sufficient, cell metabolism would face the additional demand for ATP synthesis elicited by cAMP-induced increase in sodium transport, and the PLA_2/arachidonate-monooxygenase pathway would not be triggered.

In support to this hypothesis, it was found that *1*) cAMP induced similar levels of phosphorylation of Na^+-K^+-ATPase α-subunit in hypoxic and in well-oxygenated MTALs, and *2*) mepacrine abolished the inhibitory effect of cAMP on Na^+-K^+-ATPase activity in hypoxic MTALs but did not alter cAMP-induced phosphorylation of its α-subunit (467). Altogether, these findings indicate that the inhibitory mechanism triggered by PLA_2 stimulation applies to Na^+-K^+-ATPase units that had been phosphorylated through PKA stimulation beforehand.

2. Na^+-K^+-2Cl^- cotransporter

As expected, cAMP analogs also stimulated the Na^+-K^+-2Cl^- cotransport activity measured either as bumetanide-sensitive rubidium uptake (19) or bumetanide-sensitive intracellular acidification in response to exposure to NH_4Cl (ammonium being transported by Na^+-K^+-2Cl^- cotransporter as a potassium substitute) (14) in suspensions of rat MTALs. This PKA-mediated functional effect relied on a primary activation of the cotransporter, since the stimulation of the Na^+-K^+-2Cl^- cotransporter was not prevented by the inhibition of either Na^+-K^+-ATPase, apical potassium channels, or basolateral chloride channels (14). In vitro treatment with vasopressin also stimulated apical sodium entry through the Na^+-K^+-2Cl^- cotransporter (563, 779).

In mouse MTALs, it had been proposed that vasopressin altered the mode of apical sodium entry from a Na^+-Cl^- cotransport to the classical Na^+-K^+-2Cl^- cotransport (779), both modes being sensitive to furosemide. Very recently, a molecular basis has been proposed for this shift. Several alternatively spliced cDNAs encoding the murine apical furosemide-sensitive Na^+-K^+-2Cl^- cotransporter mBSC1 have been identified. A total of six isoforms with different COOH termini are expressed in the mouse kidney: three C9 forms (mBSC1-A9, -F9, and -B9) of ~150 kDa and three C4 forms (mBSC1–4A, -4B, and -4F) of ~120 kDa due to truncation of COOH termini (570). When expressed in *Xenopus* oocytes, the three C9 isoforms induced a furosemide-sensitive Na^+-K^+-2Cl^- cotransport activity that was not sensitive to PKA (640). In contrast, expression of the C4 isoforms induced no sodium transport activity unless the oocytes were preexposed to hypotonic medium for 1 h; under such conditions, furosemide-sensitive and potassium-independent Na^+-Cl^- cotransport activity appeared and was inhibited by PKA stimulation (639). Moreover, coexpression of C4 isoforms in normal *Xenopus* oocytes exerted a dominant negative effect on C9-mediated Na^+-K^+-2Cl^- cotransport activity, and this inhibitory effect was reversed by activation of PKA (640). If confirmed in mouse MTAL, this process would account for the cAMP-induced shift from Na-Cl to Na^+-K^+-2Cl^- cotransport. The sensitivity of the C4 isoforms to tonicity may also account for the inhibitory effect of hypertonicity on both basal and vasopressin-stimulated sodium chloride reabsorption (380).

In cultured cells derived from mouse MTAL, acute stimulation of Na^+-K^+-2Cl^- cotransport by forskolin or vasopressin was associated with F-actin redistribution and was impaired by stabilization of actin filaments by phalloidin (901), suggesting that vasopressin acts at least in part through actin depolymerization (739).

In addition to the short-term control of Na^+-K^+-2Cl^- cotransport activity, cAMP generation might be an important regulator of Na^+-K^+-2Cl^- cotransporter expression in TAL. Indeed, 1-wk vasopressin infusion to Brattleboro rats (which exhibit a spontaneous knockout of the vasopressin gene) or dehydratation of normal rats (which increases endogenous vasopressin secretion) increased the expression of the Na^+-K^+-2Cl^- cotranporter BSC1 (461). Furthermore, expression of adenylyl cyclase type

VI and of BSC1 decreased in mice with heterozygous disruption of the $G\alpha_s$ gene (232).

3. Potassium and chloride channels

Stimulation of apical sodium entry (Na^+-K^+-$2Cl^-$ cotransporter) and basolateral extrusion (Na^+-K^+-ATPase) is not sufficient to account for increased solute reabsorption in the TAL. Because of the tight coupling between the different transporters, it also requires stimulation of apical potassium recycling and basolateral chloride exit. Early experiments by in vitro microperfusion indicated that activation of PKA hyperpolarized the basolateral membrane (377, 714). However, this observation was interpreted differently. According to Andreoli's group (377), this was accounted for by primary stimulation of apical ion entry, involving stimulation of Na^+-K^+-$2Cl^-$ cotransport and potassium recycling through apical potassium channels, leading to increased intracellular chloride concentration and higher diffusion potential for chloride across basolateral membrane. According to Greger's group (714), it resulted from primary stimulation of basolateral chloride channels, that decreased intracellular chloride concentration which in turn activated apical ion entry and potassium recycling.

Although intracellular chloride concentration has not been measured, it seems likely that both apical ion entry, potassium recycling, and basolateral chloride exit steps are primarily activated through PKA stimulation. In any case, apical potassium channels (663, 851) and basolateral chloride channels (352, 664) may be activated through PKA stimulation.

To sum up, basal multihormonal stimulation of cAMP/PKA pathway maintains a high sodium chloride reabsorption in TAL. This tonic effect involves the stimulation of apical Na^+-K^+-$2Cl^-$ cotransporter and of potassium recycling through potassium channels associated with a coordinated increase in basolateral sodium and chloride exit through the stimulation of Na^+-K^+-ATPase and chloride channels (Fig. 18). The simultaneous regulation of apical and basolateral transporters by cAMP allows the regulation of transcellular sodium flux without altering intracellular ionic strength. When oxygen availability is restricted, this stimulatory pathway is overridden by the activation of a PLA_2-mediated pathway that inhibits Na^+-K^+-ATPase and Na^+-K^+-$2Cl^-$ cotransporter activities leading to a decrease in sodium reabsorption (253). This latter mechanism might be important to ensure cell survival under pathological conditions. Finally, this basal stimulation is downregulated by numerous factors and through various pathways, as summarized below.

C. Inhibition of Sodium Transport in TALs

As already mentioned, the physiological regulation of the transport properties of TALs is accounted for mainly by the negative modulation of the stimulatory effect of cAMP/PKA signaling pathway rather than by the stimulation itself. In fact, numerous agents and signaling pathways may inhibit sodium transport in TALs. In the following paragraphs, these are discussed according to the mechanism of inhibition rather than specific hormones. Part of the inhibition of sodium transport in TAL results from the unique properties of the adenylyl cyclase expressed in TAL. Indeed, TALs exclusively express type 6 adenylyl cyclase (141) which, along with type 5, displays the unique properties of being inhibited both by intracellular calcium ($[Ca^{2+}]_i$) and by coupling to $G\alpha_i$ (144). However, other mediators may inhibit TAL function independently of intracellular cAMP level.

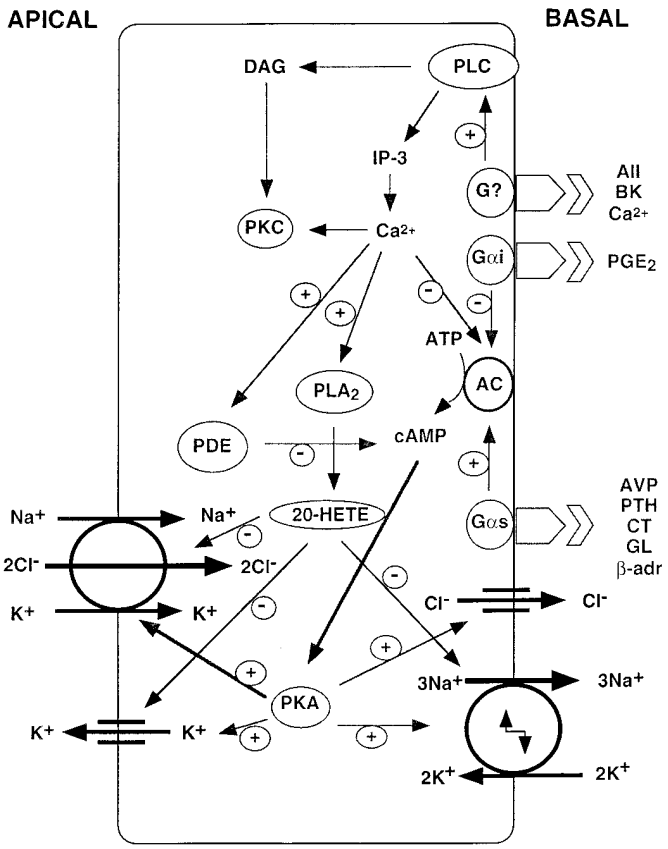

FIG. 18. Overview of the principal signaling pathways controlling the active sodium transport in cells of the thick ascending limb of Henle's loop. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets. PDE, phosphodiesterase; BK, bradykinin; CT, calcitonin; GL, glucagon.

1. Inhibition through $G\alpha_i$

Prostaglandins, particularly PGE_2, inhibit vasopressin-induced solute transport in TAL (192, 772). The role of $G\alpha_i$ activation in this inhibition was suggested by the following: *1*) PGE_2 suppressed the effect of vasopressin on mouse TAL sodium transport, but not that of forskolin

or cAMP (192); *2*) PGE_2 reduced hormone-induced accumulation of cAMP in TAL from various species (291, 814); *3*) PGE_2 effect was curtailed by pertussis toxin treatment (291, 789); and *4*) EP_3 prostaglandin receptors (coupled to $G\alpha_i$) are expressed in TAL (112), whereas there is no functional evidence for EP_1 (coupled to PLC activation) and EP_2 (coupled to adenylyl cyclase activation) receptors in rat TAL (2, 202) (Fig. 18).

2. Inhibition through increased intracellular calcium concentration

ANG II, bradykinin, and extracellular calcium increase $[Ca^{2+}]_i$ through G protein-coupled receptors and inhibit solute reabsorption in the rat TAL (15, 202, 346–348). However, $[Ca^{2+}]_i$ may exert inhibitory action through different mechanisms, namely, a direct effect of calcium on transport systems, reduction of hormone-induced accumulation of intracellular cAMP, activation of PLA_2 and arachidonate metabolism, or activation of calcium-sensitive PKCs. These differences may be related to their cortical or medullary origin in the TAL.

A direct effect of $[Ca^{2+}]_i$ was proposed in view of the inhibitory effect of cytosolic calcium on the activity of apical potassium channels in excised patches from rat TAL (94).

In MTAL, increased $[Ca^{2+}]_i$ also activates calcium-sensitive PLA_2 and thereby the production of 20-hydroxyeicosatetraenoic acid (20-HETE) through the cytochrome *P*-450 monooxygenase. 20-HETE was found to mediate the inhibition of apical potassium channel and/or Na^+-K^+-$2Cl^-$ cotransporter in response to bradykinin (347), ANG II (15, 523), and extracellular calcium (853). As already discussed above, this pathway also inhibits Na^+-K^+-ATPase activity (Fig. 18).

Finally, in the CTAL, ANG II, extracellular calcium, and bradykinin decrease $G\alpha_s$-mediated intracellular accumulation of cAMP (144, 202). This inhibition resulted from two mechanisms: *1*) stimulation of cAMP degradation by calcium-activated phosphodiesterases, and *2*) pertussis toxin-insensitive inhibition of type 6 adenylyl cyclase (202). Interestingly, the first pathway was shared by the three mediators, whereas strong inhibition of adenylyl cyclase was elicited only through activation of the Ca^{2+}-sensing receptor (202). This clearly indicates that in a same cell, increased $[Ca^{2+}]_i$ can elicit different effects according to the inducing agonist. This observation supposes an intracellular compartmentalization of $[Ca^{2+}]_i$ increases (Fig. 18).

3. Inhibition through cGMP

Cell-permeant analogs of cGMP also inhibit solute reabsorption in the TAL (592, 598). Inhibition through cGMP can be physiologically triggered by receptor guanylate cyclase in response to atrial natriuretic peptide and urodilatin (592, 598), but also through activation of soluble guanylate cyclase (for example, by cytokines), since a nitric oxide donor such as nitroprusside reduced sodium chloride reabsorption (593). Finally, sodium reabsorption was also inhibited by platelet-activating factor (PAF) through increased production of cGMP (593). However, the coupling between PAF receptor and cGMP production is not understood as yet.

4. Calcium-insensitive PKC

Calcium-insensitive PKCs (new and atypical PKCs) have been involved in the effect of endothelin and insulin in the TAL.

Endothelin inhibited solute reabsorption in mouse TAL without increasing $[Ca^{2+}]_i$, but its effect was blocked by PKC inhibitors and was mimicked by DAG analogs (201), suggesting the involvement of PKC of the new family in this process.

Insulin stimulates sodium chloride reabsorption in the rabbit and mouse MTAL (424, 534). This stimulation was blocked by genistein, by wortmannin, and by staurosporine and calphostin C, indicating the involvement of tyrosine kinase, PIK, and PKC (423). Interestingly, insulin effect on sodium transport did not require extracellular calcium, and insulin did not change $[Ca^{2+}]_i$, indicating that relevant PKC should belong to the new or atypical family (425).

Thus stimulation of these new and/or atypical PKCs in TAL can either inhibit (endothelin) or stimulate (insulin) sodium reabsorption. These opposite effects might be mediated by different subtypes of PKCs, e.g., a new PKC for the inhibitory effect of endothelin and an atypical one for insulin stimulatory action. It is worth noting that besides classical PKCs (α and βII), both new (δ and ϵ) and atypical PKCs (ζ) have been detected in TAL (24).

D. Dopamine

In contrast to the other receptors coupled to the activation of adenylyl cyclase, activation of D_1-like receptors inhibited sodium reabsorption by the in vitro microperfused rat MTAL (348). Dopamine and D_1-like agonists were active from the basolateral but not the luminal side, conversely to the proximal tubule where they were active from both sides.

Consistently with the cAMP/PKA signaling mechanism (see above), but in contrast to its reported effect on sodium reabsorption (348), dopamine stimulated the apical Na^+-K^+-$2Cl^-$ cotransport (19). Thus, according to these observations, the stimulation of apical sodium entry must be overwhelmed by an inhibition of the basolateral exit. The paradoxical dopamine-induced PKA-mediated inhibition of Na^+-K^+-ATPase V_{max} described in rat MTAL has been proposed to account for the inhibitory effect of

dopamine on sodium transport (551, 703). This inhibition would result from PKA-mediated phosphorylation of DARPP-32, which in turn would inhibit protein phosphatase-1. Activation of PKA and inhibition of protein phosphatase-1 would increase the phosphorylation level of the Na^+-K^+-ATPase α-subunit on Ser-943, i.e., the PKA phosphorylation site, and inhibit its activity (at the opposite to the demonstrated stimulatory effect). This mechanism was proposed on the basis of the following: *1*) DARPP-32, a dopamine- and cAMP-regulated protein phosphatase-1 regulator (342), is expressed in thick ascending limb from various mammalian species (310, 551); *2*) a phosphopeptide corresponding to amino acid residues 8–38 of DARPP-32 inhibited Na^+-K^+-ATPase activity in permeabilized rat MTAL cells (21); and *3*) calyculin A, a protein phosphatase-1 inhibitor, inhibited Na^+-K^+-ATPase activity in rat MTAL (495). Unfortunately, the effect of dopamine on the phosphorylation of Na^+-K^+-ATPase α-subunit has not been evaluated in these studies. Because it is now established that PKA phosphorylation of Na^+-K^+-ATPase does not inhibit its activity in MTAL (467), alternative mechanisms of inhibition of the enzyme should be considered. Also, one might consider the involvement of PKC in dopamine-induced inhibition of Na^+-K^+-ATPase since *1*) PKC activation is a pathway for inhibiting sodium transport in MTAL (see above), and *2*) D_1-like receptors are also coupled to PKC stimulation through $G\alpha_q$ (408, 922).

E. Glucocorticoids

The first piece of evidence for corticosteroid action in TAL was provided as early as 1957 by Guinnebault and Morel (353) who reported that adrenalectomy reduced the corticomedullary gradient of osmotic pressure. Decreased sodium chloride reabsorption along the TAL of ADX rats was further supported by in vivo micropuncture (387) and microperfusion experiments (578, 767) studies and was fully demonstrated by in vitro microperfusion of MTAL (900). Adrenalectomy also reduced bicarbonate reabsorption along the loop of Henle, and this effect was reversed by physiological doses of dexamethasone (832).

Adrenalectomy reduced Na^+-K^+-ATPase activity in rat and rabbit TAL (246, 247). In rabbit TAL, this decrease in Na^+-K^+-ATPase activity was accompanied by a parallel decrease in the numbers of Na^+-K^+-ATPase at the plasma membrane, as determined by specific [^{3}H]ouabain binding (249). Conversely, chronic deoxycorticosterone acetate (DOCA) treatment of adrenal-intact rats and rabbits did not alter Na^+-K^+-ATPase activity along the TAL (245, 323). Within 3 h, in vivo administration of dexamethasone to ADX rabbits increased Na^+-K^+-ATPase activity in TAL back to the level observed in normal animals, whereas aldosterone administration had no effect during the same time frame (247). However, no change in Na^+-K^+-ATPase abundance was observed during this short-term stimulation of Na^+-K^+-ATPase (245), suggesting that dexamethasone activated preexisting ATPase units. Short-term (<3 h) stimulation of Na^+-K^+-ATPase was reproduced by in vitro addition of dexamethasone, but not aldosterone, to MTAL from ADX rats (221, 662). This effect was blocked by actinomycin D and cycloheximide (221), suggesting that dexamethasone induces the expression of a protein, which in turn modulates the activity of Na^+-K^+-ATPase. It has been proposed that dexamethasone would inhibit PLA_2 activity through induction of an inhibitor, and thereby relieve Na^+-K^+-ATPase from the tonic inhibitory influence of arachidonate metabolites (219).

Curiously, no attempt was made to evaluate the role of corticosteroids on either apical Na^+-K^+-$2Cl^-$ cotransporter and potassium channel, or basolateral K^+-Cl^- cotransporter and chloride channel in TAL. Only a recent study demonstrated that the Na^+/H^+ exchanger NHE-3 was upregulated by chronic (>3 days) dexamethasone treatment in TAL of both adrenal-intact and ADX rats, although adrenalectomy had no effect (514).

VI. HORMONAL CONTROL OF SODIUM AND POTASSIUM TRANSPORT IN THE COLLECTING DUCT

A. General Transport Properties of Collecting Ducts

In mammals, the collecting duct is the final site of regulation of sodium, potassium, acid/base, and water excretion. Accordingly, the collecting duct may reabsorb between 0 and 5% of the filtered sodium, reabsorb or not water, and either reabsorb or secrete bicarbonate. Regulation of acid/base balance originates in intercalated cells and is not related to Na^+-K^+-ATPase-generated ion gradients but to H^+-ATPase- and H^+-K^+-ATPase-generated proton gradients. Therefore, the cellular and molecular mechanisms underlying acid/base transport and regulation are not discussed here. In contrast, because Na^+-K^+-ATPase drives both sodium reabsorption and potassium secretion in the principal cells, transport and regulation of these two cations is discussed.

The collecting duct constitutes a tight epithelium displaying high transepithelial resistance and very low paracellular water permeability. Active electrogenic sodium reabsorption generates a large lumen-negative transepithelial voltage (−10 to −60 mV) in the CCD. In deeper portions of the collecting duct, this transepithelial voltage decreases and turns slightly lumen positive as a result of decreased sodium reabsorption by principal cells combined with increased electrogenic proton secretion by intercalated cells.

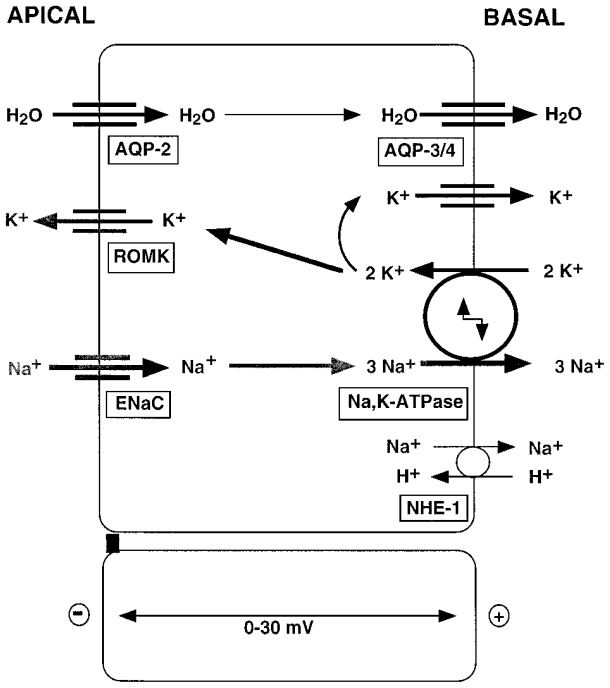

FIG. 19. Cellular mechanism of sodium, potassium, and water transport in principal cells of the collecting duct. Arrows indicate net fluxes of water and ions. The names of the currently cloned transporters are mentioned into rectangular boxes.

As summarized in Figure 19, sodium reabsorption in principal cells is linked to potassium secretion through a two-step mechanism: pumping of potassium within and sodium out of the cell by the basolateral Na^+-K^+-ATPase generates driving forces for apical sodium entry and potassium exit. Through this mechanism, potassium secretion is primarily coupled to sodium reabsorption with the $2K^+$:$3Na^+$ stoichiometry of Na^+-K^+-ATPase.

The major fraction of apical sodium entry is mediated by amiloride-sensitive ENaC, although thiazide-sensitive Na^+-Cl^- cotransporter (TSC) may contribute somewhat in the rat CCD (800). The epithelial sodium channel belongs to a new family of sodium channels displaying homologies with *Caenorhabditis elegans* degenerins (125, 506). Like all the members of the degenerin/ENaC gene superfamily, ENaC displays two hydrophobic membrane-spanning regions with intracellular NH_2 and COOH termini and a large extracellular loop with highly conserved cysteine residues (670, 755). ENaC is a multimeric channel made of homologous α-, β-, and γ-subunits (126), associated in a 2:1:1 stoichiometry (292), surrounding the channel pore (126). ENaC is potently and specifically inhibited by submicromolar concentrations of the diuretic amiloride and its derivative benzamylamiloride. For more information on the structure and function of ENaC, readers are refered to recent reviews (44, 317, 325).

Apical membrane of principal cells contains two distinct types of potassium channels, maxi potassium channels (619) that are activated by membrane depolarization and calcium and low-conductance (10–30 pS) inwardly rectifying potassium channels (304) that likely correspond to the cloned ROMK channel (389), in particular the ROMK-1 and ROMK-2 isoforms (101). Maxi potassium channels are probably not involved in potassium secretion because they are sensitive to tetraethylammonium, whereas collecting duct secretion of potassium is not (303). Basolateral membranes of the principal cells also display several types of potassium channels of various conductance (30, 85, and 140 pS) that are inhibited by intracellular acidification and activated by hyperpolarization (849). Their respective role in potassium transport is not clearly understood. However, secretion of potassium through the apical membrane is favored over recycling across the basolateral membrane because diffusive sodium entry depolarizes the apical membrane and thereby increases the driving force for potassium secretion.

In addition, collecting duct principal cells are also the site of water reabsorption through apical vasopressin-induced water channels (AQP-2) and constitutive basolateral water channels (AQP-3 and AQP-4). Although the driving force for water absorption is mainly provided by the countercurrent concentrating mechanism in the loop of Henle (see above), sodium reabsorption along the collecting duct, especially in CCD, dilutes the luminal fluid and contributes to the generation of an osmotic gradient favorable to water reabsorption. Finally, the basolateral membrane of collecting duct principal cells contains a Na^+/H^+ exchanger (NHE-1) involved in the regulation of intracellular pH, and which might be involved in the mediation of mineralocorticoid action (47).

The two major hormonal factors that positively control sodium reabsorption by the collecting duct are aldosterone and vasopressin. Insulin may play a significant role in the postprandial period. The stimulatory effects of these factors might be balanced by the negative influence of several mediators such as dopamine, α_2-adrenergic agonist, and prostaglandins.

B. Aldosterone

Concomitantly to the purification of aldosterone from adrenal cortex, clinicians isolated from the urine of patients with abnormal sodium retention a substance that later proved to be aldosterone (524), a first indication that aldosterone might be involved in the regulation of sodium handling. Later, injection of aldosterone in dog renal artery was reported to alter the electrolytic composition of their urine, identifying the kidney as a target for aldosterone (45). Aldosterone decreases urinary sodium excretion (antinatriuretic effect) and increases that of potassium (kaliuretic effect) and of protons (31, 45, 188, 731). Stop-flow experiments revealed quite early that the distal

parts of the nephron were the sites of action of aldosterone (834). Later, aldosterone was also shown to facilitate the hydrosmotic action of vasopressin (341, 659). In this review we consider only the effects of aldosterone on sodium and potassium excretion. Before analyzing these effects of aldosterone on ion transport, it is important to briefly describe the experimental models that allowed their study.

1. Experimental models to study aldosterone action

The effects of aldosterone on renal sodium and potassium handling were assessed first by clearance studies in which were recorded the changes in urine composition induced by administration of aldosterone to normal or ADX animals. Due to the restricted site of aldosterone action along the nephron, further study of the cell and molecular targets was performed on isolated collecting ducts.

Two types of in vitro experiments were attempted to elucidate the action of aldosterone on isolated collecting ducts. In most studies, the plasma level of aldosterone was manipulated in vivo (by adrenalectomy, acute or chronic administration of aldosterone, or change of the cation content of the diet), and the transport function of the tubules was determined in vitro after their isolation by microdissection. This approach supposes that the cells keep the "memory" of their in vivo steroidal environment, which is likely for hormones acting through induction of protein synthesis. In a few studies, the effects of aldosterone were evaluated after in vitro addition on nephron segments dissected from either normal or mineralocorticoid-deficient animals. This approach is limited by the short duration of in vitro survival of isolated nephron compared with the delay of steroid response.

Experimental models of tight epithelia, such as the frog skin or the urinary bladder of amphibians, palliated this limitation and were very useful to study the action of aldosterone in vitro. In addition, these are planar epithelia that can be mounted easily in Ussing's chambers for flux and electrophysiological measurements. According to Ussing-Koeffoed-Jonhson model (475), sodium flux across these epithelia can be estimated by the short-circuit current measured when the two faces of the epithelia are bathed with symmetrical solutions. A main limitation of these epithelia is their much lower capacity to reabsorb sodium, compared with renal collecting duct. This has been circumvented in part by developing cell lines such as A6 cells derived from amphibian kidney (630), which can be mounted in Ussing's chambers, when grown on filters. It should be noted, however, that all the conclusions derived from these models may not be directly transposable to the mammalian nephron. Thus efforts were made recently to develop cell lines derived from mammalian collecting duct principal cells that keep their sensitivity to corticosteroids (75, 230).

2. Effects on sodium transport

Despite the early recognition of impaired renal capacity to reabsorb sodium in patients with Addison's disease, a direct stimulatory effect of aldosterone on sodium transport was demonstrated only in 1961 by Crabbé (188) in amphibian epithelia. This report indicated that aldosterone increased the short-circuit current across the toad bladder. Further characterization of this stimulatory effect (765) indicated that it appears after a 45- to 90-min lag period, followed by an early response, lasting for ~3 h, during which sodium transport nearly doubles and the transepithelial electrical resistance decreases by about one-half. Then, sodium transport keeps on increasing for 12–24 h, whereas the electrical resistance remains low.

In mammals, aldosterone effect on renal handling of sodium has been more difficult to characterize, probably because it depends critically on the experimental conditions. Due to the presence of both GR and MR in the collecting duct, aldosterone concentrations within the physiological range (10^{-10} to 10^{-8} M) must be tested to avoid activation of GRs. Furthermore, effects of acute administration of aldosterone must be distinguished from the chronic effects that may result from secondary associated changes such as extracellular volume expansion, alteration of tubular sodium delivery, or morphological adaptations of tubular cells.

Renal action of aldosterone was evaluated in the light of these considerations in only two studies. Horisberger et al. (394) evaluated aldosterone action on anesthetized ADX and glucocorticoid-supplemented rats, whereas Campen et al. (124) studied conscious ADX rats. A reduction of urinary sodium excretion occurred 30–60 min after aldosterone injection and persisted for several hours. The maximum antinatriuretic effect was observed with <0.3 μg/100 g body wt of aldosterone, corresponding to 5 nM plasma aldosterone concentration (a concentration allowing near saturation of MRs). Aldosterone induced antinatriuresis in the presence as well as in the absence of glucocorticoids, but the magnitude of the effect was directly related to the rate of sodium excretion before aldosterone administration. Antinatriuresis was inhibited by the MR antagonist spironolactone and by inhibitors of transcription such as actinomycin D (395), confirming that aldosterone induces the synthesis of proteins that account for its antinatriuretic effect.

Most studies by in vitro microperfusion of CCD evaluated the effect of long-term corticosteroid administration on transport functions. The first report (350) indicated that the transepithelial potential difference (PD) was higher in CCDs microdissected from rabbit treated with DOCA than in CCDs from mineralocorticoid-defi-

cient animals. It was subsequently shown that DOCA treatment also increased sodium reabsorption in rat and rabbit CCD (609, 668, 722, 775). Unexpectedly, changes in transepithelial PD and sodium reabsorption were observed only after several days of treatment with DOCA, peaking after 4–18 days (415, 609). When the plasma concentration of aldosterone was varied within physiological range by modifying the cation content of the diet, both the transepithelial PD and the sodium reabsorption flux measured in vitro were correlated with the in vivo aldosterone concentration; half-maximal increases in voltage and sodium transport were observed at 0.2–0.5 nM plasma aldosterone, a concentration corresponding to the affinity of MR for aldosterone. However, here again, these changes reflect the chronic action of aldosterone, correlated with morphological changes of the principal cells of the CCD (844), and do not correspond to the early (<3 h) effect of aldosterone.

Reports of short-term effects triggered by adding aldosterone to in vitro microperfused CCDs remain few and controversial. Gross and Kokko (351) first reported that, after a 10- to 20-min latency following the addition of aldosterone to CCD from ADX rabbits, the transepithelial PD increased from near-zero control values to values in the −20 mV range after 1.5–2 h (351). Using a similar protocol for CCDs from rabbits with normal plasma aldosterone level, Schwartz and Burg (722) failed to observe any effect of aldosterone added in vitro on either transepithelial PD or sodium transport. This apparent discrepancy suggests that the latency before observing a stimulation of sodium transport by aldosterone depends on the aldosterone status of the animals from which CCD were obtained (ADX vs. normal rabbits) and/or on the basal transport capacity of the CCD before in vitro addition of hormone. Finally, Wingo et al. (886) reported that in vitro addition of aldosterone to CCD from ADX rabbits enhanced sodium reabsorption without altering the transepithelial PD (886), an observation hard to reconcile with the mechanism of sodium reabsorption usually admitted.

Only a few studies investigated the effect of aldosterone on the tubular transport of sodium by other parts of the collecting duct: aldosterone was ineffective in rabbit OMCD (350, 775), and it increased sodium reabsorption in rat IMCD (410). Thus the following discussion is restricted to the CCD.

Short-term effect of aldosterone was recently evaluated in an immortalized mouse collecting duct principal cell line (75). When grown at confluence on permeable filters, these mpkCCD_{c14} cells displayed the typical electrophysiological features of a tight epithelium with a high transepithelial electrical resistance (R_T), a negative transepithelial voltage, and a positive short-circuit current (I_{sc}). I_{sc} was fully inhibited by ENaC blockers such as amiloride and benzamylamiloride. Addition of aldosterone (10^{-7} M) increased I_{sc} and reduced R_T after a 30- to 60-min lag period, and the maximal effects on these two parameters were observed after 4–6 h. Increased I_{sc} was abolished by cycloheximide and actinomycin D. The threshold of aldosterone action was observed with 10^{-10} M aldosterone, and the effect of low concentrations of aldosterone (10^{-9} M) was not altered by RU486, a specific antagonist of GR. These last two findings strongly suggest that aldosterone is active through occupancy of MRs, the presence of which was verified in these cells by RT-PCR and binding experiments.

3. Effects on potassium transport

Addison's disease is often associated with hyperkalemia, whereas patients with hyperaldosteronism tend to display a negative potassium balance. However, whether the kaliuretic effect of aldosterone is a direct action of the hormone on tubular potassium handling or whether it is secondary to other changes is still a subject of debate. Indeed, early evaluations of the effect of mineralocorticoid administration on urinary potassium excretion led to controversial results (656). The issue to be discussed here focuses on whether the kaliuretic action of aldosterone is entirely secondary to its antinatriuretic action or whether regulation of sodium and potassium transport may be uncoupled. Here again, results are different for the short-term and the long-term effects of aldosterone.

Long-term treatment with mineralocorticoid increases potassium secretion in isolated CCDs (609, 773). In rabbits fed diets with various cation contents, not only the rate of sodium reabsorption but also that of potassium secretion measured in vitro in isolated CCDs were correlated with the in vivo aldosterone plasma levels (722). Stokes (773) carefully examined, by means of in vitro microperfusion, the relationship between sodium reabsorption and potassium secretion in CCDs from normal and DOCA-treated rabbits (773); the rate of sodium reabsorption varied in a wide range, and a linear relationship was observed between these rates and those of potassium secretion in the same tubules. The sodium over potassium flux ratio was 1.34, which is close to the 1.5 stoichiometry of Na^+-K^+-ATPase. These data suggest that long-term mineralocorticoid treatment alters sodium and potassium transport in parallel, probably through a common mechanism.

Conversely, dissociation between sodium and potassium transport was reported in some circumstances in response to short-term aldosterone treatment. For instance, despite consistent effects on sodium reabsorption, aldosterone increased potassium transport in some (309, 394) but not all studies (89, 246, 656). In addition, actinomycin D reduced aldosterone-induced changes in potassium transport in one (395) but not all studies (504, 884). In fact, several factors (including dietary potassium intake, glomerular filtration rate, acid/base status, trans-

epithelial PD, or the rate of sodium reabsorption in the distal nephron) may influence the overall potassium excretion and thereby modulate the action of aldosterone on the collecting duct. For example, stimulation of potassium excretion by aldosterone is better evidenced in potassium-depleted animals than in normal ones (290, 878), and adequate sodium supply to the distal nephron is required for observing mineralocorticoid-induced kaliuresis (399, 632).

In summary, the long-term effects of aldosterone on sodium and potassium transport in the collecting duct are tightly coupled, whereas in the short term, stimulation of potassium secretion by aldosterone may be counterbalanced by other phenomena and therefore may be dissociated from antinatriuresis.

4. Induction and activation of Na^+-K^+-ATPase

In the mammalian kidney, aldosterone induces three successive responses: an early response (0–2 h) that includes a lag period and the early onset of stimulation of sodium reabsorption, a late response (2–24 h) during which stimulation of sodium transport fully develops, and a delayed response (>2 days) that includes morphological remodeling of collecting duct. The two first responses are mainly observed either after a single administration of aldosterone to aldosterone-depleted animals or after in vitro addition of the hormone to aldosterone-deprived tissue preparations. The delayed response is observed following repeated injections of aldosterone to adrenal-intact animals. The following discussion is mainly focused on the early and late responses, and reference to long-term changes will be noted only when they highlight the short-term mechanisms.

As previously mentioned, Edelman et al. (233) have demonstrated in the early 1960s that the stimulation of sodium transport observed in response to aldosterone requires RNA and protein synthesis. Since then, most efforts focused on the characterization of the proteins specifically induced by aldosterone (aldosterone-induced proteins, AIP) and involved in sodium transport, using a candidate approach. The first candidates were Na^+-K^+-ATPase and the apical sodium channel ENaC, the two main effectors of sodium reabsorption, and the mitochondrial enzymes involved in the synthesis of ATP, the fuel required by the pump. Only the first two are discussed here, and the reader is referred to reviews for metabolic aspects (540, 678).

Adrenalectomy reduced Na^+-K^+-ATPase activity by >70% in rat, rabbit, and mouse CCD (222, 246, 247); this effect occurred after a 1- to 1.5-day lag period during which a latent pool of Na^+-K^+-ATPase was activated (50, 98), and reached its maximum after 4–5 days (222). A single injection of aldosterone (2–10 μg/kg body wt) to ADX rats and rabbits restored within 3 h Na^+-K^+-ATPase activity back to its level in CCD of adrenal-intact animals (246, 247). This effect was abolished by the MR antagonist spironolactone (247, 657). The time courses of aldosterone effect (single administration to ADX rats) on urinary sodium excretion and on Na^+-K^+-ATPase activity in CCD were similar (247). In adrenal-intact animals, mineralocorticoids also stimulated Na^+-K^+-ATPase activity in the collecting duct, but the response was delayed (onset and the maximal effects are observed within 2 days and 1 wk, respectively) (245), as was the effect on sodium transport. In fact, the latency before detecting aldosterone-induced stimulation of Na^+-K^+-ATPase is directly related to the initial ATPase activity (the lower the initial activity, the shorter the latency period) (375), suggesting that additional parameters modulate aldosterone action on Na^+-K^+-ATPase. 3,3′,5-Triiodothyronine (T_3) might be one of these parameters since in vitro incubation of CCD from normal rats with aldosterone alone failed to stimulate Na^+-K^+-ATPase activity whereas incubation with a combination of aldosterone and T_3 stimulated it within 3 h (49). This effect of T_3 is specific for mammals, since in amphibian epithelia, T_3 antagonizes the effect of aldosterone (329).

The question has long been debated whether aldosterone activates preexisting Na^+-K^+-ATPase units or whether it induces de novo synthesis of Na^+-K^+-ATPase. The following findings support that Na^+-K^+-ATPase is induced by aldosterone: *1*) aldosterone increased both the activity and the number of Na^+-K^+-ATPase units in CCD (49, 249), *2*) aldosterone-induced stimulation of Na^+-K^+-ATPase activity in CCD was blocked by actinomycin D and cycloheximide (49), *3*) adrenalectomy reduced and aldosterone restored the amount of mRNAs encoding for the α_1-subunit (but not the β_1-subunit) of Na^+-K^+-ATPase in CCD (255, 260, 818), and *4*) the 5′-flanking region of Na^+-K^+-ATPase α_1-subunit gene contains GREs (909). However, this does not mean that the early increase in activity and number of Na^+-K^+-ATPase units corresponds to membrane expression of de novo synthesized units. Indeed, in vivo administration of aldosterone to ADX rats restored a control level of α_1-subunit mRNAs after 6 h (818), whereas Na^+-K^+-ATPase activity was already restored after 2–3 h (246). Thus aldosterone rapidly triggers the transcription of Na^+-K^+-ATPase α_1-subunit (early induced mRNA), but the early stimulation in the enzyme activity is likely accounted for by the activation of preexisting pump units through induction of an unknown regulatory protein. Because the latent pool of Na^+-K^+-ATPase present in CCD from normal rats disappears after adrenalectomy (50), its activation cannot account for the early stimulation. On the other hand, the early increase in sodium affinity of Na^+-K^+-ATPase in response to aldosterone in A6 cells (78) may participate in the early stimulation but remains to be confirmed in mammalian principal cells. Interestingly, aldosterone was

shown to increase calcineurin activity within 30 min in rat CCD (822). Because inhibition of calcineurin by either cyclosporine or FK506 inhibited Na^+-K^+-ATPase activity in the rat CCD (486, 823), the early stimulation of calcineurin activity might be involved in the early stimulation of Na^+-K^+-ATPase by aldosterone. It should be noted, however, that conversely to early activation of Na^+-K^+-ATPase which was blocked by actinomycin D and cycloheximide (49), aldosterone-induced early stimulation of calcineurin was not blocked by actinomycin D (822); rather, it seems to be triggered by the heat shock protein released by MRs in response to aldosterone binding (822).

The question of whether Na^+-K^+-ATPase is an early aldosterone-induced protein has received further elements of response from studies in amphibian cell cultures. In A6 cells, aldosterone increased very early the transcription rate of the Na^+-K^+-ATPase α- and β-subunit mRNAs: stimulation occurred as early as 15–30 min after addition of aldosterone (842), and within 6 h, the amount of the mRNAs of α_1- and the β_1-subunits increased four- and twofold, respectively (843). Although aldosterone induced rapidly the transcription of Na^+-K^+-ATPase genes, the expression of the newly synthesized pump units in the basolateral membrane was only observed after 6–18 h of latency (329, 436, 487). This delayed membrane expression of the pump is accounted for by the time required for accumulation of mRNAs, translation, and assembly of the α- and β-subunits, and insertion into the membrane (252, 679). That this process takes longer in amphibian than in mammalian cells may be related to the difference in metabolic rates between poikilotherms and homeotherms.

Another long-debated question is whether induction of Na^+-K^+-ATPase is a primary effect of aldosterone or whether it is secondary to increased apical sodium entry. In addition to its actions on the recruitment of an endogenous latent pool of Na^+-K^+-ATPase (which corresponds to a nongenomic increase in pump density) and on the activation of already active pumps (by a substrate effect), increased sodium entry was thought to induce the genomic expression of Na^+-K^+-ATPase through increasing intracellular sodium concentration. In the collecting duct, this hypothesis was based on the fact that in vivo treatment of ADX rabbits with the ENaC blocker amiloride before aldosterone injection abolished Na^+-K^+-ATPase stimulation (633). However, when reevaluated in vitro, it was shown that at a concentration sufficient to block totally and specifically sodium channels (622), amiloride did not alter the induction of Na^+-K^+-ATPase by aldosterone in the CCD (49). This demonstrated that early Na^+-K^+-ATPase stimulation was independent of an increment of intracellular concentration of sodium brought about by increased apical sodium conductance. In fact, the inhibitory effect of amiloride observed in vivo might be due to inhibition of the basolateral Na^+/H^+ exchanger (NHE-1) rather than inhibition of the sodium channels. Indeed, Oberleithner et al. (601) reported that aldosterone increased intracellular pH through activation of the Na^+/H^+ exchanger in frog distal nephron cells (601). Furthermore, specific inhibition of the Na^+/H^+ exchanger by EIPA abolishes the in vitro induction of Na^+-K^+-ATPase in the rat collecting duct, and the action of EIPA was antagonized by alkalinizing the cells by incubation at higher pH (47).

In summary, although transcription of Na^+-K^+-ATPase α- and β-subunit mRNAs is triggered very early in response to aldosterone, the early increase in its activity that parallels the stimulation of sodium transport is likely accounted for by activation of preexisting units (Fig. 20). The mechanisms underlying this stimulation are not known yet, but they are not related to changes occurring at the apical cell border. Finally, the time course of the early stimulation of Na^+-K^+-ATPase varies with the initial status of the animals, in particular with their basal aldosterone and T_3 status, but other factors are likely involved.

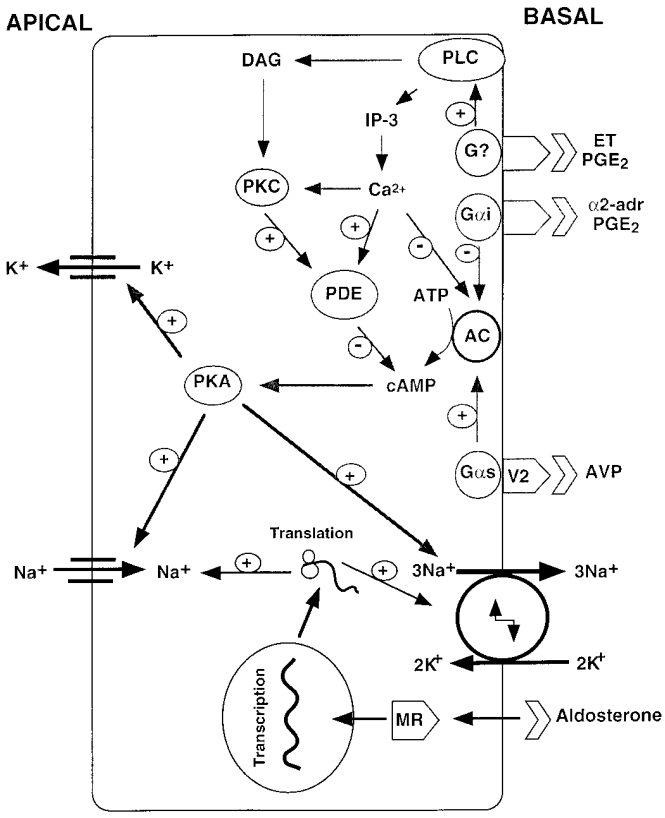

FIG. 20. Overview of the principal signaling pathways controlling the active sodium transport in principal cells of the collecting duct. Arrows indicate the direction of the signaling cascade and the resulting stimulatory (+) or inhibitory (−) effect on their targets. V_2, vasopressin V_2 receptor; MR, mineralocorticoid receptor.

5. *Induction and activation of epithelial sodium channels*

The early response of amphibian tight epithelia to aldosterone was mimicked by addition of the sodium ionophore amphotericin B at the apical pole of the cells (503). In addition, the effect of amphotericin B was not additive with that of aldosterone (189), suggesting that the early response involves an increased rate of apical sodium entry (which accounts for both increased sodium transport and decreased transepithelial resistance). Direct demonstration of aldosterone effect on apical sodium conductance in toad skin was first provided by Nagel and Crabbé (583), who showed that aldosterone increased threefold the sodium conductance of the apical membrane while sodium transport rate increased 2.7-fold. By electrophysiological analysis of the toad urinary bladder, Palmer et al. (623) reported that in vitro addition of aldosterone increased the density of active sodium channels without altering single-channel current, demonstrating the recruitment and/or the activation of preexisting but electrically silent sodium channels.

In the mammalian kidney, chronic changes in plasma aldosterone concentration also increased the apical amiloride-sensitive sodium conductance measured in vitro in rat and rabbit CCD (306, 694, 709). Cell-attached patch-clamp analysis of the apical membrane of CCD principal cells indicated that feeding rats with a low-sodium diet for 2 days markedly enhanced the density of active channels at the apical pole of principal cells (618). However, no data are available concerning the short-term effect (either in vivo or in vitro) of aldosterone on apical sodium conductance.

The mechanism underlying the increased number of active sodium channels (ENaC) at the apical membrane is not elucidated yet. It could be accounted for by either an increase in the number of channels in the membrane, as a result of de novo synthesis or of recruitment of an intracellular reservoir of channels, or by activation of silent channels already present in the membrane by aldosterone-induced regulatory factors.

Although corticosteroids stimulate the transcription of ENaC through transactivation of GREs in the 5′-flanking region of ENaC α-subunit gene (704), this transcriptional effect is likely not responsible for the early increase in channel density. Indeed, in A6 cells, the abundance of mRNAs for α-, β-, and γ-subunits of ENaC remained unchanged during the first 3 h of stimulation by aldosterone, and a marked increase was observed only after 24 h (546). In addition, the rate of synthesis of the α-subunit of ENaC increased significantly after 3 h of aldosterone treatment, whereas the amiloride-sensitive electrogenic sodium transport was stimulated about threefold after 1 h (546). Similarly, in rat collecting duct, adrenalectomy decreased the abundance of the mRNAs encoding the α-subunit (but not the β- and γ-subunits) of ENaC, but administration of aldosterone restored the expression of ENaC α-subunit mRNAs only after >2 days of treatment (30, 255). Within 2 days, aldosterone did not increase the immunodetected amount of all three subunits of ENaC (670), whereas after 10 days of dietary NaCl restriction or aldosterone administration, the abundance of ENaC α-subunit was markedly increased in rat CCD (542). These results demonstrate that aldosterone-induced early and late (up to 2 days) increases of apical sodium conductance in the mammalian CCD are not accounted for by de novo synthesis of ENaC, but rather by membrane insertion and/or activation of preexisting channels. Conversely to amphibian epithelia and rat CCD, in the mouse collecting duct principal cell line mpkCCD$_{c14}$, the time course of induction of ENaC synthesis is compatible with the early changes in I_{sc} and R_T. Indeed, the abundance of α-ENaC transcripts and the rate of synthesis of the cognate protein increased over twofold as early as 2 h after addition of aldosterone. The increased rate of synthesis of the ENaC α-subunit was blocked by actinomycin D (75). Here again, aldosterone effect was restricted to the α-subunit, since neither ENaC β- nor the γ-subunit transcripts were induced even after 24 h (75).

In fact, several results support that channels rapidly activated by aldosterone are already present in the apical membrane. For example, trypsinization of the apical sodium channels of toad bladders before aldosterone addition markedly reduced aldosterone effect on sodium transport (324), suggesting that the channels that were stimulated by aldosterone preexisted in the membrane. Also, Kleyman et al. (471) reported that aldosterone altered neither the binding of the ENaC blocker [^{3}H]benzamil (471) nor the rate of synthesis and expression of sodium channels at the apical cell surface in A6 cells.

What are the mechanisms responsible for the early activation of preexisting ENaC by aldosterone? Several factors have been proposed and investigated. Because the early response to aldosterone depends on mRNA and protein synthesis, a prerequisite is that such factors must be transcriptionally activated (Fig. 20). Several mechanisms, including changes in intracellular pH, methylation, and phosphorylation, have been postulated and are described below. These are neither exclusive nor exhaustive possibilities.

In rat CCD, intracellular alkalinization within physiological range markedly increases the open probability of apical sodium channels (622), in a manner that could account for early aldosterone effect. In addition, aldosterone was reported to rapidly (20 min) induce an intracellular alkalinization, sufficient to activate sodium channels, in both the amphibian early distal tubule (601) and the frog skin (369). This alkalinization was mediated through the activation of the Na^+/H^+ exchanger (369, 864). Whether this early stimulation of Na^+/H^+ exchange

by aldosterone is a genomic effect has not been established. However, it was mediated by MR occupancy since it was blocked by spironolactone (864). Activation of preexisting channels by cytosolic alkalinization would account for the fact that early activation of sodium channels by aldosterone was not retained in membrane vesicle preparation (29).

Incubation of apical membrane vesicles from A6 cells with the methyl donor *S*-adenosylmethionine increased the membrane methylation (both proteins and lipid) and doubled the rate of amiloride-sensitive sodium tranport. When A6 cells were pretreated with aldosterone, treatment of the vesicles with *S*-adenosylmethionine increased neither the membrane methylation nor sodium transport, which were already high, indicating that the effects of aldosterone and methylation were not additive (696). Thus aldosterone might stimulate a transmethylation reaction that participates to the activation of the apical sodium channel. The nature of the transmethylated proteins and/or lipids of importance for activation of the sodium channels is not known yet, although several proteins have been identified (reveiwed in Ref. 697). In contrast, the transmethylation pathway involved in aldosterone action has been identified in part. First, isoprenylcysteine-*O*-carboxyl methyltransferase (pcMTase) appears to be responsible for aldosterone-induced transmethylation (771). Indeed, in A6 cells, *1*) aldosterone increased pcMTase activity, without inducing its expression; *2*) inhibition of pcMTase reduced aldosterone-induced sodium transport; and *3*) overexpression of pcMTase potentiated the effect of aldosterone on sodium transport. Second, *S*-adenosyl-L-homocysteine hydrolase (SAHHase) regulates aldosterone-induced sodium transport (770). SAHHase is the only enzyme in vertebrates that is able to catabolize *S*-adenosyl-L-homocysteine, an end product and an inhibitor of transmethylation processes. In A6 cells, *1*) aldosterone stimulated SAHHase activity without inducing its expression, *2*) inhibition of SAHHase reduced sodium transport, and *3*) overexpression of SAHHase increased methylation processes and potentiated aldosterone effect on sodium transport. Thus, although the transmethylase pcMTase and the transmethylation regulator SAHHase are not induced by aldosterone, their activity is stimulated by aldosterone, and this stimulation is essential for membrane methylation and stimulation of sodium transport.

A serum- and glucocorticoid-regulated serine/threonine kinase (sgk) was identified by differential screens for glucocorticoid-inducible transcripts in a rat mammary tumor cell line (859, 860). sgk mRNAs are expressed in most rat tissues. However, in situ hybridization on kidney indicated that it was expressed at low level, and mainly in the glomeruli of ADX rats, whereas in ADX rats treated with aldosterone for 4 h, a strong expression was seen in the distal nephron (157). More recently, sgk mRNAs were shown to be induced as soon as 30 min after addition of aldosterone on rat collecting ducts, an effect which was mediated by mineralocorticoid receptors and did not require protein synthesis (586). Coexpression in *Xenopus* oocytes or rat sgk or of its amphibian ortholog with ENaC strongly stimulated ENaC-mediated sodium current (13, 157). This stimulation resulted from increased number of active ENaCs at the oocyte membrane with no change in single-channel properties (13). Although aldosterone increased the phosphorylation of the β- and γ-subunits of ENaC Madin-Darby canine kidney (MDCK) cells (733), the effect of sgk is not mediated through direct phosphorylation of ENaC subunits because deletion of the COOH termini of the three subunits did not prevent sgk action (13).

Analysis of cDNAs generated by differential display PCR from aldosterone-treated or untreated A6 cells allowed the characterization of several adrenal steroid upregulated RNAs (ASURs) (764). Among them was the *Xenopus* ortholog of the mammalian monomeric G protein K-Ras2. When coexpressed with ENaC in *Xenopus* oocytes, a constitutively active mutant of K-Ras2 was found to decrease, through activation of endocytosis, the number of ENaCs present at the membrane, but also to increase markedly the intrinsic activity of the remaining surface-expressed channels (543). The pathway underlying physiological activation of K-Ras during aldosterone stimulation is not yet known.

In summary, aldosterone-induced late increase in apical sodium conductance is likely mediated through membrane insertion of newly synthetized ENaCs, whereas the early stimulation is mediated by activation of preexisting channels. This early activation is controled by multiple factors, including intracellular pH, methylation, and phosphorylation cascades as well as small G proteins.

6. Regulation of potassium channels

Increased activity of apical ENaC and basolateral Na^+-K^+-ATPase is theoretically sufficient to account for both aldosterone-induced sodium reabsorption and potassium secretion. Indeed, stimulation of Na^+-K^+-ATPase increases intracellular potassium concentration and hyperpolarizes the basolateral membrane, and activation of ENaC depolarizes the apical membrane, thereby favoring apical potassium extrusion. Nonetheless, aldosterone may also control apical and basolateral potassium channels, which would permit some dissociation between the effects of aldosterone on sodium and on potassium transport.

Within 4 days, DOCA treatment doubled the apical potassium conductance in rabbit CCD (694). In ADX rabbits, this stimulatory effect of mineralocorticoids occurred as early as 3 h (693). Thus aldosterone would not only increase the driving force for potassium secretion

across the apical membrane, but also the conductance of this membrane. However, no stimulation of apical potassium conductance was found in CCDs from rats either chronically treated with DOCA treatment (707, 708) or fed a low-salt diet (304). Nonetheless, expression in the kidney cortex of mRNAs encoding the apical potassium channel ROMK was downregulated in ADX rats and upregulated in response to aldosterone (65, 845).

Because amiloride abolished the aldosterone-induced early increase in apical potassium conductance in rabbit CCD, it was proposed that this increase was secondary to increased sodium reabsorption (693). However, as already discussed for Na^+-K^+-ATPase, the inhibitory effect of amiloride is likely to be secondary to inhibition of the basolateral Na^+/H^+ exchanger. In MDCK cells, a dog kidney-derived cell line exhibiting some properties of collecting duct cells, aldosterone stimulated the Na^+/H^+ exchanger and alkalinized the cells, and thereby increased potassium secretion via the setting of preexisting potassium channels in the apical membrane (600). In the frog distal renal tubule, aldosterone-enhanced density of apical potassium channels was mimicked by increasing intracellular pH and was reduced by amiloride, consistent with the involvement of a stimulation of the Na^+/H^+ exchanger (850). Thus early regulation of apical potassium channels by aldosterone results from intracellular alkalinization-mediated recruitment of inactive channels, whereas late regulation might be accounted for by de novo synthesis of new channels.

Adrenalectomy decreased (708) and chronic DOCA treatment increased (695) the basolateral potassium conductance of rabbit CCD. However, during chronic DOCA treatment, the marked induction of Na^+-K^+-ATPase hyperpolarizes basolateral membrane so that the electrochemical gradient becomes favorable to potassium entry and not exit (774). Thus, coupled to the stimulation of Na^+-K^+-ATPase and the enhanced apical potassium conductance, changes at the basolateral border contribute to increase potassium secretion.

According to the molecular events underlying aldosterone action on potassium transport, it appears that part of the kaliuretic effect (that accounted for by increased driving force across the apical membrane) is tightly coupled to the antinatriuretic effect, whereas the part resulting from increased potassium conductance of the two cell membranes is independent from increased sodium transport.

C. Vasopressin and Activation of the cAMP/PKA Signaling Pathway

In collecting ducts principal cells, the cAMP/PKA signaling pathway is mainly stimulated by vasopressin (through its V_2 receptors coupled to adenylyl cyclase) and by glucagon. In collecting ducts, the main actions of vasopressin are to permeabilize the apical membrane to water through expression of apical AQP-2 and to increase urea permeability of IMCD. However, vasopressin also stimulates sodium reabsorption and potassium secretion along the CCD and OMCD. The following discussion focuses exclusively on the regulation of these two cation transports.

1. Effect on sodium transport

In rat and rabbit CCDs, in vitro microperfusion studies demonstrated that cell-permeant cAMP analogs stimulate sodium reabsorption (111, 371). In contrast, CCDs from rat and rabbits responded differently when triggered by vasopressin. In the in vitro microperfused rat CCD, vasopressin induced a sustained increase in the lumen-negative transepithelial voltage (588, 667), reflecting the stimulation of sodium reabsorption (668, 812). In contrast, in the rabbit in vitro microperfused CCD, vasopressin produced a transient increase in sodium reabsorption followed by a sustained inhibition (159, 302, 391). As discussed below, the inhibitory effect of vasopressin in the rabbit CCD most likely results from increased synthesis of prostaglandins E_2 (391). The lack of inhibitory effect of cAMP analogs in rabbit CCD suggests that the inhibitory effect of prostaglandins occurs through inhibition of cAMP production (see below). Finally, it should be mentioned that the stimulatory effect of vasopressin on sodium transport by the rat CCD and in A6 cells is potentiated by in vivo mineralocorticoid treatment (148, 372, 668, 839).

Vasopressin also increases renal potassium excretion (287, 762), secondarily to an increase in potassium secretion in late distal tubule (287) and CCD (706, 812) associated with an inhibition of potassium reabsorption by the OMCD (762). In the in vitro microperfused rat CCD, vasopressin induced a sustained stimulation of potassium secretion (812).

2. Stimulation of Na^+-K^+-ATPase

Although stimulation of Na^+-K^+-ATPase is a prerequisite for increasing sodium reabsorption in the CCD, initial studies by Satoh et al. (700) concluded to an inhibition of the maximal hydrolytic activity of Na^+-K^+-ATPase in response to forskolin and to cell-permeant cAMP analogs in isolated rat CCD. This effect of cAMP was indirect and relied on the PLA_2/arachidonate/cytochrome *P*-450-monooxygenase pathway (700, 701). However, recent unpublished results from our laboratories indicate that in CCD as in MTAL (see above), this inhibitory pathway is promoted by artefactual metabolic stress. Indeed, in well-oxygenated isolated rat CCDs, cell-permeant analog of cAMP induced a twofold stimulation of both the transport and the hydrolytic activity of Na^+-K^+-ATPase.

This effect was associated with a twofold increase in the cell surface expression of Na^+-K^+-ATPase, suggesting that cAMP induces the recruitment of active Na^+-K^+-ATPase units from intracellular stores to the plasma membrane, a mechanism previously evidenced in the rat proximal tubule (136). These findings indicate that increased Na^+-K^+-ATPase activity in response to the activation of PKA participates in the stimulation of sodium reabsorption (Fig. 20).

The stimulatory effect of the cAMP/PKA pathway on Na^+-K^+-ATPase is also promoted by vasopressin. In vivo infusion and in vitro addition of vasopressin increased the V_{max} of Na^+-K^+-ATPase in rat (812) and in mouse (96, 187) CCD, respectively. In mouse CCDs, increased V_{max} was paralleled by increased number of cell surface active Na^+-K^+-ATPase units, as measured by [^{3}H]ouabain binding (187). This stimulatory effect of vasopressin is mediated through V_2 receptor (187) and requires protein phosphatase activity (96). In addition, as previously shown for the stimulation of sodium reabsorption, the effects of vasopressin and aldosterone on Na^+-K^+-ATPase were synergistic (187). This observation may indicate that aldosterone sensitizes the principal cells of the collecting duct to the action of vasopressin.

In addition to its short-term effect on Na^+-K^+-ATPase activity, vasopressin also increases the translational rate of the α-subunit in cultured cells derived from rat CCD (215). Altogether, these observations suggest that vasopressin controls both Na^+-K^+-ATPase activity and expression level in principal cells of the mammalian CCD.

3. ENaC

Vasopressin and/or cAMP stimulation of sodium transport in the CCD is associated with a depolarization of the apical membrane (588, 709) and an increase in apical membrane sodium conductance (708, 716). The stimulatory effect of cAMP on ENaC (Fig. 20) was confirmed by patch-clamping the apical membrane of principal cells from isolated rat CCDs (305, 308); cAMP treatment before formation of the patch increases the density of active channels (308).

The mechanism of activation/recruitment of ENaC by the vasopressin/cAMP/PKA pathway was further studied in A6 cells. Vasopressin, forskolin, and cAMP analogs increased the density of active ENaCs in A6 cells (250, 472, 538). This increase in ENaC density was prevented by pretreatment with brefeldin A (472), a drug that disrupts the Golgi apparatus and prevents delivery of newly synthezised or recycling proteins from this intracellular compartment. Taken together with the stimulatory effect of vasotocin, i.e., the amphibian analog of vasopressin, on apical membrane exocytosis (840) and with the requirement of PIK activity (234), these results suggest that vasopressin induces a translocation of ENaC from an intracellular pool to the plasma membrane. This hypothesis is further supported by the inhibitory effect of microtubules and actin microfilaments disruption on the vasopressin-stimulated sodium transport and apical exocytosis (841). Indeed, in polarized renal epithelial cells, apical membrane trafficking events are facilitated by microtubules and actin microfilaments (115, 362).

In addition, cAMP may also activate preexisting sodium channels, since incubation of excised inside-out patches from A6 cells in the presence of ATP and the catalytic subunit of PKA increased ENaC activity (646). The activation of these preexisting channels by ATP and PKA required phosphorylation of actin filaments (647) and may also involve direct phosphorylation of the β- and γ-subunits of ENaC by PKA (733).

In addition to its short-term (minutes) effect on apical sodium conductance, long-term (hours) vasopressin treatment increased amiloride-sensitive sodium transport, cell surface expression of ENaC, and the translation rate of the β- and γ-subunits of ENaC in cultured cells derived from rat CCD (215).

These results suggest that vasopressin controls both the synthesis, the membrane recruitment, and the activity of ENaC in principal cells of the mammalian collecting duct.

4. Potassium transporters

Stimulation of apical sodium reabsorption by vasopressin (709) increases the driving force for potassium secretion through apical potassium channels (706). In addition, vasopressin and cAMP increased the density and/or activated apical low-conductance potassium channel (ROMK) in rat CCD (138, 364, 852) (Fig. 20). Whether vasopressin induces translocation of potassium channels from an intracellular pool or activates silent plasma membrane potassium channels remains to be determined.

D. Negative Modulation of Vasopressin Action

The stimulatory effects of vasopressin on the collecting duct are negatively modulated by several factors such as prostaglandins, adenosine, α_2-adrenergic agonists, endothelin, or bradykinin. Most of these mediators modulate the intracellular concentration of cAMP at the level of its production and/or degradation. A general feature of these secondary modulations is their cellular and species specificity as they often differ between rat and rabbit as well as between CCD and OMCD.

1. Prostanglandins

In rat CCD, PGE_2 did not alter either vasopressin-dependent cAMP content (146) or vasopressin-stimulated

sodium transport and transepithelial voltage (158). In contrast, PGE_2 reduced vasopressin-dependent cAMP content in rat OMCD (1). This resulted mainly from an increased rate of cAMP degradation by calcium-activated phosphodiesterases (146) mediated by a pertussis toxin-insensitive increase in intracellular calcium concentration (1). Curiously, this effect was blocked by phorbol esters (2). However, the functional effect of PGE_2 on cation transport and water permeability has not been evaluated in rat OMCD.

In rabbit CCD, PGE_2 inhibited vasopressin-stimulated synthesis of cAMP (349) and vasopressin-induced water permeability (378) through pertussis toxin-sensitive inhibition of adenylyl cyclase ($G\alpha_i$). PGE_2 also inhibited vasopressin-stimulated sodium reabsorption and transepithelial voltage in the rabbit CCD (158, 413, 776), but apparently through another mechanism. Indeed, inhibition of sodium reabsorption was mimicked by phorbol esters (18), in agreement with its mediation by pertussis toxin-insensitive increase in intracellular calcium and PKC (378). The pertussis toxin-sensitive and -insensitive signaling pathways triggered by PGE_2 in the rabbit CCD are activated through different receptors (Fig. 20) with distinct affinities for PGE_2 and its analog sulprostone. The role of PKC in the inhibitory effect of PGE_2 on sodium transport is further supported by the sustained stimulatory effect of vasopressin on sodium transport observed after downregulation of the novel PKC-ϵ by antisense oligonucleotides in primary cultures of rabbit CCD cells (197).

In agreement with the stimulatory effect of vasopressin on Na^+-K^+-ATPase activity, chronic inhibition of PGE_2 synthesis by indomethacin treatment increased Na^+-K^+-ATPase activity in rabbit CCD (185). An in vitro inhibitory effect of PGE_2 on Na^+-K^+-ATPase was also reported in rabbit IMCD (427); however, the very high doses of PGE_2 used (micromolar range) preclude any physiological relevance of this effect.

Both rabbit and rat CCD (104, 419, 432, 468, 717) and interstitial cells (100, 485) synthesize PGE_2, and this process is stimulated by vasopressin (468, 717) likely through V_1 receptors (433). Thus vasopressin-induced synthesis of prostaglandins is part of a regulatory feedback mechanism that limits vasopressin action. This may be more important for limiting water transport rather than cation transport, as it may prevent excessive swelling and dilution of cell compartment. Nonetheless, we already mentioned that such feedback regulation also applies to sodium transport in the rabbit CCD. Indeed, inhibition of prostaglandin synthesis by meclofenamate suppressed the fall in sodium transport consecutive to its transient stimulation by vasopressin (391).

2. Bradykinin

Like prostaglandins, bradykinin had no effect alone but inhibited the vasopressin-induced water permeability in rabbit CCD (721). Bradykinin was also reported to inhibit sodium reabsorption in rat CCD by some (813) but not all authors (371). Curiously, in the study of Tomita et al. (813), bradykinin antagonized the vasopressin-induced sodium reabsorption but did not alter vasopressin-induced rise in transepithelial voltage and potassium secretion. In the rabbit CCD, the effects of bradykinin are likely mediated by a stimulation of prostaglandin synthesis by CCD and interstitial cells (934) and the subsequent inhibition of vasopressin response by PGE_2 (see above). The rat CCD principal cells, however, are insensitive to PGE_2, and therefore, another mechanism may account for the inhibitory effect of bradykinin. As mentioned before for PGE_2, bradykinin may also constitute the efferent limb of a negative-feedback mechanism, since vasopressin stimulates the renal kallikrein-kinin system (267).

3. α_2-Adrenergic agonists

The site of increased urinary sodium and water excretion observed in response to an α_2-adrenergic receptor agonist was localized to the collecting duct by micropuncture studies in normal rat (768). This result was confirmed by in vitro microperfusion studies performed in rat CCD, in which epinephrine and the α_2-agonist clonidine antagonized vasopressin-induced stimulation of sodium and water reabsorption (158, 371). Conversely, clonidine altered neither water permeability nor sodium reabsorption promoted by vasopressin in the rabbit CCD (158). This species difference is consistent with the pattern of inhibition of vasopressin-dependent cAMP accumulation in rat and rabbit CCD; clonidine inhibited cAMP production in rat but not in rabbit CCD (140).

Inhibition of cAMP production by α_2-adrenergic agonists in rat collecting duct largely results from $G\alpha_i$-mediated inhibition of adenylyl cyclase (1, 145) (Fig. 20). However, because epinephrine also antagonized the stimulatory effect of a cell-permeant cAMP analog on sodium transport (371), a post cAMP effect may also be involved in the functional inhibition.

4. Dopamine

Dopamine has no effect per se on sodium transport, but it inhibits vasopressin-induced sodium reabsorption in the in vitro microperfused rat CCD (781). This effect is mediated through D_4 receptors (D_2-like) and is likely secondary to a decrease in cAMP generation in response to vasopressin (497). It should be mentioned that in contrast to the D_1-like agonist fenoldopam (606), dopamine did not increase the intracellular cAMP content in the rat CCD (497). These observations can be accounted for by a

balanced activation of D_1-like and D_2-like receptors, which are positively and negatively coupled to adenylyl cyclase, respectively. An inhibition of Na^+-K^+-ATPase activity in response to dopamine and fenoldopam has been reported in isolated rat CCD (790).

5. Endothelin

The stimulatory effects of vasopressin on both water and cation reabsorption are also antagonized by endothelin (235, 481, 811) through its ET_B receptors (235). Endothelin reduces vasopressin-induced cAMP production (235, 810) through a calcium-dependent (481, 587) stimulation of PKC (810) (Fig. 20). This mechanism seems to be shared by rat (810, 811), rabbit (481), and mouse CCD (587).

In the rabbit CCD, the inhibitory effect of endothelin was accompanied by an inhibition of ENaC (481).

E. Insulin

In contrast to its inhibitory effect on potassium secretion and sodium reabsorption reported in the in vitro perfused rabbit CCD (316), insulin increases Na^+-K^+-ATPase-mediated cation transport in a time- and concentration-dependent manner in isolated rat CCDs (281). The reason for this discrepancy remains unexplained, since in every system studied so far, including the A6 cells (286), insulin stimulated Na^+-K^+-ATPase-coupled sodium transport (178, 196, 266, 355, 520, 671).

The mechanism of the stimulation of Na^+-K^+-ATPase transport activity by insulin has been studied in isolated rat CCD and OMCD (282). It is independent of apical sodium entry through amiloride-sensitive sodium channels. In contrast to PCT, the transport activity of Na^+-K^+-ATPase was stimulated both under rate-limiting and saturating sodium concentrations, revealing a V_{max} effect. Because insulin did not alter the number of active pump units, an increase in turnover of Na^+-K^+-ATPase activity has been proposed. In addition, the effect of insulin was abolished by permeabilization of cells, suggesting the requirement of soluble cofactors. These results point out the cellular specificity of the mechanisms of control of Na^+-K^+-ATPase activity by insulin. In A6 cells, insulin also stimulated the apical sodium entry through ENaC (251, 539).

VII. CONCLUSION AND PERSPECTIVES

Along this review, we summarized the current knowledge of the hormonal regulatory mechanisms that control Na^+-K^+-ATPase-dependent sodium transport along the kidney tubule. During the past decade, tremendous efforts were made to identify the molecular players that account for the transport properties of individual nephron segments. Currently, the major sodium transporters expressed along the kidney tubule have been cloned, their expression pattern along the kidney tubule has been established, and their major intrinsic properties regarding ion transport kinetics have been determined. In parallel, numerous hormone receptors expressed along the kidney tubule as well as many signaling intermediates have been identified at the molecular level. New approaches, including SAGE, DNA microarrays, and sequencing the genome of entire organisms, will undoubtedly lead to the identification of the remaining molecular players.

The identification of hormones involved in the control of active sodium reabsorption by the kidney tubule and the delineation of their sites of action lead to the discovery of a highly complex regulatory network. Among the factors increasing the complexity of this hormonal control, one can underline the following: *1*) a given hormone can bind to distinct molecular subtypes of receptors that couple to different signaling pathways with different effects on target transporters (e.g., vasopressin V_{1a} and V_2 receptor coupled to PKA and PKC, respectively); *2*) a molecular receptor species generally couples to more than one signaling pathway (e.g., activation of PKA and PKCs by PTH-PTHrP receptor); *3*) activation of basolateral and apical receptors can generate different effects (e.g., ANG II); *4*) the final effect of a hormone on active sodium transport is dependent on the concentration of the hormone (e.g., ANG II), the cellular context (e.g., PTH in proximal tubule and TAL), and external factors (e.g., oxygen supply, metabolic substrate availability); and *5*) the effect of a hormone can be modulated by the presence of other hormones or local factors (e.g., interactions between vasopressin and prostaglandins). Thus a "physiological effect" can be viewed as the result of a transient imbalance between numerous positive and negative influences exerted from the cell surface and from the interior of the cell. However, some general conclusions can be drawn. Several hormones (e.g., aldosterone) can exert long-term effects (within hours or days) that generally alter the expression level of sodium transporters and thereby reset the reabsorption capacity of the target nephron segment. Many hormones can also acutely (within seconds or minutes) modulate the activity of presynthesized sodium transporters to ensure homeostatic adjustments. This short-term control can be achieved either through an alteration in kinetics of transporters and/or changes in their cell surface expression. Another emerging picture illustrated in this review is the coordinated control of apical and basolateral sodium transport systems. This coordination (cross-talk) minimizes the changes in intracellular ion concentrations, intracellular pH, and cell volume that would otherwise alter many cellular functions.

Although the major hormonal and paracrine/auto-

crine factors influencing tubular sodium transport and the initial steps of intracellular signaling initiated by the activation of their cognate receptors are currently identified, many intermediate signaling molecules remain to be found. In addition, the identification of regulatory sites (e.g., phosphorylation sites, protein-protein interaction sites) in the currently cloned sodium transporters is an area of intensive research that will shed some light on the molecular mechanisms that modulate the function and govern the subcellular distribution of these transporters. The study of the interactions between ion transporters, signaling molecules, cytoskeleton, and vesicular transport machinery is another exciting area for future research in cell physiology. Finally, the generation of transgenic animals will allow the in vivo validation of the in vitro models. However, the most important challenge will be to put molecules together to get a coherent dynamic picture of the regulation of the system at the cellular, the organ, and the whole organism levels so as to understand how the dysfunction of one player may interfere with the function of the whole machinery.

We are grateful to D. Chabardès, M. Imbert-Teboul, J. Caverzasio, S. Gonin, and F. Roger for critical advice and careful review of this manuscript.

Works from our laboratories were supported by grants from the Centre National de la Recherche Scientifique and the Commissariat à l'Energie Atomique (to A. Doucet), from the Swiss National Science Foundation (31–50643.97 and 31–56830.99; to E. Féraille), and from the Société Académique de Genève (to E. Féraille).

Address for reprint requests and other correspondence: E. Féraille, Div. of Nephrology, Geneva University Hospital, CH-1211 Geneva 14, Switzerland (E-mail: feraille@cmu.unige.ch).

REFERENCES

1. Aarab L, Montégut M, Siaume-Perez S, Imbert-Teboul M, and Chabardès D. PGE_2-induced inhibition of AVP-dependent cAMP accumulation in the OMCD of the rat kidney is cumulative with respect to the effects of α_2-adrenergic and A_1-adenosine agonists, insensitive to pertussis toxin and dependent on extracellular calcium. *Pflügers Arch* 423: 397–405, 1993.
2. Aarab L, Siaume-Perez S, and Chabardès D. The activation of protein kinase C prevents PGE_2-induced inhibition of AVP-dependent cAMP accumulation in the rat outer medullary collecting tubule. *Pflügers Arch* 425: 417–425, 1993.
3. Abdulnour-Nakhoul S, Khuri RN, and Nakhoul N. Effect of norepinephrine on cellular sodium transport in *Ambystoma* kidney proximal tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F725–F736, 1994.
4. Adachi S, Uchida S, Ito H, Hata M, Hiroe M, Marumo F, and Sasaki S. Two isoforms of a chloride channel predominantly expressed in thick ascending limb of Henle's loop and collecting ducts of rat kidney. *J Biol Chem* 269: 17677–17683, 1994.
5. Aharonovitz O and Granot Y. Stimulation of mitogen-activated protein kinase and Na^+/H^+ exchanger in human platelets. Differential effect of phorbol ester and vasopressin. *J Biol Chem* 271: 16494–16499, 1996.
6. Ahn KY, Madsen KM, Tisher CC, and Kone BC. Differential expression and cellular distribution of mRNAs encoding α- and β-isoforms of Na^+-K^+-ATPase in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F792–F801, 1993.
7. Ahn S, Maudsley S, Luttrell LM, Lefkowitz RJ, and Daaka Y. Src-mediated tyrosine phosphorylation of dynamin is required for β_2-adrenergic receptor internalization and mitogen-activated protein kinase signaling. *J Biol Chem* 274: 1185–1188, 1999.
8. Al-Awqati Q, Vijayakumar S, Hikita C, Chen J, and Takito J. Phenotypic plasticity in the intercalated cell: the hensin pathway. *Am J Physiol Renal Physiol* 275: F183–F190, 1998.
9. Albiston AL, Obeyesekere VR, Smith RE, and Krozowski ZS. Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* 105: R11–R17, 1994.
10. Alblas J, Van Corven EJ, Hordijk PL, Milligan G, and Moolenaar WH. G_i-mediated activation of the $p21^{ras}$-mitogen-activated protein kinase pathway by α_2-adrenergic receptors. *J Biol Chem* 268: 22235–22238, 1993.
11. Alkhunaizi AM, Yaqoob MM, Edelstein CL, Gengaro PE, Burke TJ, Nemenoff RA, and Schrier RW. Arachidonic acid protects against hypoxic injury in rat proximal tubules. *Kidney Int* 49: 620–625, 1996.
12. Allera A and Wildt L. Glucocorticoid-recognizing and -effector sites in rat liver plasma membrane. Kinetics of corticosterone uptake by isolated vesicles. I. Binding and transport. *J Steroid Biochem Mol Biol* 42: 737–756, 1992.
13. Alvarez De La Rosa D, Zhang P, Naray-Fejes-Toth A, Fejes-Toth G, and Canessa C. The serum and glucocorticoid kinase sgk increases the abundance of epithelial sodium channels in the plasma membrane of *Xenopus* oocytes. *J Biol Chem.* In press.
14. Amlal H, Legoff C, Vernimmen C, Paillard M, and Bichara M. Na^+-K^+-(NH_4^+)-$2Cl^-$ cotransport in medullary thick ascending limb: control by PKA, PKC, and 20-HETE. *Am J Physiol Cell Physiol* 271: C455–C463, 1996.
15. Amlal H, Legoff C, Vernimmen C, Soleimani M, Paillard M, and Bichara M. Angiotensin II controls Na^+-K^+-(NH_4^+)-$2Cl^-$ cotransport via 20-HETE and PKC in medullary thick ascending limb. *Am J Physiol Cell Physiol* 274: C1047–C1056, 1998.
16. Ammar A, Schmidt A, Semmekrot B, Roseau S, and Butlen D. Receptors for neurohypophyseal hormones along the rat nephron: ^{125}I-labelled $d(CH_2)5Tyr(Me)_2$, Thr4, Orn8, Tyr-NH_2 vasotocin binding in microdissected tubules. *Pflügers Arch* 418: 220–227, 1991.
17. Ando Y and Asano Y. Functional evidence for an apical V_1 receptor in rabbit cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F467–F471, 1993.
18. Ando Y, Jacobson HR, and Breyer MD. Phorbol myristate acetate, dioctanoylglycerol, and phosphatidic acid inhibit the hydroosmotic effect of vasopressin on rabbit cortical collecting tubule. *J Clin Invest* 80: 590–593, 1987.
19. Aoki Y, Albrecht FE, Bergman KR, and Jose PA. Stimulation of Na^+-K^+-$2Cl^-$ cotransport in rat medullary thick ascending limb by dopamine. *Am J Physiol Regulatory Integrative Comp Physiol* 271: R1561–R1567, 1996.
20. Aperia A, Bertorello A, and Seri I. Dopamine causes inhibition of Na^+-K^+-ATPase activity in rat proximal convoluted tubule segments. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F39–F45, 1987.
21. Aperia A, Fryckstedt J, Svensson L, Hemmings HC Jr, Nairn AC, and Greengard P. Phosphorylated M_r 32,000 dopamine- and cAMP-regulated phosphoprotein inhibits Na^+,K^+-ATPase activity in renal tubule cells. *Proc Natl Acad Sci USA* 88: 2798–2801, 1991.
22. Aperia A, Holtbäck U, Syrén ML, Svenson LB, Fryckstedt J, and Greengard P. Activation/deactivation of renal Na^+,K^+-ATPase: a final common pathway for regulation of natriuresis. *FASEB J* 8: 336–439, 1994.
23. Aperia A, Ibarra B, Svensson F, Klee C, and Greengard P. Calcineurin mediates α-adrenergic stimulation of Na^+-K^+-ATPase activity in renal tubule cells. *Proc Natl Acad Sci USA* 89: 7394–7397, 1992.
24. Aristimudo PC and Good DW. PKC isoforms in rat medullary thick ascending limb: selective activation of the δ-isoform by PGE_2. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F624–F631, 1997.
25. Aronson PS. Role of ion exchangers in mediating NaCl transport in the proximal tubule. *Kidney Int* 49: 1665–1670, 1996.
26. Aronson PS and Giebisch G. Mechanisms of chloride transport in proximal tubule. *Am J Physiol Renal Physiol* 273: F179–F192, 1997.

27. ARRIZA JL, WEINBERGER C, CERELLI G, GLASER TM, HANDELIN BL, HOUSMAN DE, AND EVANS RE. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* 237: 268–275, 1987.
28. ARYSTARKHOVA E, WETZEL RK, ASINOVSKI NK, AND SWEADNER KJ. The γ subunit modulates Na^+ and K^+ affinity of the renal Na,K-ATPase. *J Biol Chem* 274: 33183–33185, 1999.
29. ASHER C AND GARTY H. Aldosterone increases the apical permeability of toad bladder by two different mechanisms. *Proc Natl Acad Sci USA* 85: 7413–7417, 1988.
30. ASHER C, WALD H, ROSSIER BC, AND GARTY H. Aldosterone-induced increase in the abundance of Na^+ channel subunits. *Am J Physiol Cell Physiol* 271: C605–C611, 1996.
31. AUGUST C, NELSON D, AND THORN GW. Response of normal subjects to large amounts of aldosterone. *J Clin Invest* 37: 1549–1555, 1958.
32. AUKLAND K AND KROG J. Renal oxygen tension. *Nature* 188: 671, 1960.
33. AZARANI A, GOLTZMAN D, AND ORLOWSKI J. PTH and PTH-related peptide inhibit the apical Na^+/H^+ exchanger NHE3 isoform in renal cells (OK) via dual signaling cascade involving protein kinase A and C. *J Biol Chem* 270: 20004–20010, 1995.
34. AZARANI A, GOLTZMAN D, AND ORLOWSKI J. Structurally diverse N-terminal peptides of PTH (PTH) and PTH-related peptide (PTHRP) inhibit the Na^+/H^+ exchanger NHE3 isoform by binding to the PTH/PTHRP receptor type I and activating distinct signaling pathways. *J Biol Chem* 271: 14931–14936, 1996.
35. BACKER JM, MYERS MG, SUN XJ, CHIN DJ, SHOELSON SE, MIRALPEIX M, AND WHITE MF. Association of IRS1 with the insulin receptor and the phosphatidylinositol 3′-kinase. Formation of binary and ternary signalling complexes in intact cells. *J Biol Chem* 268: 8204–8212, 1993.
36. BAILLY C, IMBERT-TEBOUL M, ROINEL N, AND AMIEL C. Isoproterenol increases Ca, Mg, and NaCl reabsorption in mouse thick ascending limb. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F1224–F1231, 1990.
37. BAILLY C, ROINEL N, AND AMIEL C. Pth-like glucagon stimulation of Ca and Mg reabsorption in Henle's loop of the rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 246: F205–F212, 1984.
38. BAINES AD AND CHAN W. Production of urine free dopamine from DOPA: a micropuncture study. *Life Sci* 26: 253–259, 1980.
39. BAINES AD, DRANGOVA R, AND HO P. Role of diacylglycerol in adrenergic-stimulated ^{86}Rb uptake by proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F1133–F1138, 1990.
40. BAINES AD AND HO P. Specific α_1-, α_2-, and β-responses to norepinephrine in pyruvate-perfused rat kidneys. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F170–F176, 1987.
41. BAINES AD, HO P, AND DRANGOVA R. Proximal tubular dopamine production regulates basolateral Na-K-ATPase. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F566–F571, 1992.
42. BALBOA MA AND INSEL PA. Stimulation of phospholipase D via α_1-adrenergic receptors in Madin-Darby canine kidney cells is independent of PKC-α and -ϵ activation. *Mol Pharmacol* 53: 221–227, 1998.
43. BANK N AND AYNEDJIAN HS. A micropuncture study of the effect of parathyroid hormone on renal bicarbonate reabsorption. *J Clin Invest* 58: 336–344, 1976.
44. BARBRY P AND HOFMAN P. Molecular biology of Na^+ absorption. *Am J Physiol Gastrointest Liver Physiol* 273: G571–G585, 1997.
45. BARGER AC, BERLIN RD, AND TULENKO JF. Infusion of aldosterone 9-α-fluoro-hydrocortisone and antidiuretic hormone into the renal artery of normal and adrenalectomized, unanesthetized dogs: effect on electrolyte and water excretion. *Endocrinology* 62: 804–814, 1958.
46. BARLET-BAS C, ARYSTARKHOVA E, CHEVAL L, MARSY S, SWEADNER K, MODYA-NOV N, AND DOUCET A. Are there several isoforms of Na,K-ATPase α subunit in the rabbit nephron? *J Biol Chem* 268: 11512–11515, 1993.
47. BARLET-BAS C, CHEVAL L, FÉRAILLE E, MARSY S, AND DOUCET A. Regulation of tubular Na-K-ATPase. In: *Nephrology*, edited by Hatano M. Tokyo: Springer-Verlag, 1991, p. 419–434.
48. BARLET-BAS C, CHEVAL L, KHADOURI C, MARSY S, AND DOUCET A. Difference in the Na^+ affinity of Na^+-K^+-ATPase along the rabbit nephron: modulation by K. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F246–F250, 1990.
49. BARLET-BAS C, KHADOURI C, MARSY S, AND DOUCET A. Sodium-independent in vitro induction of Na^+,K^+-ATPase by aldosterone in renal target cells: permissive effect of triiodothyronine. *Proc Natl Acad Sci USA* 85: 1707–1711, 1988.
50. BARLET-BAS C, KHADOURI C, MARSY S, AND DOUCET A. Enhanced intracellular sodium concentration in kidney cells recruits a latent pool of Na-K-ATPase whose size is modulated by corticosteroids. *J Biol Chem* 265: 7799–7803, 1990.
51. BAUM M. Insulin stimulates volume absorption in the rabbit proximal convoluted tubule. *J Clin Invest* 79: 1104–1109, 1987.
52. BAUM M, AMEMIYA M, DWARAKANATH V, ALPERN RJ, AND MOE OW. Glucocorticoids regulate NHE-3 transcription in OKP cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F164–F169, 1996.
53. BAUM M, CANO A, AND ALPERN RJ. Glucocorticoids stimulate Na^+/H^+ antiporter in OKP cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F1027–F1031, 1993.
54. BAUM M AND HAYS SR. Phorbol myristate acetate and dioctanoylglycerol inhibit transport in rabbit proximal convoluted tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 254: F9–F14, 1988.
55. BAUM M, MOE OW, GENTRY DL, AND ALPERN RJ. Effect of glucocorticoids on renal NHE-3 and NHE-1 mRNA. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F437–F432, 1994.
56. BAUM M AND QUIGLEY R. Glucocorticoids stimulate rabbit proximal convoluted tubule acidification. *J Clin Invest* 91: 110–114, 1993.
57. BAUM M AND QUIGLEY R. Inhibition of proximal convoluted tubule transport by dopamine. *Kidney Int* 54: 1593–1600, 1998.
58. BAUM M, QUIGLEY R, AND QUAN A. Effect of luminal angiotensin II on rabbit proximal convoluted tubule bicarbonate absorption. *Am J Physiol Renal Physiol* 273: F595–F600, 1997.
59. BAUMANN K, CHAN YL, BODE MF, AND PAPAVASSILIOU F. Effect of parathyroid hormone and cyclic adenosine 3′,5′-monophosphate on isotonic fluid reabsorption: polarity of proximal tubular cells. *Kidney Int* 11: 77–85, 1977.
60. BEACH RE, SCHWAB SJ, BRAZY PC, AND DENNIS VW. Norepinephrine increases Na-K-ATPase and solute transport in rabbit proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F215–F220, 1987.
61. BEATO M. Gene regulation by steroid hormones. *Cell* 89: 335–344, 1989.
62. BECK JS, BURGESS WJ, AND BALMENT RJ. Interaction between adrenergic agonists and forskolin on adenylate cyclase activity in the rabbit proximal tubule. *Renal Physiol Biochem* 18: 231–236, 1995.
63. BECK JS, MARSOLAIS M, NOËL J, BRETON S, AND LAPRADE R. Dibutyryl cyclic adenosine monophosphate stimulates the sodium pump in rabbit renal cortical tubules. *Renal Physiol Biochem* 18: 21–26, 1995.
64. BECKER BN, CHENG HF, AND HARRIS RC. Apical ANG II-stimulated PLA_2 activity and Na^+ flux: a potential role for Ca^{2+}-independent PLA_2. *Am J Physiol Renal Physiol* 273: F554–F562, 1997.
65. BEESLEY AH, HORNBY D, AND WHITE SJ. Regulation of distal nephron K^+ channels (ROMK) mRNA expression by aldosterone in rat kidney. *J Physiol (Lond)* 509: 629–634, 1998.
66. BÉGUIN P, BEGGAH AT, CHIBALIN AV, BURGENER-KAIRUZ P, JAISSER F, MATHEWS PM, ROSSIER BC, COTECCHIA S, AND GEERING K. Phosphorylation of the Na,K-ATPase α-subunit by protein kinase A and C in vitro and in intact cells. Identification of a novel motif for PKC-mediated phosphorylation. *J Biol Chem* 269: 24437–24445, 1994.
67. BÉGUIN P, BEGGAH AT, COTECCHIA S, AND GEERING K. Adrenergic, dopaminergic, and muscarinic receptor stimulation leads to PKA phosphorylation of Na-K-ATPase. *Am J Physiol Cell Physiol* 270: C131–C137, 1996.
68. BÉGUIN P, PEITSCH MC, AND GEERING K. α_1 but not α_2 or α_3 isoforms of Na,K-ATPase are efficiently phosphorylated in a novel protein kinase C motif. *Biochemistry* 35: 14098–14108, 1996.
69. BÉGUIN P, WANG X, FIRSOV D, PUOTI A, CLAEYS D, HORISBERGER JD, AND GEERING K. The γ subunit is a specific component of the Na,K-ATPase and modulates its transport function. *EMBO J* 16: 4250–4260, 1997.
70. BEHAR V, PINES M, NAKAMOTO C, GREENBERG Z, BISELLO A, STUECKLE SM, BESSALLE R, USDIN TB, CHOREV M, ROSENBLATT M, AND SUVA LJ. The human PTH2 receptor: binding and signal transduction prop-

erties of the stably expressed recombinant receptor. *Endocrinology* 137: 2748–2757, 1996.
71. Bello-Reuss E. Effect of catecholamines on fluid reabsorption by the isolated proximal convoluted tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 238: F347–F352, 1980.
72. Bello-Reuss E, Higashi Y, and Kaneda Y. Dopamine decreases fluid reabsorption in straight portions of rabbit proximal tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 242: F634–F640, 1982.
73. Belusa R, Wang ZM, Matsubara T, Sahlgen B, Dulubova I, Nairn AC, Ruoslahti E, Greengard P, and Aperia A. Mutation of the protein kinase C phosphorylation site on rat α_1 Na^+,K^+-ATPase alters regulation of intracellular Na^+ and pH and influences cell shape and adhesiveness. *J Biol Chem* 272: 20179–20184, 1997.
74. Bennett AM, Hausdorff SF, O'Reilly AM, Freeman RM Jr, and Neel BG. Multiple requirements for SHPTP2 in epidermal growth factor-mediated cell cycle progression. *Mol Cell Biol* 16: 1189–1202, 1996.
75. Bens M, Vallet V, Cluzeaud F, Pascual-Letallec L, Kahn A, Rafestin-Oblin ME, Rossier BC, and Vandewalle A. Corticosteroid-dependent sodium transport in a novel immortalized mouse collecting duct principal cell line. *J Am Soc Nephrol* 10: 923–934, 1999.
76. Bernardo AA, Kear FT, Santos AVP, Ma J, Steplock D, Robey RB, and Weinman EJ. Basolateral Na^+/HCO_3^- cotransport activity is regulated by the dissociable Na^+/H^+ exchanger regulatory factor. *J Clin Invest* 104: 195–201, 1999.
77. Beron J, Forster I, Béguin P, Geering K, and Verrey F. Phorbol 12-myristate 13-acetate down-regulates Na,K-ATPase independent of its protein kinase C site: decrease in basolateral cell surface area. *Mol Biol Cell* 8: 387–398, 1997.
78. Beron J, Mastroberardino L, Spillman A, and Verrey F. Aldosterone modulates sodium kinetics of Na,K-ATPase containing an α_1 subunit in A6 kidney cell epithelia. *Mol Biol Cell* 6: 261–271, 1995.
79. Bertorello A. Diacylglycerol activation of protein kinase C results in a dual effect on Na^+,K^+-ATPase activity from intact proximal tubule cells. *J Cell Sci* 101: 343–347, 1992.
80. Bertorello A and Aperia A. Regulation of Na^+-K^+-ATPase activity in kidney proximal tubules: involvement of GTP binding proteins. *Am J Physiol Renal Fluid Electrolyte Physiol* 256: F57–F62, 1989.
81. Bertorello A and Aperia A. Na-K-ATPase is an effector protein for protein kinase C in renal proximal tubule cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 256: F370–F373, 1989.
82. Bertorello A and Aperia A. Inhibition of proximal tubule Na-K-ATPase activity requires simultaneous activation of DA_1 and DA_2 receptors. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F924–F928, 1990.
83. Bertorello A, Aperia A, Walaas SI, Nairn AC, and Greengard P. Phosphorylation of the catalytic subunit of Na^+,K^+-ATPase inhibits the activity of the enzyme. *Proc Natl Acad Sci USA* 88: 11359–11362, 1991.
84. Bertorello A, Hopfield JF, Aperia A, and Greengard P. Inhibition by dopamine of (Na+K)ATPase activity in neostriatal neurons through D_1 and D_2 dopamine receptor synergism. *Nature* 347: 386–388, 1990.
85. Bertorello A, Hökfelt T, Goldstein M, and Aperia A. Proximal tubule Na-K-ATPase activity is inhibited during high-salt diet: evidence for DA-mediated effect. *Am J Physiol Renal Fluid Electrolyte Physiol* 254: F795–F801, 1988.
86. Bertorello AM, Ridge KM, Chibalin AV, Katz AI, and Sznajder JI. Isoproterenol increases Na^+-K^+-ATPase activity by membrane insertion of α-subunits in lung alveolar cells. *Am J Physiol Lung Cell Mol Physiol* 276: L20–L27, 1999.
87. Besseghir K, Trimble ME, and Stoner L. Action of ADH on isolated medullary thick ascending limb of the Brattleboro rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 251: F271–F277, 1986.
88. Bharatula M, Hussain T, and Lokhandwala MF. Angiotensin II AT1 receptor/signaling mechanisms in the biphasic effect of the peptide on proximal tubular Na^+,K^+-ATPase. *Clin Exp Hypertens* 20: 465–480, 1998.
89. Bia MF, Karen T, and Defronzo RA. The effect of dexamethasone on renal electrolyte excretion in the adrenalectomized rat. *Endocrinology* 111: 882–888, 1982.
90. Bidet M, Mérot J, Tauc M, and Poujeol P. Na^+-H^+ exchanger in proximal cells from kidney. II. Short-term regulation by glucocorticoids. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F945–F951, 1987.
91. Biemesderfer D, Rutherford PA, Nagy T, Pizzonia JH, Abu-Alfa A, and Aronson PS. Monoclonal antibodies for high-resolution localization of NHE3 in adult and neonatal rat kidney. *Am J Physiol Renal Physiol* 273: F289–F299, 1997.
92. Binswanger U, Helme-Kolb C, Forgo J, Mrkic B, and Murer H. Rapid stimulation of Na^+/H^+ exchange by 1,25-dihydroxyvitamin D_3; interaction with parathyroid-hormone-dependent inhibition. *Pflügers Arch* 424: 391–397, 1993.
93. Blaikie P, Immanuel D, Wu J, Li N, Yajnik V, and Margolis B. A region in Shc distinct from the SH2 domain can bind tyrosine-phosphorylated growth factor receptors. *J Biol Chem* 269: 32031–32034, 1994.
94. Bleich M, Schlatter E, and Greger R. The luminal K^+ channel of the thick ascending limb of Henle's loop. *Pflügers Arch* 415: 449–460, 1990.
95. Bloch RD, Zikos D, Fisher KA, Schleicher L, Oyama M, Cheng JC, Skopicki HA, Sukowski EJ, Cragoe EJ, and Peterson DR. Activation of proximal tubular Na-H exchange by angiotensin II. *Am. J Physiol Renal Fluid Electrolyte Physiol* 263: F135–F143, 1992.
96. Blot-Chabaud M, Coutry N, Laplace M, Bonvalet JP, and Farman N. Role of protein phosphatase in the regulation of Na^+-K^+-ATPase by vasopressin in the cortical collecting duct. *J Membr Biol* 153: 233–239, 1996.
97. Blot-Chabaud M, Jaisser F, Gingold-P Bonvalet M, and Farman N. Na-K-ATPase-dependent sodium flux in cortical collecting tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 255: F605–F613, 1988.
98. Blot-Chabaud M, Wanstok P, Bonvalet F, and Farman N. Cell sodium-induced recruitment of Na^+-K^+-ATPase pumps in rabbit cortical collecting tubules is aldosterone dependent. *J Biol Chem* 265: 11676–11681, 1990.
99. Boer WH, Braam B, Fransen R, Boer P, and Koomans HA. Effects of reduced renal perfusion pressure and acute volume expansion on proximal tubule and whole kidney angiotensin II content in the rat. *Kidney Int* 51: 44–49, 1997.
100. Bohman SO. Demonstration of prostaglandin synthesis in collecting ducts and other cell types of the rabbit renal medulla. *Prostaglandins* 14: 729–744, 1977.
101. Boim MA, Ho K, Shuck ME, Bienkowski MJ, Block JH, Slightom JL, Yang Y, Brenner BM, and Hebert SC. Romk inwardly rectifying ATP-sensitive K^+ channel. II. Cloning and distribution of alternative forms. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F1132–F1140, 1995.
102. Bonvalet P. Binding and action of aldosterone, dexamethasone, 1–25$(OH)_2D_3$, and estradiol along the nephron. *J Steroid Biochem* 27: 953–961, 1987.
103. Bonvalet P, Doignon I, Blot-Chabaud M, Pradelles P, and Farman N. Distribution of 11β-hydroxysteroid dehydrogenase along the rabbit nephron. *J Clin Invest* 86: 832–837, 1990.
104. Bonvalet P, Pradelles P, and Farman N. Segmental synthesis and actions of protaglandins along the nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F377–F387, 1987.
105. Borghini I, Geering K, Gjilovci A, Wollheim CB, and Pralong WF. In vivo phosphorylation of the Na,K-ATPase α subunit in sciatic nerves of control and diabetic rats: effects of protein kinase modulators. *Proc Natl Acad Sci USA* 91: 6211–6215, 1994.
106. Bossuyt X, Muller M, Hagenbuch B, and Meier JP. Polyspecific drug and steroid clearance by an organic anion transporter of mammalian liver. *J Pharmacol Exp Ther* 276: 891–896, 1996.
107. Bouby N, Bankir L, Trinh-Trang-Tan MM, Minuth WW, and Kriz W. Selective ADH-induced hypertrophy of the medullary thick ascending limb in Brattleboro rats. *Kidney Int* 28: 456–466, 1985.
108. Bouby N, Hus-Citharel A, Marchetti J, Bankir L, Corvol P, and Llorens-Cortes C. Expression of type I angiotensin II receptor subtypes and angiotensin II-induced calcium mobilization along the rat nephron. *J Am Soc Nephrol* 8: 1658–1667, 1997.
109. Braam B, Mitchell KD, Fox J, and Navar LG. Proximal tubular secretion of angiotensin II in rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F891–F898, 1993.
110. Breton S, Beck JS, and Laprade R. cAMP stimulates proximal

convoluted tubule Na^+-K^+-ATPase activity. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F400–F410, 1994.
111. Breyer MD. Feedback inhibition of cyclic adenosine monophosphate-stimulated Na^+ transport in the rabbit cortical collecting duct via Na^+-dependent basolateral Ca^{2+} entry. *J Clin Invest* 88: 1502–1510, 1991.
112. Breyer MD, Jacobson HR, Harris LS, and Breyer RM. In situ hybridization and localization of mRNA for the rabbit prostaglandin EP_3 receptor. *Kidney Int* 43: 1372–1378, 1993.
113. Brezis M, Agmon Y, and Epstein FH. Determinant of intrarenal oxygenation. I. Effects of diuretics. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F1059–F1062, 1994.
114. Brinson AE, Harding T, Diliberto PA, He Y, Li X, Hunter D, Herman B, Earp HS, and Graves LM. Regulation of a calcium-dependent tyrosine kinase in vascular smooth muscle cells by angiotensin II and platelet-derived growth factor. Dependence on calcium and the actin cytoskeleton. *J Biol Chem* 273: 1711–1718, 1998.
115. Brown D and Stow JL. Protein trafficking and polarity in kidney epithelium: from cell biology to physiology. *Physiol Rev* 76: 245–297, 1996.
116. Bruneval P, Hinglais N, Alhenc-Gelas F, Tricottet V, Corvol P, Menard J, Camilleri JP, and Bariety J. Angiotensin I converting enzyme in human intestine and kidney. Ultrastructural immunohistochemical localization. *Histochemistry* 85: 73–80, 1986.
117. Brunskill NJ, Stuart J, Tobin AB, Walls J, and Nahorski S. Receptor-mediated endocytosis of albumin by kidney proximal tubule cells is regulated by phosphatidylinositide 3-kinase. *J Clin Invest* 101: 2140–2150, 1998.
118. Buffin-Meyer B, Verbavatz JM, Cheval L, Marsy S, Younes-Ibrahim M, Le Moal C, and Doucet A. Regulation of Na^+,K^+-ATPase in the rat outer medullary collecting duct during potassium depletion. *J Am Soc Nephrol* 9: 538–550, 1998.
119. Buffin-Meyer B, Younes-Ibrahim M, Barlet-Bas C, Cheval L, Marsy S, and Doucet A. K depletion modifies the properties of Sch-28080-sensitive K-ATPase in rat collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F124–F131, 1997.
120. Buisson B, Laflamme L, Bottari SP, De Gasparo M, Gallo-Payet N, and Payet MD. A G protein is involved in the angiotensin AT_2 receptor inhibition of the T-type calcium current in non-differentiated NG108–15 cells. *J Biol Chem* 270: 1670–1674, 1995.
121. Bunnachak D, Almeida ARP, Wetzels JFM, Gengaro P, Nemenoff RA, Burke TJ, and Schrier RW. Ca^{2+} uptake, fatty acid, and LDH release during proximal tubule hypoxia: effects of mepacrine and dibucaine. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F196–F201, 1994.
122. Burnatowska-Hledin MA and Spielman WS. Vasopressin V_1 receptors on the principal cells of the rabbit cortical collecting tubule. *J Clin Invest* 83: 84–89, 1989.
123. Burns KD, Inagami T, and Harris RC. Cloning of a rabbit kidney cortex AT_1 angiotensin II receptor that is present in proximal tubule epithelium. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F645–F654, 1993.
124. Campen TJ, Vaughn DA, and Fanestil DD. Mineralo- and glucocorticoid effects on renal excretion of electrolytes. *Pflügers Arch* 399: 93–101, 1983.
125. Canessa CM, Horisberger JD, and Rossier BC. Epithelial sodium channel related to proteins involved in neurodegeneration. *Nature* 361: 467–470, 1993.
126. Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, and Rossier BC. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature* 367: 463–467, 1994.
127. Cano A. Characterization of the rat NHE_3 promoter. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F629–F636, 1996.
128. Cano A, Preisig PA, and Alpern RJ. Cyclic adenosine monophosphate acutely inhibits and chronically stimulates Na/H antiporter in OKP cells. *J Clin Invest* 92: 1632–1638, 1993.
129. Cantley LG, Fuhro R, and Silva P. Isolated MTAL cells produce an inhibitor of ouabain-sensitive oxygen consumption. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F210–F215, 1991.
130. Cantley LC, Josephson L, Warner R, Yanagisawa M, Lechêne C, and Guidotti G. Vanadate is a potent (Na^+ + K^+) ATPase inhibitor found in ATP derived from muscle. *J Biol Chem* 252: 7421–7423, 1977.
131. Caplan MJ. Membrane polarity in epithelial cells: protein sorting and establishment of polarized domains. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F425–F429, 1997.
132. Carpenter CL and Cantley LC. Phosphoinositide kinases. *Curr Opin Cell Biol* 8: 153–158, 1996.
133. Carpentier JL, Paccaud JP, Gorden P, Rutter WJ, and Orci L. Insulin-induced surface redistribution regulates internalization of the insulin receptor and requires its autophosphorylation. *Proc Natl Acad Sci USA* 89: 162–166, 1992.
134. Carranza ML, Féraille E, and Favre H. Protein kinase C-dependent phosphorylation of the Na^+,K^+-ATPase α-subunit in rat kidney cortical tubules. *Am J Physiol Cell Physiol* 271: C136–C143, 1996.
135. Carranza ML, Féraille E, Kiroytcheva M, Rousselot M, and Favre H. Stimulation of ouabain-sensitive $^{86}Rb^+$ uptake and Na^+,K^+-ATPase α-subunit phosphorylation by a cAMP-dependent signaling pathway in intact cells from rat kidney cortex. *FEBS Lett* 396: 309–314, 1996.
136. Carranza ML, Rousselot M, Chibalin AV, Bertorello AM, Favre H, and Féraille E. Protein kinase A induces recruitment of active Na^+,K^+-ATPase units to the plasma membrane of rat proximal convoluted tubule. *J Physiol (Lond)* 511: 235–243, 1998.
137. Casarini DE, Boim MA, Stella RC, Krieger-Azzolini MH, Krieger JE, and Schor N. Angiotensin I-converting enzyme activity in tubular fluid along the rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F405–F409, 1997.
138. Cassola AC, Giebisch G, and Wang W. Vasopressin increases density of apical low-conductance K channels in rat CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F502–F509, 1993.
139. Cervenka L, Wang CT, and Navar LG. Effects of acute AT_1 receptor blockade by candesartan on arterial pressure and renal function in rats. *Am J Physiol Renal Physiol* 274: F940–F945, 1998.
140. Chabardès D, Brick-Ghannam C, Montégut M, and Siaume-Perez S. Effect of PGE_2 and α-adrenergic agonists on AVP-dependent cAMP levels in rabbit and rat CCT. *Am J Physiol Renal Fluid Electrolyte Physiol* 255: F43–F48, 1988.
141. Chabardès D, Firsov D, Aarab L, Clabecq A, Bellanger AC, Siaume-Perez S, and Elalouf JM. Localization of mRNAs encoding Ca^{2+}-inhibitable adenylyl cyclases along the renal tubule. Functional consequences for regulation of the cAMP content. *J Biol Chem* 271: 19264–19271, 1996.
142. Chabardès D, Gagnan-Brunette M, Imbert-Teboul M, Gontcharevskaia O, Montégut M, Clique A, and Morel F. Adenylate cyclase responsiveness to hormones in various portions of the human nephron. *J Clin Invest* 65: 439–448, 1980.
143. Chabardès D, Imbert M, Clique A, Montégut M, and Morel F. PTH sensitive adenyl cyclase activity in different segments of the rabbit nephron. *Pflügers Arch* 354: 229–239, 1975.
144. Chabardès D, Imbert-Teboul M, and Elalouf JM. Functional properties of Ca^{2+}-inhibitable type 5 and type 6 adenylyl cyclases and role of Ca^{2+} increase in the inhibition of intracellular cAMP content. *Cell Signal* 11: 651–663, 1999.
145. Chabardès D, Montégut M, Imbert-Teboul M, and Morel F. Inhibition of α_2-adrenergic agonists on AVP-induced cAMP accumulation in isolated collecting tubule of the rat kidney. *Mol Cell Endocrinol* 37: 263–275, 1984.
146. Chabardès D, Montégut M, Zhou Y, and Siaume-Perez S. Two mechanisms of inhibition by prostaglandin E_2 of hormone-dependent cell cAMP in the rat collecting tubule. *Mol Cell Endocrinol* 73: 111–121, 1990.
147. Chabre O, Conklin BR, Brandon S, Bourne HR, and Limbird LE. Coupling of the α_{2A}-adrenergic receptor to multiple G-proteins. *J Biol Chem* 269: 5730–5734, 1994.
148. Chalfant ML, Coupaye-Gerard B, and Kleyman TR. Distinct regulation of Na reabsorption and Cl secretion by arginine vasopressin in the amphibian cell line A6. *Am J Physiol Cell Physiol* 264: C1480–C1488, 1993.
149. Chambrey R, Warnock DG, Podevin RA, Bruneval P, Mandet C, Belair MF, Bariety J, and Paillard M. Immunolocalization of the Na^+/H^+ exchanger isoform NHE2 in rat kidney. *Am J Physiol Renal Physiol* 275: F379–F386, 1998.
150. Champigneulle A, Siga E, Vassent G, and Imbert-Teboul M. V_2-like vasopressin receptor mobilizes intracellular Ca^{2+} in rat medullary

collecting tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F35–F45, 1993.
151. CHAMPIGNEULLE A, SIGA E, VASSENT G, AND IMBERT-TEBOUL M. Relationship between extra- and intracellular calcium in distal segments of the renal tubule. Role of the Ca^{2+} receptor RaKCaR. *J Membr Biol* 156: 117–129, 1997.
152. CHAN YL. The role of norepinephrine in the regulation of fluid absorption in the rat proximal tubule. *J Pharmacol Exp Ther* 215: 65–70, 1980.
153. CHARLTON JA AND BAYLIS PH. Stimulation of rat renal medullary Na^+/K^+-ATPase by arginine vasopressin is mediated by the V2 receptor. *J Endocrinol* 127: 213–216, 1990.
154. CHASE LR AND AURBACH GD. Parathyroid function and the renal excretion of 3′5′-adenylic acid. *Proc Natl Acad Sci USA* 58: 518–525, 1967.
155. CHASE LR AND AURBACH GD. Renal adenyl cyclase: anatomically separate sites for parathyroid hormone and vasopressin. *Science* 159: 545–547, 1968.
156. CHATSUDTHIPONG V AND CHAN YL. Inhibitory effect of angiotensin II on renal tubular transport. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F340–F346, 1991.
157. CHEN SY, BHARGAVA A, MASTROBERARDINO L, MEIJER OC, WANG J, BUSE P, FIRESTONE GL, VERREY F, AND PEARCE D. Epithelium sodium channel regulated by aldosterone-induced protein sgk. *Proc Natl Acad Sci USA* 96: 2514–2519, 1999.
158. CHEN L, REIF MC, AND SCHAFER JA. Clonidine and PGE_2 have different effects on Na^+ and water transport in rat and rabbit CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 261: F126–F136, 1991.
159. CHEN L, WILLIAMS SK, AND SCHAFER JA. Differences in synergistic actions of vasopressin and deoxycorticosterone in rat and rabbit CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F147–F156, 1990.
160. CHEN X, LI W, YOSHIDA H, TSUCHUDA S, NISHIMURA H, TAKEMOTO F, OKUBO S, FOGO A, MATSUSAKA T, AND ICHIKAWA I. Targeting deletion of angiotensin type 1B receptor gene in the mouse. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F299–F304, 1997.
161. CHENG SXJ, AIZMAN O, NAIRN AC, GREENGARD P, AND APERIA A. $[Ca^{2+}]_i$ determines the effects of protein kinases A and C on activity of rat renal Na^+,K^+-ATPase. *J Physiol (Lond)* 518: 37–46, 1999.
162. CHEVAL L, BARLET-BAS C, AND DOUCET A. Characterization of the molecular isoforms of Na^+/K^+-ATPase subunits along the rat nephron by polymerase chain reaction. In: *The Sodium Pump*, edited by Bamberg E and Schoner W. Darmstadt, Germany: Steinkopff, 1994, p. 704–709.
163. CHEVAL L AND DOUCET A. Measurement of Na-K-ATPase-mediated rubidium influx in single segments of rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F111–F121, 1990.
164. CHIBALIN AV, KATZ AI, BERGGREN PO, AND BERTORELLO AM. Receptor-mediated inhibition of renal Na^+-K^+-ATPase is associated with endocytosis of its α- and β-subunits. *Am J Physiol Cell Physiol* 273: C1458–C1465, 1997.
165. CHIBALIN AV, OGIMOTO G, PEDEMONTE CH, PRESSLEY TA, KATZ AI, FÉRAILLE E, BERGGREN PO, AND BERTORELLO AM. Dopamine-induced endocytosis of Na^+,K^+-ATPase is initiated by phosphorylation of Ser^{18} in the rat α-subunit and is responsible for the decreased activity in epithelial cells. *J Biol Chem* 274: 1920–1927, 1999.
166. CHIBALIN AV, PEDEMONTE CH, KATZ AI, FÉRAILLE E, BERGGREN PO, AND BERTORELLO AM. Phosphorylation of the catalytic α-subunit constitutes a triggering signal for Na^+,K^+-ATPase endocytosis. *J Biol Chem* 273: 8814–8819, 1998.
167. CHIBALIN AV, VASILETS LA, HENNEKES H, PRALONG D, AND GEERING K. Phosphorylation of Na,K-ATPase α-subunits in microsomes and in homogenates of *Xenopus* oocytes resulting from the stimulation of protein kinase A and protein kinase C. *J Biol Chem* 267: 22378–22384, 1992.
168. CHIBALIN AV, ZIERATH JR, KATZ AI, BERGGREN PO, AND BERTORELLO AM. Phosphatidylinositol 3-kinase-mediated endocytosis of renal Na^+,K^+-ATPase α subunit in response to dopamine. *Mol Biol Cell* 9: 1209–1220, 1998.
169. CHINI CC, GRANDE JP, CHINI EN, AND DOUSA TP. Compartmentalization of cAMP signaling in mesangial cells by phosphodiesterase isozymes PDE3 and PDE4. Regulation of superoxidation and mitogenesis. *J Biol Chem* 272: 9854–9859, 1997.
170. CHOI KH, EDELSTEIN CL, GENGARO P, SCHRIER RW, AND NEMENOFF RA. Hypoxia induces changes in phospholipase A_2 in rat proximal tubules: evidence for multiple forms. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F846–F853, 1995.
171. CHOU CL, RAPKO SI, AND KNEPPER MA. Phosphoinositide signaling in rat inner medullary collecting duct. *Am J Physiol Renal Physiol* 274: F564–F572, 1998.
172. CHRISTENSEN EI AND BIRN H. Hormone, growth factor, and vitamin handling by proximal tubule cells. *Curr Opin Nephrol Hypertens* 6: 20–27, 1997.
173. CHRISTOFORIDIS S, MIACZYNSKA M, ASHMAN K, WILM M, ZHAO L, YIP S, WATERFIELD MD, BACKER JM, AND ZERIAL M. Phosphatidylinositol-3-OH kinases are Rab5 effectors. *Nature Cell Biol* 1: 249–252, 1999.
174. CICIRELLI MF, TONKS NK, DILTZ CD, WEIEL JE, FISCHER EH, AND KREBS EG. Microinjection of a protein-tyrosine-phosphatase inhibits insulin action in *Xenopus* oocytes. *Proc Natl Acad Sci USA* 87: 5514–5518, 1990.
175. CIUFFO GM, HEEMSKERK FMJ, AND SAAVEDRA JM. Purification and characterization of angiotensin II AT_2 receptors from neonatal rat kidney. *Proc Natl Acad Sci USA* 90: 11009–11013, 1993.
176. CLAPP WL, BOWMAN P, SHAW GS, PATEL P, AND KONE BC. Segmental localization of mRNAs encoding Na^+-K^+-ATPase α- and β-subunit isoforms in rat kidney using RT-PCR. *Kidney Int* 46: 627–638, 1994.
177. CLARKE JD, CRAGOE EJ, AND LIMBIRD LE. α_2-Adrenergic receptors regulate Na-H exchange via a cAMP-dependent mechanism. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F977–F985, 1990.
178. CLAUSEN T AND FLATMAN JA. Effects of insulin and epinephrine on Na^+-K^+ and glucose transport in soleus muscle. *Am J Physiol Endocrinol Metab* 252: E492–E499, 1987.
179. COGHLAN VM, PERRINO BA, HOWARD M, LANGEBERG LK, HICKS JB, GALLATIN WM, AND SCOTT JD. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science* 267: 108–111, 1995.
180. COHEN GB, REN R, AND BALTIMORE D. Modular binding domains in signal transduction proteins. *Cell* 80: 237–248, 1995.
181. COHN DE, KLAHR S, AND HAMMERMAN MR. Metabolic acidosis and parathyroidectomy increases Na^+-H^+ exchange in brush border vesicles. *Am J Physiol Renal Fluid Electrolyte Physiol* 245: F217–F222, 1983.
182. COOPER GJ AND BORON WF. Effect of PCMBS on CO_2 permeability of *Xenopus* oocytes expressing aquaporin 1 or its C189S mutant. *Am J Physiol Cell Physiol* 275: C1481–C1486, 1998.
183. COPPOLA S AND FRÖMTER E. An electrophysiological study of angiotensin II regulation of Na-HCO_3 cotransport and K conductance in renal proximal tubules. *Pflügers Arch* 427: 143–150, 1994.
184. COPPOLA S AND FRÖMTER E. An electrophysiological study of angiotensin II regulation of Na-HCO_3 cotransport and K conductance in renal proximal tubules. II. Effect of micromolar concentrations. *Pflügers Arch* 427: 151–156, 1994.
185. CORDOVA HR, KOKKO JP, AND MARVER D. Chronic indomethacin increases rabbit cortical collecting tubule Na^+-K^+-ATPase activity. *Am J Physiol Renal Fluid Electrolyte Physiol* 256: F570–F576, 1989.
186. CORNELIUS F AND LOGVINENKO N. Functional regulation of reconstituted Na,K-ATPase by protein kinase A phosphorylation. *FEBS Lett* 380: 277–280, 1996.
187. COUTRY N, FARMAN P, BONVALET N, AND BLOT-CHABAUD M. Synergistic action of vasopressin and aldosterone on basolateral Na^+-K^+-ATPase in the cortical collecting duct. *J Membr Biol* 145: 99–106, 1995.
188. CRABBÉ J. Stimulation of active sodium transport by the isolated toad bladder with aldosterone in vitro. *J Clin Invest* 40: 2103–2110, 1961.
189. CRABBÉ J. Suppression by amphotericin B of the effect exerted by aldosterone on active sodium transport. *Arch Int Physiol Biochim* 75: 342–345, 1967.
190. CRAMBERT G, HASLER U, BEGGAH AT, YU C, MODYANOV NN, HORISBERGER JD, LELIEVRE L, AND GEERING K. Transport and pharmacological properties of 9 human Na,K-ATPase isozymes. *J Biol Chem.* In press.
191. CRESPO P, XU N, SIMMONDS WS, AND GUTKIND JS. Ras-dependent

activation of MAP kinase pathway mediated by G-protein $\beta\gamma$ subunits. *Nature* 369: 418–420, 1994.
192. CULPEPPER RM AND ANDREOLI TE. Interactions among prostaglandin E_2, antidiuretic hormone and cyclic adenosine monophosphate in modulating Cl^- absorption along single mouse medullary thick ascending limb of Henle. *J Clin Invest* 71: 1588–1601, 1983.
193. DAAKA Y, LUTTRELL LM, AHN S, DELLA ROCCA G, FERGUSON SSG, CARON MG, AND LEFKOWITZ RJ. Essential role for G protein-coupled receptor endocytosis in the activation of mitogen activated kinase. *J Biol Chem* 273: 685–688, 1998.
194. DAI LJ AND QUAMME GA. Hormone-mediated Ca^{2+} transients in isolated renal cortical thick ascending limb cells. *Pflügers Arch* 427: 1–8, 1994.
195. DAWIRS RR AND TEUCHERT-NOODT G. Demonstration of dopamine-immunoreactive cells in the proximal convoluted tubule of gerbil (*Meriones unguiculatus*) kidney. *J Histochem Cytochem* 40: 1685–1691, 1992.
196. DEACHAPUNYA C, PALMER-DENSMORE M, AND O'GRADY SM. Insulin stimulates transepithelial sodium transport by activation of a protein phosphatase that increases Na-K ATPase activity in endometrial epithelial cells. *J Gen Physiol* 114: 561–574, 1999.
197. DECOY DL, SNAPPER JR, AND BREYER MD. Anti sense DNA downregulates protein kinase C-ϵ and enhances vasopressin-stimulated Na^+ absorption in rabbit cortical collecting duct. *J Clin Invest* 95: 2749–2756, 1995.
198. DEFRONZO RA, COOKE CR, ANDRES R, FALOONA GR, AND DAVIS PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest* 55: 845–855, 1975.
199. DEFRONZO RA, GOLDBERG M, AND AGUS ZS. The effects of glucose and insulin on renal electrolyte transport. *J Clin Invest* 58: 83–90, 1976.
200. DE JESUS FERREIRA MC AND BAILLY C. Luminal and basolateral endothelin inhibit chloride reabsorption in the mouse thick ascending limb via a Ca^{2+}-independent pathway. *J Physiol (Lond)* 503: 749–758, 1997.
201. DE JESUS FERREIRA MC AND BAILLY C. Extracellular Ca^{2+} decreases chloride reabsorption in rat CTAL by inhibiting cAMP pathways. *Am J Physiol Renal Physiol* 275: F198–F203, 1998.
202. DE JESUS FERREIRA MC, HÉLIÈS-TOUSSAINT C, IMBERT-TEBOUL M, BAILLY-M VERBAVATZ C, BELLANGER AC, AND CHABARDÈS D. Co-expression of a Ca^{2+}-inhibitable adenylyl cyclase and of a Ca^{2+}-sensing receptor in the thick ascending limb cell of the rat kidney. Inhibition of hormone-dependent cAMP accumulation by extracellular Ca^{2+}. *J Biol Chem* 273: 15192–15202, 1998.
203. DELL'ACQUA ML AND SCOTT JD. Protein kinase A anchoring. *J Biol Chem* 272: 12881–12884, 1997.
204. DELLA ROCCA GJ, VAN BIESEN T, DAAKA Y, LUTTRELL DK, LUTTRELL LM, AND LEFKOWITZ RJ. Ras-dependent mitogen-activated protein kinase activation by G protein-coupled receptors. Convergence of G_i- and G_q-mediated pathways on calcium/calmodulin, Pyk2, and src kinase. *J Biol Chem* 272: 19125–19132, 1997.
205. DENNING MF, DLUGOSTZ AA, THREADGILL DW, MAGNUSON T, AND YUSPA SH. Activation of the epidermal growth factor receptor signal transduction pathway stimulates tyrosine phosphorylation of protein kinase Cδ. *J Biol Chem* 271: 5325–5331, 1996.
206. DENNIS VW. Influence of bicarbonate on parathyroid hormone-induced changes in fluid absorption by the proximal tubule. *Kidney Int* 10: 373–380, 1976.
207. DE ROOIJ J, ZWARTKRUIS FJT, VERHEIJEN MHG, COOL RH, NIJMAN SMB, WITTINGHOFER A, AND BOS JL. Epac is a Rap 1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396: 474–477, 1998.
208. DERRICKSON BH AND MANDEL LJ. Parathyroid hormone inhibits Na^+-K^+-ATPase through G_q/G_{11} and the calcium-independent phospholipase A_2. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F781–F788, 1997.
209. DE SMET P, ERLIJ D, AND VAN DRIESSCHE W. Insulin effects on ouabain binding in A6 renal cells. *Pflügers Arch* 434: 11–18, 1997.
210. DI STEFANO A, ROINEL N, DE ROUFFIGNAC C, AND WITTNER M. Transepithelial Ca^{2+} and Mg^{2+} transport in the cortical thick ascending limb of Henle's loop of the mouse is a voltage-dependent process. *Renal Physiol Biochem* 16: 157–166, 1993.
211. DI STEFANO A, WITTNER M, NITSCHKE R, BRAITSCH R, GREGER R, BAILLY C, AMIEL C, ELALOUF JM, ROINEL N, AND DE ROUFFIGNAC C. Effects of glucagon on Na, Cl, K, Mg and Ca transports in cortical and medullary thick ascending limbs of mouse kidney. *Pflügers Arch* 414: 640–646, 1989.
212. DI STEFANO A, WITTNER M, NITSCHKE R, BRAITSCH R, GREGER R, BAILLY C, AMIEL C, ROINEL N, AND DE ROUFFIGNAC C. Effects of parathyroid hormone and calcitonin on Na^+, Cl^-, K^+, Mg^{2+} and Ca^{2+} transport in cortical and medullary thick ascending limbs of mouse kidney. *Pflügers Arch* 417: 161–167, 1990.
213. DIXON BS, BRECKON R, FORTUNE J, SUTHERLAND E, SIMON FR, AND ANDERSON RJ. Bradykinin activates protein kinase C in cultured cortical collecting tubular cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 257: F808–F817, 1989.
214. DIXON RA, KOBILKA BK, STRADER DJ, BENOVIC JL, DOHLMAN HG, FRIELLE T, BOLANOWSKI MA, BENNETT CD, RANDS E, DIEHL RE, MUMFORD RA, SLATER EE, SIGAL IS, CARON MG, LEFKOWITZ RJ, AND STRADA CD. Cloning of the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin. *Nature* 321: 75–79, 1986.
215. DJELIDI S, FAY M, CLUZEAUD F, ESCOUBET B, EUGENE E, CAPURRO C, BONVALET JP, FARMAN N, AND BLOT-CHABAUD M. Transcriptional regulation of sodium transport by vasopressin in renal cells. *J Biol Chem* 272: 32919–32924, 1997.
216. DOCHERTY JR. Subtypes of functional α_1- and α_2-adrenoreceptors. *Eur J Pharmacol* 361: 1–15, 1998.
217. DOLSON GM, HISE MK, AND WEINMAN EJ. Relationship among parathyroid hormone, cAMP, and calcium on proximal tubule sodium transport. *Am J Physiol Renal Fluid Electrolyte Physiol* 249: F409–F416, 1985.
218. DOMINGUEZ JH, SNOWDOWNE KW, FREUDENRICH CC, BROWN T, AND BORLE AB. Intracellular messenger for action of angiotensin II on fluid transport in rabbit proximal tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F423–F428, 1987.
219. DOUCET A. Multiple hormonal control of the Na/K-ATPase activity in the thick ascending limb. In: *Nephrology*, edited by Davison AM. London: Baillères Tindall, 1988, p. 247–254.
220. DOUCET A AND BARLET C. Evidence for differences in the sensitivity to ouabain of Na,K-ATPase along the rabbit nephron. *J Biol Chem* 261: 993–995, 1986.
221. DOUCET A, HUS-CITHAREL A, AND MOREL F. In vitro stimulation of Na-K-ATPase in rat thick ascending limb by dexamethasone. *Am J Physiol Renal Fluid Electrolyte Physiol* 251: F851–F857, 1986.
222. DOUCET A AND KATZ AI. Short-term effects of aldosterone on Na-K-ATPase in single nephron segments. *Am J Physiol Renal Fluid Electrolyte Physiol* 241: F273–F278, 1981.
223. DOUCET A AND KATZ AI. Mineralocorticoid receptors along the nephron: [^{3}H]aldosterone binding in rabbit tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 241: F605–F611, 1981.
224. DOUCET A, KATZ AI, AND MOREL F. Determination of Na-K-ATPase activity in single segments of the mammalian nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 237: F105–F113, 1979.
225. DUBLINEAU I, PRADELLES P, DE ROUFFIGNAC C, AND ELALOUF M. In vitro desensitization of isolated nephron segments to vasopressin. *Proc Natl Acad Sci USA* 87: 7583–7587, 1990.
226. DUBROVSKY AHE, NAIR RC, BYERS MK, AND LEVINE DZ. Renal and acid excretion in the adrenalectomized rat. *Kidney Int* 19: 516–528, 1981.
227. DUFF JL, MARRERO MB, PAXTON WG, CHARLES CH, LAU LF, BERNSTEIN KE, AND BERK BC. Angiotensin II induces 3CH134, a protein-tyrosine phosphatase, in vascular smooth muscle cells. *J Biol Chem* 268: 26037–26040, 1993.
228. DULIN NO, ALEXANDER LD, HARWARKAR S, FALK JR, AND DOUGLAS JG. Phospholipase A_2-mediated activation of mitogen-activated protein kinase by angiotensin II. *Proc Natl Acad Sci USA* 95: 8098–8102, 1998.
229. DUNLAY R AND HRUSKA K. PTH receptor coupling to phospholipase C is an alternate pathway of signal transduction in bone and kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F223–F231, 1990.
230. DUONG VAN HUYEN J, BENS M, AND VANDEWALLE A. Differential effects of aldosterone and vasopressin on chloride fluxes in transimmortalized mouse cortical collecting duct cells. *J Membr Biol* 164: 79–90, 1998.
231. ECELBARGER CA, CHOU CL, LOLAIT SJ, KNEPPER MA, AND DIGIOVANNI

SR. Evidence for dual signaling pathways for V_2 vasopressin receptor in rat inner medullary collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F623–F633, 1996.
232. ECELBARGER CA, YU S, LEE AJ, WEINSTEIN LS, AND KNEPPER MA. Decreased renal Na-K-2Cl cotransporter abundance in mice with heterozygous disruption of the $G\alpha_s$ gene. *Am J Physiol Renal Physiol* 277: F235–F244, 1999.
233. EDELMAN IS, BOGOROCH R, AND PORTER GA. On the mechanism of action of aldosterone on sodium transport: the role of protein synthesis. *Proc Natl Acad Sci USA* 50: 1169–1177, 1963.
234. EDINGER RS, ROKAW MD, AND JOHNSON JP. Vasopressin stimulates sodium transport in A6 cells via a phosphatidylinositide 3-kinase-dependent pathway. *Am J Physiol Renal Physiol* 277: F575–F579, 1999.
235. EDWARDS RM, STACK EJ, PULLEN M, AND NAMBI P. Endothelin inhibits vasopressin action in rat inner medullary collecting duct via ET_B receptor. *J Pharmacol Exp Ther* 267: 1028–1033, 1993.
236. EFENDIEV R, BERTORELLO AM, AND PEDEMONTE CH. Pkc-β and PKC-ζ mediate opposing effects on proximal tubule Na^+,K^+-ATPase activity. *FEBS Lett* 456: 45–46, 1999.
237. EGUCHI S, IWASAKI H, INAGAMI T, NUMAGUCH K, YAMAKAWA T, MOTLEY ED, OWADA KM, MARUMO F, AND HIRATA Y. Involvement of PYK2 in angiotensin II signaling of vascular smooth muscle cells. *Hypertension* 33: 201–206, 1999.
238. EGUCHI S, MATSUMOTO T, MOTLEY ED, UTSUNOMIYA H, AND INAGAMI T. Identification of an essential signaling cascade for mitogen-activated protein kinase activation by angiotensin II in cultured rat vascular smooth muscle cells. Possible requirement of G_q-mediated p21ras activation coupled to a Ca^{2+}/calmodulin-sensitive tyrosine kinase. *J Biol Chem* 271: 14169–14175, 1996.
239. EGUCHI S, NUMAGUCHI K, IWASAKI H, MATSUMOTO T, YAMAKAWA T, UTSUNOMIYA H, MOTLEY ED, KAWAKATSU H, OWADA KM, HIRATA Y, MARUMO F, AND INAGAMI T. Calcium-dependent epidermal growth factor receptor transactivation mediates the angiotensin II-induced mitogen-activated protein kinase activation in vascular smooth muscle cells. *J Biol Chem* 273: 8890–8896, 1998.
240. EIAM-ONG S, HILDEN SA, JOHNS CA, AND MADIAS NE. Stimulation of basolateral Na-HCO_3^- cotransporter by angiotensin II in rabbit renal cortex. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F195–F203, 1993.
241. EKLÖF AC, HOLTBÄCK U, SUNDELÖF M, CHEN S, AND APERIA A. Inhibition of COMT induces dopamine-dependent natriuresis and inhibition of proximal tubular Na^+,K^+-ATPase. *Kidney Int* 52: 742–747, 1997.
242. ELALOUF M, ROINEL N, AND DE ROUFFIGNAC C. ADH-like effects of calcitonin transport by Henle's loop of rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 246: F213–F220, 1984.
243. ELALOUF JM, ROINEL N, AND DE ROUFFIGNAC C. Effects of glucagon and PTH on the loop of Henle of rat juxtamedullary nephrons. *Kidney Int* 29: 807–813, 1986.
244. ELHAWARY AM AND PANG CC. α_{1b}-Adrenoceptors mediate renal tubular sodium and water reabsorption in the rat. *Br J Pharmacol* 111: 819–824, 1994.
245. EL MERNISSI G, CHABARDES D, DOUCET A, HUS-CITHAREL A, IMBERT-TEBOUL M, LE BOUFFANT F, MONTEGUT M, SIAUME S, AND MOREL F. Changes in tubular basolateral membrane markers after chronic DOCA treatment. *Am J Physiol Renal Fluid Electrolyte Physiol* 245: F100–F109, 1983.
246. EL MERNISSI G AND DOUCET A. Short-term effect of aldosterone on rat sodium transport and tubular Na-K-ATPase in the rat. *Pflügers Arch* 399: 139–146, 1983.
247. EL MERNISSI G AND DOUCET A. Short-term effects of aldosterone and dexamethasone on Na-K-ATPase along the rabbit nephron. *Pflügers Arch* 399: 147–151, 1983.
248. EL MERNISSI G AND DOUCET A. Quantitation of [^{3}H]ouabain binding and turnover of Na-K-ATPase along the rabbit nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 247: F158–F167, 1984.
249. EL MERNISSI G AND DOUCET A. Specific activity of Na-K-ATPase after adrenalectomy and hormone replacement along the rabbit nephron. *Pflügers Arch* 402: 258–263, 1984.
250. ERLIJ D, DE SMET P, MESOTTEN D, AND VAN DRIESSCHE W. Forskolin increases apical sodium conductance in cultured toad kidney cells (A6) by stimulating membrane insertion. *Pflügers Arch* 438: 195–204, 1999.
251. ERLIJ D, DE SMET P, AND VAN DRIESSCHE W. Insulin increases area and Na^+ channel density of apical membrane of cultured kidney cells. *J Physiol (Lond)* 481: 533–542, 1994.
252. ERNST SA, GAEGGELER HP, GEERING K, KRAEHENBÜHL JP, AND ROSSIER BC. Differential effect of aldosterone on expression of apical membrane proteins and of basolateral membrane β-subunit of Na,K-ATPase (Abstract). *J Cell Biol* 103: 465a, 1986.
253. ESCALANTE B, ERLIJ D, FALCK JR, AND MCGIFF JC. Effect of cytochrome *P*-450 arachidonate metabolites on ion transport in rabbit kidney loop of Henle. *Science* 251: 799–801, 1991.
254. ESCOUBET B, COUREAU C, BLOT-CHABAUD P, BONVALET M, AND FARMAN N. Corticosteroid receptor mRNA expression is unaffected by corticosteroids in rat kidney, heart, and colon. *Am J Physiol Cell Physiol* 270: C1343–C1353, 1996.
255. ESCOUBET B, COUREAU C, BONVALET JP, AND FARMAN N. Noncoordinate regulation of epithelial Na channel and Na pump subunit mRNAs in kidney and colon by aldosterone. *Am J Physiol Cell Physiol* 272: C1482–C1491, 1997.
256. EVANS RM. The steroid and thyroid hormone receptor superfamily. *Science* 240: 889–895, 1988.
257. FAN L, WIEDERKEHR MR, COLLAZO R, WANG H, CROWDER LA, AND MOE OW. Dual mechanisms of regulation of Na/H exchanger NHE-3 by parathyroid hormone in rat kidney. *J Biol Chem* 274: 11289–11295, 1999.
258. FANTL WJ, ESCOBEDO JA, MARTIN GA, TURCK CW, DEL ROSARIO M, AND WILLIAMS LT. Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signalling pathways. *Cell* 69: 413–423, 1992.
259. FARMAN N. Molecular and cellular determinants of mineralocorticoid selectivity. *Curr Opin Nephrol Hypertens* 8: 45–51, 1999.
260. FARMAN N, COUTRY N, LOGVINENKO N, BLOT-CHABAUD M, BOUR-BOUZE R, AND BONVALET P. Adrenalectomy reduces α_1- and not β_1-Na^+-K^+-ATPase mRNA expression in rat distal nephron. *Am J Physiol Cell Physiol* 263: C810–C817, 1992.
261. FARMAN N, OBLIN ME, LOMBES M, DELAHAYE F, WESTPHAL P, BONVALET HM, AND GASC M. Immunolocalization of gluco- and mineralocorticoid receptors in rabbit kidney. *Am J Physiol Cell Physiol* 260: C226–C233, 1991.
262. FARMAN N, VANDEWALLE A, AND BONVALET P. Binding of aldosterone to cytoplasmic and nuclear receptors of the rabbit kidney. *Am J Physiol Cell Physiol* 240: C20–C27, 1981.
263. FAURE R, BAQUIRAN G, BERGERON JJM, AND POSNER BI. The dephosphorylation of insulin and epidermal growth factor receptors. *J Biol Chem* 267: 11215–11221, 1992.
264. FAWELL SE, LEES JA, WHITE R, AND PARKER MG. Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. *Cell* 60: 953–962, 1990.
265. FEENER EP, BACKER JM, KING GL, WILDEN PA, SUN XJ, KAHN CR, AND WHITE MF. Insulin stimulates serine and tyrosine phosphorylation in the juxtamembrane region of the insulin receptor. *J Biol Chem* 268: 11256–11264, 1993.
266. FEHLMANN M AND FREYCHET P. Insulin and glucagon stimulation of (Na^+-K^+)-ATPase transport activity in isolated rat hepatocytes. *J Biol Chem* 256: 7449–7453, 1981.
267. FEJES-TOTH G, ZAHAJSKY T, AND FILEP J. Effect of vasopressin on renal kallikrein excretion. *Am J Physiol Renal Fluid Electrolyte Physiol* 239: F388–F392, 1980.
268. FELDER CC, ALBRECHT FE, CAMPBELL T, EISNER GM, AND JOSE PA. cAMP-independent, G protein-linked inhibition of Na/H exchange in renal brush by D_1 dopamine agonists. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F1032–F1037, 1993.
269. FELDER RA, BLECHER M, CALCAGNO PL, AND JOSE PA. Dopamine receptors in the proximal tubule of the rabbit. *Am J Physiol Renal Fluid Electrolyte Physiol* 247: F499–F505, 1984.
270. FELDER CC, CAMPELL T, ALBRECHT F, AND JOSE PA. Dopamine inhibits Na-H exchanger activity in renal BBMV by stimulation of adenylate cyclase. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F297–F303, 1990.
271. FELDER CC, MCKELVEY AM, GITLER MS, MEISNER G, AND JOSE PA. Dopamine receptor subtypes in renal brush border and basolateral membranes. *Kidney Int* 36: 183–193, 1989.

272. FELDMAN D, FUNDER JW, AND EDELMAN IS. Evidence for a new class of corticosterone receptors in the rat kidney. *Endocrinology* 92: 1429–1441, 1973.
273. FENG F, ABEL PW, SCOFIELD M, LIU F, WOLFF DW, AND JEFFRIES WB. Heterogeneous expression of α_1-adrenoceptor subtypes among rat nephron segments. *Mol Pharmacol* 44: 926–933, 1993.
274. FENG F, PETTINGER WA, ABEL PW, AND JEFFRIES WB. Regional distribution of α_1-adrenoceptor subtypes in rat kidney. *J Pharmacol Exp Ther* 258: 263–268, 1991.
275. FÉRAILLE E, BARLET-BAS C, CHEVAL L, ROUSSELOT L, CARRANZA M, DREHER D, ARYSTARKHOVA E, DOUCET A, AND FAVRE H. Presence of two isoforms of Na,K-ATPase with different pharmacological and immunological properties in the rat kidney. *Pflügers Arch* 430: 205–212, 1995.
276. FÉRAILLE E, BÉGUIN P, CARRANZA ML, GONIN S, ROUSSELOT M, MARTIN PY, FAVRE H, AND GEERING K. Is phosphorylation of the α_1 subunit at Ser-16 involved in the control of Na,K-ATPase activity by phorbol ester-activated protein kinase C. *Mol Biol Cell* 11: 39–50, 2000.
277. FÉRAILLE E, CARRANZA ML, BUFFIN-MEYER B, ROUSSELOT M, DOUCET A, AND FAVRE H. Protein kinase C-dependent stimulation of Na^+-K^+-ATPase in rat proximal convoluted tubules. *Am J Physiol Cell Physiol* 268: C1277–C1283, 1995.
278. FÉRAILLE E, CARRANZA M-L, GONIN S, BÉGUIN P, PEDEMONTE C, ROUSSELOT M, CAVERZASIO J, GEERING K, MARTIN PY, AND FAVRE H. Insulin-induced stimulation of Na^+,K^+-ATPase activity in kidney proximal tubule cells depends on phosphorylation of the α-subunit at Tyr-10. *Mol Biol Cell* 10: 2847–2859, 1999.
279. FÉRAILLE E, CARRANZA M-L, ROUSSELOT M, AND FAVRE H. Insulin enhances sodium sensivity of Na-K-ATPase in isolated rat proximal convoluted tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F55–F62, 1994.
280. FÉRAILLE E, CARRANZA M-L, ROUSSELOT M, AND FAVRE H. Modulation of Na^+,K^+-ATPase activity by tyrosine phosphorylation process in rat proximal convoluted tubule. *J Physiol (Lond)* 498: 99–108, 1997.
281. FÉRAILLE E, MARSY S, CHEVAL L, BARLET-BAS C, KHADOURI C, FAVRE H, AND DOUCET A. Sites of antinatriuretic action of insulin along rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 263: F175–F179, 1992.
282. FÉRAILLE E, ROUSSELOT M, RAJERISON R, AND FAVRE H. Effect of insulin on Na^+,K^+-ATPase in rat collecting duct. *J Physiol (Lond)* 488: 171–180, 1995.
283. FESCHENKO MS AND SWEADNER KJ. Conformation-dependent phosphorylation of Na,K-ATPase by protein kinase A and protein kinase C. *J Biol Chem* 269: 30436–30444, 1994.
284. FESCHENKO MS AND SWEADNER KJ. Structural basis for species-specific differences in the phosphorylation of Na,K-ATPase by protein kinase C. *J Biol Chem* 270: 14072–14077, 1995.
285. FESCHENKO MS AND SWEADNER KJ. Phosphorylation of Na,K-ATPase by protein kinase C at Ser^{18} occurs in intact cells but does not result in direct inhibition of ATP hydrolysis. *J Biol Chem* 272: 17726–17733, 1997.
286. FIDELMAN ML, MAY JM, BIBER TUL, AND WATLINGTON CO. Insulin stimulation of Na^+ transport and glucose metabolism in cultured kidney cells. *Am J Physiol Cell Physiol* 242: C121–C123, 1982.
287. FIELD MJ, STANTON BA, AND GIEBISCH GH. Influence of ADH on renal potassium handling: a micropuncture and microperfusion study. *Kidney Int* 25: 502–511, 1984.
288. FILBURN CR AND HARRISON S. Parathyroid hormone regulation of cytosolic Ca in rat proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F545–F552, 1990.
289. FILHOL O, CHAMBAZ EM, GILL GN, AND COCHET C. Epidermal growth factor stimulates a protein tyrosine kinase which is separable from epidermal growth factor receptor. *J Biol Chem* 268: 26978–26982, 1993.
290. FIMOGNARI GM, FANESTIL DD, AND EDELMAN IS. Induction of RNA and protein synthesis in the action of aldosterone in the rat. *Am J Physiol* 213: 954–962, 1967.
291. FIRSOV D, AARAB L, MANDON B, SIAUME-PEREZ S, DE ROUFFIGNAC C, AND CHABARDÈS D. Arachidonic acid inhibits hormone-stimulated cAMP accumulation in the medullary thick ascending limb of the rat kidney by a mechanism sensitive to pertussis toxin. *Pflügers Arch* 429: 636–646, 1995.
292. FIRSOV D, GAUTSCHI I, MERILLAT AM, ROSSIER BC, AND SCHILD L. The heterotetrameric architecture of the epithelial sodium channel (ENaC). *EMBO J* 17: 344–352, 1998.
293. FIRSOV D, MANDON B, MOREL A, MÉROT J, LE MAOUT S, BELLANGER AC, DE ROUFFIGNAC M, ELALOUF C, AND BUHLER M. Molecular analysis of vasopressin receptors in the rat nephron. Evidence for alternative splicing of the V_2 receptor. *Pflügers Arch* 429: 79–89, 1994.
294. FISONE G, CHENG SXJ, NAIRN AC, CZERNIK AJ, HEMMINGS HC JR, HÖÖG JO, BERTORELLO AM, KAISER R, BERGMAN T, JÖRNVALL H, APERIA A, AND GREENGARD P. Identification of the phosphorylation site for cAMP-dependent protein kinase on Na^+,K^+-ATPase and effects of site-directed mutagenesis. *J Biol Chem* 269: 9368–9373, 1994.
295. FISONE G, SNYDER GL, FRYCKSTEDT J, CAPLAN MJ, APERIA A, AND GREENGARD P. Na^+,K^+-ATPase in the choroid plexus. Regulation by serotonin/protein kinase C pathway. *J Biol Chem* 270: 2427–2430, 1995.
296. FORBUSH B III, KAPLAN JH, AND HOFFMAN JF. Characterization of a new photoaffinity derivative of ouabain: labeling of the large polypeptide and of a proteolipid component of the Na,K-ATPase. *Biochemistry* 17: 3667–3676, 1978.
297. FRANKE TF, YANG SI, CHAN TO, DATTA K, KAZLAUSKAS A, MORRISON DK, KAPLAN DR, AND TSICHLIS PN. The protein kinase encoded by the akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* 81: 727–736, 1995.
298. FREIBERG JM, KINSELLA J, AND SACKTOR B. Glucocorticoids increase the Na^+-H^+ gradient-dependent phosphate-uptake systems in renal brush border membrane vesicles. *Proc Natl Acad Sci USA* 79: 4932–4936, 1982.
299. FRIEDLANDER G. Regulation of renal phosphate handling: recent findings. *Curr Opin Nephrol Hypertens* 5: 316–320, 1996.
300. FRIEDLANDER G AND AMIEL C. Cellular mode of action of parathyroid hormone. *Adv Nephrol Necker Hosp* 23: 265–279, 1994.
301. FRIELLE T, COLLINS S, DANIEL KW, CARON MG, LEFKOWITZ RJ, AND KOBILKA BK. Cloning of the cDNA for the human β_1-adrenergic receptor. *Proc Natl Acad Sci USA* 84: 7920–7924, 1987.
302. FRINDT G AND BURG MB. Effect of vasopressin on sodium transport in renal cortical collecting tubules. *Kidney Int* 1: 224–231, 1972.
303. FRINDT G AND PALMER LG. Ca-activated K channels in apical membrane of mammalian CCT, and their role in K secretion. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F458–F467, 1987.
304. FRINDT G AND PALMER LG. Low-conductance K channels in apical membrane of rat cortical collecting tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 256: F143–F151, 1989.
305. FRINDT G AND PALMER LG. Regulation of Na channels in the rat cortical collecting tubule: effects of cAMP and methyl donors. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F1086–F1092, 1996.
306. FRINDT G, SACKIN H, AND PALMER LG. Whole-cell currents in rat cortical collecting tubule: low-Na diet increases amiloride-sensitive conductance. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F562–F567, 1990.
307. FRINDT G, SILVER RB, WINDHAGER EE, AND PALMER LG. Feedback regulation of Na channels in rat CCT. II. Effects of inhibition of Na entry. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F565–F574, 1993.
308. FRINDT G, SILVER RB, WINDHAGER EE, AND PALMER LG. Feedback regulation of Na channels in rat CCT. III. Response to cAMP. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F480–F489, 1995.
309. FRÖMTER E. Solute transport across epithelia: what we can learn from micropuncture studies on kidney tubules. *J Physiol (Lond)* 288: 1–31, 1979.
310. FRYCKSTEDT J, APERIA A, SNYDER G, AND MEISTER B. Distribution of dopamine- and cAMP-dependent phosphoprotein (DARPP-32) in the developing and mature kidney. *Kidney Int* 44: 495–502, 1993.
311. FUJIMORI A, MIYAUCHI A, HRUSKA KA, MARTIN KJ, AVIOLI LV, AND CIVITELLI R. Desensitization of calcium messenger system in parathyroid hormone-stimulated opossum kidney cells. *Am J Physiol Endocrinol Metab* 264: E918–E924, 1993.
312. FULLER PJ. The steroid receptor superfamily: mechanisms of diversity. *FASEB J* 5: 3092–3099, 1991.
313. FUNDER JW. Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. *Annu Rev Med* 48: 231–240, 1997.
314. FUNDER JW, FELDMAN D, AND EDELMAN IS. Specific aldosterone binding in rat kidney and parotid. *J Steroid Biochem* 3: 209–215, 1972.

315. FUNDER JW, PEARCE PT, SMITH R, AND SMITH AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 242: 583–585, 1988.
316. FURUYA H, TABEI K, MUTO S, AND ASANO Y. Effect of insulin on potassium secretion in rabbit cortical collecting ducts. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F30–F35, 1992.
317. FYFE GK, QUINN A, AND CANESSA CM. Structure and function of the Mec-ENaC family of ion channels. *Semin Nephrol* 18: 138–151, 1998.
318. GADSBY DC AND NAKAO M. Steady-state current-voltage relationship of the Na/K pump in guinea-pig ventricular myocytes. *J Gen Physiol* 94: 511–537, 1989.
319. GAMBA G, MIYANOSHITA A, LOMBARDI M, LYTTON J, LEE WS, HEDIGER MA, AND HEBERT SC. Molecular cloning, primary structure, and characterization of two members of the mammalian electroneutral Na-K-Cl cotransporter family expressed in kidney. *J Biol Chem* 269: 17713–17722, 1994.
320. GAMM DM, BAUDE EJ, AND UHLER MD. The major catalytic subunit isoforms of cAMP-dependent protein kinase have distinct biochemical properties in vitro and in vivo. *J Biol Chem* 271: 15736–15742, 1996.
321. GAO DQ, CANESSA LM, MOURADIAN MM, AND JOSE PA. Expression of the D_2 subfamily of dopamine receptor genes in kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F646–F650, 1994.
322. GAO B AND GILMAN AG. Cloning and expression of a widely distributed (type IV) adenylyl cyclase. *Proc Natl Acad Sci USA* 88: 10178–10182, 1991.
323. GARG LC, KNEPPER MA, AND BURG MB. Mineralocorticoid effects on Na-K-ATPase in individual nephron segments. *Am J Physiol Renal Fluid Electrolyte Physiol* 240: F536–F544, 1981.
324. GARTY H AND EDELMAN IS. Amiloride-sensitive trypsinization of apical sodium channels. Analysis of hormonal regulation of sodium transport in toad bladder. *J Gen Physiol* 81: 785–803, 1983.
325. GARTY H AND PALMER LG. Epithelial sodium channels: function, structure, and regulation. *Physiol Rev* 77: 359–396, 1997.
326. GARVIN JL. Angiotensin stimulates glucose and fluid absorption by rat proximal straight tubules. *J Am Soc Nephrol* 1: 272–277, 1990.
327. GARVIN JL. Angiotensin stimulates bicarbonate transport and Na^+/K^+ ATPase in rat proximal straight tubule. *J Am Soc Nephrol* 1: 1146–1152, 1991.
328. GEERING K, BEGGAH A, GOOD P, GIRARDET S, ROY S, SCHAER D, AND JAUNIN P. Oligomerization and maturation of Na,K-ATPase: functional interaction of the cytoplasmic NH_2 terminus of the β subunit with the α subunit. *J Cell Biol* 133: 1193–1204, 1996.
329. GEERING K, GIRARDET M, BRON C, KRAEHENBÜHL JP, AND ROSSIER BC. Hormonal regulation of (Na^+,K^+)-ATPase biosynthesis in the toad bladder. Effect of aldosterone and 3,5,3′-triiodo-L-thyronine. *J Biol Chem* 257: 10338–10343, 1982.
330. GEERING K, THEULAZ I, VERREY F, HAÜPTLE MT, AND ROSSIER BC. A role for the β-subunit in the expression of functional Na-K-ATPase in *Xenopus* oocytes. *Am J Physiol Cell Physiol* 257: C851–C858, 1989.
331. GEIBEL J, GIEBISCH G, AND BORON WF. Angiotensin II stimulates both Na-H exchange and Na/HCO_3 cotransport in the rabbit proximal tubule. *Proc Natl Acad Sci USA* 87: 7917–7920, 1990.
332. GESEK FA. α_2-Adrenergic receptors activate phospholipase C in renal epithelial cells. *Mol Pharmacol* 50: 407–414, 1996.
333. GESEK FA, CRAGOE EJ JR, AND STRANDHOY JW. Synergistic α_1 and α_2 adrenergic stimulation of rat proximal nephron Na^+/H^+ exchange. *J Pharmacol Exp Ther* 249: 694–700, 1989.
334. GESEK FA AND SCHOOLWERTH AC. Effects of α-adrenergic agonists on intracellular and intramitochondrial pH in rat proximal nephrons. *Am J Physiol Renal Fluid Electrolyte Physiol* 257: F623–F630, 1989.
335. GESEK FA AND SCHOOLWERTH AC. Hormonal interactions with the proximal Na-H exchanger. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F514–F521, 1990.
336. GESEK FA AND SCHOOLWERTH AC. Insulin increases Na^+-H^+ exchange activity in proximal tubules from normotensive and hypertensive rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F695–F703, 1991.
337. GIESEN EM, IMBS JL, GRIMA M, SCHMIDT M, AND SCHWARTZ J. Modulation of renal ATPase activities by cAMP. *Biochem Biophys Res Commun* 120: 619–624, 1984.
338. GILLEN CM, BRILL S, PAYNE JA, AND FORBUSH B III. Molecular cloning and functional expression of the K-Cl cotransporter from rabbit, rat, and human. A new member of the cation-chloride cotransporter family. *J Biol Chem* 271: 16237–16244, 1996.
339. GONZALEZ CB, FIGUEROA CD, REYES CE, CAORSI CE, TRONCOSO S, AND MENZEL D. Immunolocalization of V_1 vasopressin receptors in the rat kidney using anti-receptor antibodies. *Kidney Int* 52: 1206–1215, 1997.
340. GOPALAKRISHNAN SM, CHEN C, AND LOKHANDWALA MF. α_1-Adrenoreceptor subtypes mediating stimulation of Na^+,K^+-ATPase activity in rat renal proximal tubules. *Eur J Pharmacol* 288: 139–147, 1995.
341. GREEN HH, HARRINGTON AR, AND VALTIN H. On the role of antidiuretic hormone in the inhibition of acute water diuresis in adrenal insufficiency and the effects of gluco- and mineralocorticoids in reversing the inhibition. *J Clin Invest* 49: 1724–1736, 1970.
342. GREENGARD P, ALLEN PB, AND NAIRN AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23: 435–447, 1999.
343. GREGER R. Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiol Rev* 65: 760–797, 1985.
344. GREGER R AND SCHLATTER E. Properties of the basolateral membrane of the cortical thick ascending limb of Henle's loop of rabbit kidney. A model for secondary active chloride transport. *Pflügers Arch* 396: 325–334, 1983.
345. GRENADER A AND HEALY DP. Locally formed dopamine stimulates cAMP accumulation in LLC-PK_1 cells via a DA_1 dopamine receptor. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F906–F912, 1991.
346. GRIDER JS, FALCONE JC, KILPATRICK EL, OTT CE, AND JACKSON BA. Effect of bradykinin on NaCl transport in the medullary thick ascending limb of the rat. *Pflügers Arch* 287: 101–104, 1995.
347. GRIDER JS, FALCONE JC, KILPATRICK EL, OTT CE, AND JACKSON BA. *P*450 arachidonate metabolites mediate bradykinin-dependent inhibition of NaCl transport in the rat thick ascending limb. *Can J Physiol Pharmacol* 75: 91–96, 1997.
348. GRIDER J, KILPATRICK E, OTT C, AND JACKSON B. Effect of dopamine on NaCl transport in the medullary thick ascending limb of the rat. *Eur J Pharmacol* 342: 281–284, 1998.
349. GRIFFITHS NM, BRICK-HANNAM C, SIAUME-PEREZ S, AND CHABARDES D. Effect of prostaglandin E_2 on agonist-stimulated cAMP accumulation in the distal convoluted tubule isolated from the rabbit kidney. *Pflügers Arch* 422: 577–584, 1993.
350. GROSS JB, IMAI M, AND KOKKO JP. A functional comparison of the cortical collecting tubule and the distal convoluted tubule. *J Clin Invest* 55: 1284–1294, 1975.
351. GROSS JB AND KOKKO JP. Effects of aldosterone and potassium sparing diuretics on electrical potential difference across the distal nephron. *J Clin Invest* 59: 82–89, 1977.
352. GUINAMARD R, CHRAIBI A, AND TEULON J. A small-conductance Cl^- channel in the mouse thick ascending limb that is activated by ATP and protein kinase A. *J Physiol (Lond)* 485: 97–112, 1995.
353. GUINNEBAULT M AND MOREL F. Rôle de la surrénale dans les mécanismes de concentration de l'urine. *C R Soc Biol* 244: 2741–2745, 1957.
354. GUO J, LIU BY, AND BRINGHURST FR. Mechanisms of homologous and heterologous desensitization of PTH/PTHrP receptor signaling in LLC-PK_1 cells. *Am J Physiol Endocrinol Metab* 273: E383–E393, 1997.
355. GUPTA S, PHIPPS K, AND RUDERMAN NB. Differential stimulation of Na^+ pump activity by insulin and nitric oxide in rabbit aorta. *Am J Physiol Heart Circ Physiol* 270: H1287–H1293, 1996.
356. GUPTA S, RUDERMAN NB, CRAGOE EJ, AND SUSSMAN I. Endothelin stimulates Na-K-ATPase activity by a protein kinase C-dependent pathway in rabbit aorta. *Am J Physiol Heart Circ Physiol* 261: H38–H45, 1991.
357. GUSTAFSON TA, HE W, CRAPARO A, SCHAUB CD, AND O'NEILL TJ. Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 via a novel non-SH2 domain. *Mol Cell Biol* 15: 2500–2508, 1995.

358. HABERMANN E. Palytoxin acts through Na^+,K^+-ATPase. *Toxicon* 27: 1171–1187, 1989.
359. HAGEGE J AND RICHET G. Proximal tubule dopamine histofluorescence in renal slices incubated with L-dopa. *Kidney Int* 27: 3–8, 1985.
360. HALL DA AND VARNEY DM. Effect of vasopressin on electrical potential difference and chloride transport in mouse medullary thick ascending limb of Henle. *J Clin Invest* 66: 792–802, 1980.
361. HALL RA, PREMONT RT, CHOW CW, BLITZER JT, PITCHER JA, CLAING A, STOFFEL RH, BARAK LS, SHELIKONAR S, WEINMAN EJ, GRINSTEIN S, AND LEFKOWITZ RJ. The β_2-adrenergic receptor interacts with the Na^+/H^+ exchanger regulatory factor to control Na^+/H^+ exchange. *Nature* 382: 626–630, 1998.
362. HAM-ALVAREZ SF AND SHEETZ MP. Microtubule-dependent vesicle transport: modulation of channel and transporter activity in liver and kidney. *Physiol Rev* 78: 1109–1129, 1998.
363. HAMBURGER RJ, LAWSON NL, AND SCHWARTZ JH. Response to parathyroid hormone in defined segments of proximal tubule. *Am J Physiol* 230: 286–290, 1976.
364. HAMILTON KL AND EATON DC. cAMP-induced potassium channel activity in apical membrane of cultured A6 kidney cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 261: F1055–F1062, 1991.
365. HANSON AS AND LINAS SL. β-Adrenergic receptor function in rat proximal tubule epithelial cells in culture. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F553–F560, 1995.
366. HARRIS PJ. Stimulation of proximal tubular sodium reabsorption by Ile5 angiotensin II in the rat kidney. *Pflügers Arch* 381: 83–85, 1979.
367. HARRIS PJ AND YOUNG JA. Dose-dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pflügers Arch* 367: 295–297, 1977.
368. HARRISON-BERNARD LM, NAVAR LG, HO MM, VINSO GP, AND EL-DAHR SS. Immunohistochemical localization of ANG II AT_1 receptor in adult rat kidney using a monoclonal antibody. *Am J Physiol Renal Physiol* 273: F170–F177, 1997.
369. HARVEY BJ AND EHRENFELD J. Role of Na^+/H^+ exchange in the control of intracellular pH and cell membrane conductance in frog skin epithelium. *J Gen Physiol* 92: 793–810, 1988.
370. HARWALKAR S, CHANG CH, DULIN NO, AND DOUGLAS JG. Role of phospholipase A_2 isozymes in agonist-mediated signaling in proximal tubular epithelium. *Hypertension* 31: 809–814: 1998.
371. HAWK CT, KUDO LH, ROUCH AJ, AND SCHAFER JA. Inhibition by epinephrine of AVP- and cAMP-stimulated Na and water transport in Dahl rat CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F449–F460, 1993.
372. HAWK CT, LI L, AND SCHAFER JA. AVP and aldosterone at physiological concentrations have synergistic effects on Na^+ transport in rat CCD. *Kidney Int* 50: S35–S41, 1996.
373. HAWK CT AND SCHAFER JA. Clonidine, but not bradykinin or ANP, inhibits Na^+ and water transport in Dahl SS rat CCD. *Kidney Int* 44: 30–35, 1993.
374. HAYASHI M, YAMAJI Y, IYORI M, KITAJIMA W, AND SARUTA T. Effect of isoproterenol on intracellular pH of the intercalated cells in the rabbit cortical collecting ducts. *J Clin Invest* 87: 1153–1157, 1991.
375. HAYHURST RA AND O'NEIL R. Time-dependent actions of aldosterone and amiloride on Na^+-K^+-ATPase of cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 254: F689–F696, 1988.
376. HEASLEY LE, SENKFOR SI, WINITZ S, STRASHEIM A, TEITELBAUM I, AND BERL T. Hormonal regulation of MAP kinase in cultured rat inner medullary collecting tubule cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F366–F373, 1994.
377. HEBERT SC AND ANDREOLI TE. Effects of antidiuretic hormone on cellular conductive pathways in mouse medullary thick ascending limbs of Henle. II. Determinants of the ADH-mediated increases in transepithelial voltage and in net Cl^- absorption. *J Membr Biol* 80: 221–233, 1984.
378. HÉBERT RL, JACOBSON HR, FREDIN D, AND BREYER MD. Evidence that separate PGE_2 receptors modulate water and sodium transport in rabbit cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F643–F650, 1993.
379. HEBERT SC, CULPEPPER RM, AND ANDREOLI TE. NaCl transport in mouse medullary thick ascending limb of Henle. I. Functional nephron heterogeneity and ADH-stimulated NaCl cotransport. *Am J Physiol Renal Fluid Electrolyte Physiol* 241: F412–F431, 1981.
380. HEBERT SC, CULPEPPER RM, AND ANDREOLI TE. NaCl transport in the mouse thick ascending limbs. III. Modulation of the ADH effect by peritubular osmolarity. *Am J Physiol Renal Fluid Electrolyte Physiol* 241: F443–F451, 1981.
381. HEIN L, BARSH GS, PRATT RE, DZAU VJ, AND KOBILKA BK. Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice. *Nature* 377: 744–747, 1995.
382. HELDIN CH. Dimerization of cell surface receptors in signal transduction. *Cell* 80: 213–223, 1995.
383. HELMLE-KOLB C, MONTROSE MH, AND MURER H. Parathyroid hormone regulation of Na^+/H^+ exchange in opossum kidney cells: polarity and mechanisms. *Pflügers Arch* 416: 615–623, 1990.
384. HELMLE-KOLB C, MONTROSE MH, STANGE G, AND MURER H. Regulation of Na/H exchange in opossum kidney cells by parathyroid hormone, cyclic AMP and phorbol esters. *Pflügers Arch* 415: 461–470, 1990.
385. HENDRY LB. Stereochemical complementary of DNA and steroid agonists and antagonists. *J Steroid Biochem* 31: 493–523, 1988.
386. HENSLEY CB, BRADLEY ME, AND MIRCHEFF AK. Parathyroid hormone-induced translocation of Na-H antiporters in rat proximal tubules. *Am J Physiol Cell Physiol* 257: C637–C645, 1989.
387. HIERHOLZER K, WIEDERHOLT M, HOLZGREVE H, GIEBISCH G, KLOSE RM, AND WINDHAGER EE. Micropuncture study of renal transtubular concentration gradients of sodium and potassium in adrenalectomized rats. *Pflügers Arch* 285: 193–210, 1965.
388. HILPERT J, NYKJAER A, JACOBSEN C, WALLUKAT G, NIELSEN R, MOESTRUP SK, HALLER H, LUFT FC, CHRISTENSEN EI, AND WILLNOW TE. Megalin antagonizes activation of the parathyroid hormone receptor. *J Biol Chem* 274: 5620–5625, 1999.
389. HO K, NICHOLS CG, LEDERER WJ, LYTTON J, VASSILEV PM, KANAZIRSKA MV, AND HEBERT SC. Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. *Nature* 362: 31–38, 1993.
390. HOLLENBERG SM, WEINBERGER C, ONG ES, CERELLI G, ORO A, LEBO R, THOMPSON EB, ROSENFELD MG, AND EVANS RM. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* 318: 635–641, 1985.
391. HOLT WF AND LECHENE C. Adh-PGE_2 interactions in cortical collecting tubule. I. Depression of sodium transport. *Am J Physiol Renal Fluid Electrolyte Physiol* 241: F452–F460, 1981.
392. HOOGERWERF WA, TSAO SC, DEVUYST O, LEVINE SA, YUN CH, YIP JW, COHEN ME, WILSONA PD. LAZENBY J, TSE CM, AND DONOWITZ M. NHE_2 and NHE_3 are human and rabbit intestinal brush-border proteins. *Am J Physiol Gastrointest Liver Physiol* 270: G29–G41, 1996.
393. HOOTMAN SR, BROWN ME, AND WILLIAMS JA. Phorbol esters and A23187 regulate Na-K pump activity in pancreatic acinar cells. *Am J Physiol Gastrointest Liver Physiol* 252: G499–G505, 1987.
394. HORISBERGER JD AND DIEZI J. Effects of mineralocorticoids on Na^+ and K^+ excretion in the adrenalectomized rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 254: F89–F99, 1983.
395. HORISBERGER JD AND DIEZI J. Inhibition of aldosterone-induced antinatriuresis and kaliuresis by actinomycin D. *Am J Physiol Renal Fluid Electrolyte Physiol* 246: F201–F204, 1984.
396. HORISBERGER JD AND DOUCET A. Renal ion-translocating ATPases: the P-type family. In: *The Kidney, Physiology and Pathophysiology*, edited by Seldin DW and Giebisch G. New York: Raven. In press.
397. HORISBERGER JD AND GIEBISCH G. Na-K pump current in the *Amphiuma* collecting duct. *J Gen Physiol* 94: 493–510, 1984.
398. HOUILLIER P, CHAMBREY R, ACHARD JM, FROISSARD M, POGGIOLI J, AND PAILLARD M. Signaling pathways in the biphasic effect of angiotensin II on apical Na/H antiport activity in proximal tubule. *Kidney Int* 50: 1496–1505, 1996.
399. HOWELL DS AND DAVIS JO. Relationship of sodium retention to potassium excretion by the kidney during administration of desoxycorticosterone acetate to dogs. *Am J Physiol* 179: 359–363, 1954.
400. HRUSKA KA, GOLIGORSKY M, SCOBLE J, TSUTSUMI M, WESTBROOK S, AND MOSKOWITZ D. Effects of parathyroid hormone on cytosolic calcium in renal proximal tubule primary cultures. *Am J Physiol Renal Fluid Electrolyte Physiol* 251: F188–F198, 1986.
401. HRUSKA KA, MOSKOWITZ D, ESBRIT P, CIVITELLI R, WESTBROOK S, AND

HUSKEY M. Stimulation of inositol triphosphate and diacylglycerol production in renal tubular cells by parathyroid hormone. *J Clin Invest* 79: 230–239, 1987.
402. HUANG XC, RICHARDS EM, AND SUMNERS C. Mitogen-activated protein kinases in rat brain neuronal cultures are activated by angiotensin II type 1 receptors and inhibited by angiotensin II type 2 receptors. *J Biol Chem* 271: 15635–15641, 1996.
403. HUANG L, WEI YY, MOMOSE-HOTOKEZAKA A, DICKEY J, AND OKUSA MD. A_{2b}-adrenergic receptors: immunolocalization and regulation by potassium depletion in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F1015–F1026, 1996.
404. HULTER HN, LICHT JH, BONNER EL JR, GLYNN RD, AND SEBASTIAN A. Effects of glucocorticoid steroids on renal and systemic acid-base metabolism. *Am J Physiol Renal Fluid Electrolyte Physiol* 239: F30–F43, 1980.
405. HULTER HN, SIGALA JF, AND SEBASTIAN A. Effects of dexamethasone on renal and systemic acid-base metabolism. *Kidney Int* 20: 43–49, 1981.
406. HUO T AND HEALY DP. Autoradiographic localization of dopamine DA_1 receptors in rat kidney with [^{3}H]Sch 23390. *Am J Physiol Renal Fluid Electrolyte Physiol* 257: F414–F423, 1989.
407. HUSSAIN T, ABDUL-WAHAB R, AND LOKHANDWALA MF. Bromocriptine stimulates Na^+,K^+-ATPase in renal proximal tubules via the cAMP pathway. *Eur J Pharmacol* 321: 259–263, 1997.
408. HUSSAIN T AND LOKHANDWALA MF. Renal dopamine DA_1 receptor coupling with G_s and $G_{q/11}$ proteins in spontaneously hypertensive rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F339–F346, 1997.
409. HUSSAIN T AND LOCKHANDWALA MF. Renal dopamine receptor function in hypertension. *Hypertension* 32: 187–197, 1998.
410. HUSTED RF, LAPLACE JR, AND STOKES JB. Enhancement of electrogenic Na^+ transport across rat inner medullary collecting duct by glucocorticoid and mineralocorticoid hormones. *J Clin Invest* 86: 498–506, 1990.
411. IBARRA F, APERIA A, SVENSSON LB, EKLÖF AC, AND GREENGARD P. Bidirectional regulation of Na,K-ATPase activity by dopamine and an α-adrenergic agonist. *Proc Natl Acad Sci USA* 90: 21–24, 1993.
412. IINO Y. Effect of parathyroid hormone on bicarbonate absorption by proximal tubules in vitro. *Am J Physiol Renal Fluid Electrolyte Physiol* 236: F387–F391, 1979.
413. IINO Y AND IMAI M. Effects of prostaglandins on Na transport in isolated collecting tubules. *Pflügers Arch* 373: 125–132, 1978.
414. IKEDA M, YOSHITOMI K, IMAI M, AND KUROKAWA K. Cell Ca^{2+} response to luminal vasopressin in cortical collecting tubule principal cells. *Kidney Int* 45: 811–816, 1994.
415. IMAI M. The connecting tubule: a functional subdivision of the rabbit distal segments. *Kidney Int* 15: 346–356, 1979.
416. IMBERT M, CHABARDÈS D, MONTÉGUT M, CLIQUE A, AND MOREL F. Adenylate cyclase activity along the rabbit nephron as measured in single isolated segments. *Pflügers Arch* 354: 213–228, 1975.
417. IMBERT M, CHABARDÈS D, MONTÉGUT M, CLIQUE A, AND MOREL F. Vasopressin dependent adenylate cyclase in single segments of rabbit kidney tubule. *Pflügers Arch* 357: 173–186, 1975.
418. IMBERT-TEBOUL M, CHABARDÈS D, MONTÉGUT M, CLIQUE A, AND MOREL F. Vasopressin-dependent adenylate cyclase activities in the rat kidney medulla: evidence for two separate sites of action. *Endocrinology* 102: 1254–1261, 1978.
419. IMBERT-TEBOUL M, SIAUME-PEREZ S, AND MOREL F. Sites of prostaglandin E_2 (PGE_2) synthesis along the rabbit nephron. *Mol Cell Endocrinol* 45: 1–10, 1986.
420. INGELFINGER JR, ZUO WM, FON EA, ELLISON KE, AND DZAU VJ. In situ hybridization evidence for angiotensinogen messenger RNA in the rat proximal tubule. An hypothesis for the intrarenal renin angiotensin system. *J Clin Invest* 85: 417–423, 1990.
421. ISHIDA M, ISHIDA T, THOMAS SM, AND BERK BC. Activation of extracellular signal-regulated kinases (ERK1/2) by angiotensin II is dependent on c-Src in vascular smooth muscle cells. *Circ Res* 82: 7–12, 1998.
422. ISHIKAWA SE, OKADA K, AND SAITO T. Arginine vasopressin increases cellular free calcium concentration and adenosine 3′,5′-monophosphate production in rat renal papillary collecting tubule cells in culture. *Endocrinology* 123: 1376–1384, 1988.
423. ITO O, KONDO Y, OBA N, TAKAHASHI M, OMATA K, AND ABE K. Tyrosine kinase, phosphatidylinositol 3-kinase, and protein kinase C regulate insulin-stimulated NaCl absorption in the thick ascending limb. *Kidney Int* 51: 1037–1041, 1997.
424. ITO O, KONDO Y, TAKAHASHI N, KUDO K, IGARASHI Y, OMATA K, IMAI Y, AND ABE K. Insulin stimulates NaCl transport in isolated perfused MTAL of Henle's loop of rabbit kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F265–F270, 1994.
425. ITO O, KONDO Y, TAKAHASHI N, OMATA K, AND ABE K. Role of calcium in insulin-stimulated NaCl transport in medullary thick ascending limb. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F236–F241, 1995.
426. ITO M, OLIVERIO MI, MANNON PJ, BEST CF, MAEDA N, SMITHIES O, AND COFFMAN TM. Regulation of blood pressure by the type 1A angiotensin II receptor gene. *Proc Natl Acad Sci USA* 92: 3521–3525, 1995.
427. JABS K, ZEIDEL ML, AND SILVA P. Prostaglandin E_2 inhibits Na^+-K^+-ATPase activity in the inner medullary collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 257: F424–F430, 1989.
428. JACOBS L AND DOUGLAS JG. Angiotensin II type 2 receptor subtype mediates phospholipase A_2-dependent signaling in rabbit proximal tubular epithelial cells. *Hypertension* 28: 663–668, 1996.
429. JACOBS WR AND CHAN YL. Evidence for the presence of functional β-adrenoceptor along the proximal tubule of the rat kidney. *Biochem Biophys Res Commun* 141: 334–339, 1986.
430. JADHAV AL AND LIU Q. DA_1 receptor mediated regulation of Na^+-H^+ antiport activity in rat renal cortical brush border membrane vesicles. *Clin Exp Hypertens* 14: 653–666, 1992.
431. JAISSER F AND BEGGAH AT. The nongastric H^+-K^+-ATPases: molecular and functional properties. *Am J Physiol Renal Physiol* 276: F812–F824, 1999.
432. JAISSER F, BLOT-CHABAUD M, PRADELLES P, BONVALET P, AND FARMAN N. Antidiuretic hormone reduces the high PGE_2 synthesis in papillary collecting ducts of DI rats. *Pflügers Arch* 414: 464–468, 1989.
433. JAISSER F, BUGEON L, BLOT-CHABAUD M, BONVALET JP, AND FARMAN N. Effects of AVP and dDAVP on PGE_2 synthesis in superfused cortical collecting tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 256: F1044–F1050, 1989.
434. JANUARY B, SEIBOLD A, WHALEY B, HIPKIN RW, LIN D, SCHONBRUNN A, BARBER R, AND CLARK RB. β_2-Adrenergic receptor desensitization, internalization, and phosphorylation in response to full and partial agonists. *J Biol Chem* 272: 23871–23879, 1997.
435. JENSEN RE AND BERNDT WO. Characterization of adrenergic receptors on proximal tubular basolateral membranes. *Life Sci* 43: 1473–1478, 1988.
436. JOHNSON JP, JONES D, AND WIESMANN WP. Hormonal regulation of Na^+-K^+-ATPase in cultured epithelial cells. *Am J Physiol Cell Physiol* 251: C186–C190, 1986.
437. JOLY M, KAZLAULAS A, FAY FS, AND CORVERA S. Disruption of PDGF receptor trafficking by mutation of its PI-3 kinase binding sites. *Science* 263: 684–687, 1994.
438. JORGENSEN PL. Isolation and characterization of the components of the sodium pump. *Q Rev Biophys* 7: 239–274, 1975.
439. JORGENSEN PL. Sodium and potassium ion pump in kidney tubules. *Physiol Rev* 60: 864–917, 1980.
440. JORGENSEN PL AND COLLINS JH. Tryptic and chemotryptic cleavage sites in sequence of α-subunit of (Na^++K^+)-ATPase from outer medulla of mammalian kidney. *Biochim Biophys Acta* 860: 570–576, 1986.
441. JOSE PA, EISNER GM, AND FELDER RA. Renal dopamine receptors in health and hypertension. *Pharmacol Ther* 80: 149–182, 1998.
442. JOVER B, SALADINI D, NAFRIALDI N, DUPONT M, AND MIMRAN A. Effect of losartan and enalapril on renal adaptation to sodium restriction in rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F281–F288, 1994.
443. JUNG KY AND ENDOU H. Biphasic increasing effect of angiotensin II on intracellular free calcium in isolated rat early proximal tubule. *Biochem Biophys Res Commun* 165: 1221–1228, 1989.
444. JUNG KY, UCHIDA S, AND ENDOU H. Nephrotoxicity assessment by measuring cellular ATP content. I. Substrate specificities in the maintenance of ATP content in isolated rat nephron segments. *Toxicol Appl Pharmacol* 100: 369–382, 1989.
445. KAHN AM, DOLSON GM, HISE MK, BENNETT SC, AND WEINMAN EJ. Parathyroid hormone and dibutyryl cAMP inhibit Na^+/H^+ ex-

change in renal brush border vesicles. *Am J Physiol Renal Fluid Electrolyte Physiol* 248: F212–F218, 1985.
446. KAMBAYASHI Y, BARDHAN S, TAKAHASHI K, TSUZUKI S, INUI H, HAMAKUBO T, AND INAGAMI T. Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition. *J Biol Chem* 268: 24543–24546, 1993.
447. KANDASAMY RA AND ORLOWSKI J. Genomic organization and glucocorticoid transcriptional activation of the rat Na^+/H^+ exchanger NHE3 gene. *J Biol Chem* 271: 10551–10559, 1996.
448. KANSRA V, CHEN C, AND LOKHANDWALA MF. Dopamine causes stimulation of protein kinase C in rat renal proximal tubules by activating dopamine D_1 receptors. *Eur J Pharmacol* 289: 391–394, 1995.
449. KAPLAN JH. Ion movements through the sodium pump. *Annu Rev Physiol* 47: 535–544, 1985.
450. KAPLAN MR, PLOTKIN MD, LEE WS, ZU ZC, LYTTON J, AND HEBERT SC. Apical localization of the Na-K-2Cl cotransporter, rBSC1, on rat thick ascending limbs. *Kidney Int* 49: 40–47, 1996.
451. KARIM Z, DEFONTAINE N, PAILLARD M, AND POGGIOLI J. Protein kinase C isoforms in rat kidney proximal tubule: acute effect of angiotensin II. *Am. J Physiol Cell Physiol* 269: C134–C140, 1995.
452. KASHISHIAN A, KAZLAUSKAS A, AND COOPER JA. Phosphorylation sites in the PDGF receptor with different specificities for binding GAP and PI3 kinase in vivo. *EMBO J* 11: 1373–1382, 1992.
453. KATADA T, KUSAKABE K, OINUMA M, AND UI M. A novel mechanism for the inhibition of adenylate cyclase via inhibitory GTP-binding proteins. Calmodulin-dependent inhibition of the cyclase catalyst by the $\beta\gamma$ subunits of GTP-binding proteins. *J Biol Chem* 262: 11897–11900, 1987.
454. KATZ AI, DOUCET A, AND MOREL F. Na,K-ATPase activity along the rabbit, rat and mouse nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 237: F114–F120, 1979.
455. KAVANAUGH MW AND WILLIAMS LT. An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. *Science* 266: 1862–1865, 1994.
456. KAWAKAMI K, NOJIMA H, OHTA T, AND NAGANO K. Molecular cloning and sequence analysis of human Na,K-ATPase β-subunit. *Nucleic Acids Res* 14: 2833–2844, 1986.
457. KAWASAKI H, SPRINGETT GM, MOCHIZUKI N, TOKI S, NAKAYA M, MATSUDA M, HOUSMAN DE, AND GRAYBIEL AM. A family of cAMP-binding proteins that directly activate Rap 1. *Science* 282: 2275–2279, 1998.
458. KAZLAUSKAS A. Receptor tyrosine kinases and their targets. *Curr Opin Genet Dev* 4: 5–14, 1994.
459. KEUSCH I, TRAEBERT M, LÖTSCHER M, KAISSLING B, MURER H, AND BIBER J. Parathyroid hormone and dietary phosphate provoke a lysosomal routing of the proximal tubular Na/P_i-cotransporter type II. *Kidney Int* 54: 1224–1232, 1998.
460. KIKERI D, SUN A, ZEIDEL ML, AND HEBERT SC. Cell membranes impermeable to NH_3. *Nature* 339: 478–480, 1989.
461. KIM GH, ECELBARGER CA, MITCHELL C, PACKER RK, WADE JB, AND KNEPPER MA. Vasopressin increases Na-K-2Cl cotransporter expression in thick asending limb of Henle's loop. *Am J Physiol Renal Physiol* 276: F96–F103, 1999.
462. KINOSHITA S, OHLSTEIN EH, AND FELDER RA. Dopamine-1 receptors in rat proximal convoluted tubule: regulation by intrarenal dopamine. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F1068–F1074, 1990.
463. KINSELLA JL. Action of glucocorticoids on proximal tubule transport systems. *Semin Nephrol* 10: 330–338, 1990.
464. KINSELLA J, CUJDIK T, AND SACKTOR B. Na^+-H^+ exchange activity in renal brush border membrane vesicles in response to metabolic acidosis: the role of glucocorticoids. *Proc Natl Acad Sci USA* 81: 630–634, 1984.
465. KINSELLA J, FREIBERG JM, AND SACKTOR B. Glucocorticoid activation of Na^+/H^+ exchange in renal brush border vesicles: kinetic effects. *Am J Physiol Renal Fluid Electrolyte Physiol* 248: F233–F239, 1985.
466. KIRCHNER KA. Insulin increases loop segment chloride reabsorption in the euglycemic rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 255: F1206–F1213, 1988.
467. KIROYTCHEVA M, CHEVAL L, CARRANZA ML, MARTIN PY, FAVRE H, DOUCET A, AND FÉRAILLE E. Effect of cAMP on the activity and the phosphorylation of Na^+,K^+-ATPase in rat thick ascending limb of Henle. *Kidney Int* 55: 1819–1831, 1999.
468. KIRSCHENBAUM MA, LOWE AG, TRIZNA W, AND FINE LG. Regulation of vasopressin action by prostaglandin synthesis in the rabbit cortical collecting tubule. *J Clin Invest* 70: 1193–1204, 1982.
469. KLAUCK TM, FAUX MC, LABUDDA K, LANGEBERG LK, JAKEN S, AND SCOTT JD. Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. *Science* 271: 1589–1592, 1996.
470. KLEYMAN TR, COUPAYE-GERARD B, AND ERNST SA. Aldosterone does not alter apical cell-surface expression of epithelial Na^+ channels in the amphibian cell line A6. *J Biol Chem* 267: 9622–9628, 1992.
471. KLEYMAN TR, CRAGOE EJ JR, AND KRAEHENBÜHL JP. The cellular pool of Na^+ channels in the amphibian cell line A6 is not altered by mineralocorticoids. Analysis using a new photoactive amiloride analog in combination with anti-amiloride antibodies. *J Biol Chem* 264: 11995–12000, 1989.
472. KLEYMAN TR, ERNST SA, AND COUPAYE-GERARD B. Arginine vasopressin and forskolin regulate apical cell surface expression of epithelial Na channels in A6 cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F506–F511, 1994.
473. KLINE RL AND MERCER PF. Contribution of renal nerves to the natriuretic and diuretic effect of α_2 adrenergic receptor activation. *J Pharmacol Exp Ther* 253: 266–271, 1990.
474. KLINGHOFFER RA, DUCKWORTH B, VALIUS M, CANTLEY L, AND KAZLAUSKAS A. Platelet-derived growth factor-dependent activation of phosphatidylinositol 3-kinase is regulated by receptor binding of SH2-domain-containing proteins which influence Ras activity. *Mol Cell Biol* 16: 5905–5914, 1996.
475. KOEFFOED-JOHNSON V AND USSING HH. The nature of frog skin potential. *Acta Physiol Scand* 43: 298–308, 1958.
476. KONE BC AND BAYLIS C. Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F561–F578, 1997.
477. KONISHI H, TANAKA M, TAKEMURA Y, MATSUSAKI H, ONO Y, KIKKAWA U, AND NISHIZUKA Y. Activation of protein kinase C by tyrosine phosphorylation in response to H_2O_2. *Proc Natl Acad Sci USA* 94: 11233–11237, 1997.
478. KROZOWSKI ZS, PROVENCHER PH, SMITH RE, OBEYESEKERE VR, MERCER WR, AND ALBISTON AL. Isozymes of 11β-hydroxysteroid dehydrogenase: which enzyme endows mineralocorticoid specificity. *Steroids* 59: 116–120, 1994.
479. KROZOWSKI ZS, RUNDLE SE, WALLACE C, CASTELL MJ, SHEN JH, DOWLING J, FUNDER JW, AND SMITH AI. Immunolocalization of renal mineralocorticoid receptors with an antiserum against a peptide deduced from the complementary deoxyribonucleic acid sequence. *Endocrinology* 125: 192–198, 1989.
480. KURASHIMA K, SZABO EZ, LUKACS G, ORLOWSKI J, AND GRINSTEIN S. Endosomal recycling of the Na^+/H^+ exchanger NHE3 isoform is regulated by the phosphatidylinositol 3-kinase pathway. *J Biol Chem* 273: 20828–20836, 1998.
481. KUROKAWA K, YOSHITOMI K, IKEDA M, UCHIDA S, NARUSE M, AND IMAI M. Regulation of cortical collecting duct function: effect of endothelin. *Am Heart J* 125: 582–588, 1993.
482. KUROKI DW, MINDEN A, SANCHEZ I, AND WATTENBERG EV. Regulation of a c-Jun amino-terminal kinase/stress-activated protein kinase cascade by a sodium-dependent signal transduction pathway. *J Biol Chem* 272: 23905–23911, 1997.
483. KÜSTER B, SHAINSKAYA A, MANN M, AND KARLISH SJD. Mass spectrometric analysis of the γ subunit of Na,K-ATPase. In: *Proceedings of the IXth International Conference on Na,K-ATPase.* Amsterdam: Elsevier. In press.
484. LAMPRECHT G, WEINMAN EJ, AND YUN HC. The role of NHERF and E3KARP in the cAMP-mediated inhibition of NHE3. *J Biol Chem* 273: 29972–29978, 1998.
485. LARSSON C AND ANGGARD E. Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur J Pharmacol* 21: 30–36, 1973.
486. LEA JP, SANDS JM, MCMAHON SJ, AND TUMLIN JA. Evidence that the inhibition of Na^+/K^+-ATPase activity by FK506 involves calcineurin. *Kidney Int* 46: 647–652, 1994.
487. LEAL T AND CRABBÉ J. Effects of aldosterone on ($Na^+ + K^+$)-ATPase of amphibian sodium-transporting epithelial cells (A6) in culture. *J Steroid Biochem* 34: 581–584, 1989.
488. LECHLEDER RJ, SUGIMOTO S, BENNETT AM, KASHISHIAN AS, COOPER JA, SHOELSON SE, WALSH CT, AND NEEL BG. Activation of SH2-containing

phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet derived growth factor receptor β. *J Biol Chem* 268: 21478–21481, 1993.
489. LECLAIRE MM, BERNDT TJ, AND KNOX FG. Effect of renal interstitial infusion of L-dopa on sodium and phosphate excretions. *J Lab Clin Med* 132: 308–312, 1998.
490. LEE K, BROWN D, URENA P, ARDAILLOU N, ARDAILLOU R, DEEDS J, AND SEGRE GV. Localization of parathyroid hormone/parathyroid hormone-related peptide receptor mRNA in kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F186–F191, 1996.
491. LEE SMK, CHEKAL MA, AND KATZ AI. Corticosterone binding sites along the rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 244: F504–F509, 1983.
492. LEICHTWEISS HP, LUBBERS DW, WEISS C, BAUMGARTL H, AND RESCHKE W. The oxygen supply of rat kidney: measurement of intrarenal Po_2. *Pflügers Arch* 309: 328–349, 1960.
493. LEVINE SA, MONTROSE MH, TSE CM, AND DONOWITZ M. Kinetics and regulation of three cloned mammalian Na/H exchangers stably expressed in a fibroblast cell line. *J Biol Chem* 268: 25527–25535, 1993.
494. LEW R AND SUMMERS RJ. Autoradiographic localization of β-adrenoceptor subtypes in guinea-pig kidney. *Br J Pharmacol* 85: 341–348, 1985.
495. LI D, APERIA A, CELSI G, DA CRUZ E SILVA EF, GREENGARD P, AND MEISTER B. Protein phosphatase-1 in the kidney: evidence for a role in the regulation of medullary Na^+-K^+-ATPase. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F673–F680, 1995.
496. LI D, CHENG SXJ, FISONE G, CAPLAN MJ, OHTOMO Y, AND APERIA A. Effects of okadaic acid, calyculin A, and PDBu on state of phosphorylation of rat renal Na^+-K^+-ATPase. *Am J Physiol Renal Physiol* 275: F863–F869, 1998.
497. LI L AND SCHAFER JA. Dopamine inhibits vasopressin-dependent cAMP production in the rat cortical collecting duct. *Am J Physiol Renal Physiol* 275: F62–F67, 1998.
498. LI L, WANG YP, CAPPARELLI AW, JO OD, AND YANAGAWA N. Effect of luminal angiotensin II on proximal tubule fluid transport: role of apical phospholipase A_2. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F202–F209, 1994.
499. LI RY, GAITS F, RAGAB A, RAGAB-THOMAS JMF, AND CHAP H. Tyrosine phosphorylation of an SH2-containing protein tyrosine phosphatase is coupled to platelet thrombin receptor via a pertussis toxin-sensitive heterotrimeric G-protein. *EMBO J* 14: 2519–2526, 1995.
500. LIANG H, VENEMA VJ, WANG X, JU H, VENEMA RC, AND MARRERO MB. Regulation of angiotensin II-induced phosphorylation of STAT3 in vascular smooth muscle cells. *J Biol Chem* 274: 19846–19851, 1999.
501. LIAO DF, MONIA B, DEAN N, AND BERK BC. Protein kinase C-ζ mediates angiotensin II activation of ERK1/2 in vascular smooth muscle cells. *J Biol Chem* 272: 6146–6150, 1997.
502. LIAPIS H, NAG M, AND KAJI DM. K-Cl cotransporter expression in the human kidney. *Am J Physiol Cell Physiol* 275: C1432–C1437, 1998.
503. LICHTENSTEIN NS AND LEAF A. Effect of amphotericin B on the permeability of the toad bladder. *J Clin Invest* 44: 1328–1342, 1965.
504. LIFSCHITZ MD, SCHRIER RW, AND EDELMAN IS. Effect of actinomycin D on aldosterone mediated changes in electrolyte excretion. *Am J Physiol* 224: 376–380, 1973.
505. LIM YP, LOW BC, LIM J, WONG ESM, AND GUY GR. Association of atypical protein kinase C isotypes with the docker protein FRS2 in fibroblast growth factor signaling. *J Biol Chem* 274: 19025–19034, 1999.
506. LINGUEGLIA E, VOILLEY N, WALDMANN R, LAZDUNSKI M, AND BARBRY P. Expression cloning of an epithelial amiloride-sensitive Na^+ channel. A new channel type with homologies to *Caenorhabditis elegans* degenerins. *FEBS Lett* 318: 95–99, 1993.
506a. LIU F, NESBITT T, DREZNER MK, FRIEDMAN PA, AND GESEK FA. Proximal nephron Na^+/H^+ exchange is regulated by α_{1A}- and α_{1B}-adrenergic receptor subtypes. *Mol Pharmacol* 52: 1010–1018, 1997.
507. LIU FY AND COGAN MG. Angiotensin II: a potent regulator of acidification in the rat early proximal convoluted tubule. *J Clin Invest* 80: 272–275, 1987.
508. LIU FY AND COGAN MG. Angiotensin II stimulation of hydrogen ion secretion in the rat early proximal tubule. *J Clin Invest* 82: 601–607, 1988.
509. LIU FY AND COGAN MG. Angiotensin II stimulates early proximal bicarbonate absorption in the rat by decreasing cyclic adenosine monophosphate. *J Clin Invest* 84: 83–91, 1989.
510. LIU FY AND COGAN MG. Role of protein kinase C in proximal bicarbonate absorption and angiotensin signaling. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F927–F933, 1990.
511. LIU WS AND HECKMAN CA. The sevenfold way of PKC regulation. *Cell Signal* 10: 529–542, 1998.
513. LO M, LIU KL, LANTELME P, AND SASSARD J. Subtype 2 of angiotensin II receptors controls pressure-natriuresis in rats. *J Clin Invest* 95: 1394–1397, 1995.
514. LOFFING J, LÖTSCHER M, KAISSLING B, BIBER J, MURER H, SEIKALY M, ALPERN RJ, LEVI M, BAUM M, AND MOE OW. Renal Na/H exchanger NHE-3 and Na-PO_4 cotransporter NaP_i-2 protein expression in glucocorticoid excess and deficient states. *J Am Soc Nephrol* 9: 1560–1567, 1998.
515. LOGVINENKO NS, DULUBOVA I, FEDOSOVA N, LARSSON SH, NAIRN AC, ESMANN M, GREENGARD P, AND APERIA A. Phosphorylation by protein kinase C of serine-23 of the α-1 subunit of rat Na^+,K^+-ATPase affects its conformational equilibrium. *Proc Natl Acad Sci USA* 93: 9132–9137, 1996.
516. LOHSE MJ, BENOVIC JL, CODINA J, CARON MG, AND LEFKOWITZ RJ. β-Arrestin: a protein that regulates β-adrenergic receptor function. *Science* 245: 1547–1549, 1990.
517. LOLAIT SJ, O'CARROLL AM, MCBRIDE OW, KONIG M, MOREL A, AND BROWNSTEIN MJ. Cloning and characterization of a vasopressin V_2 receptor and possible link to nephrogenic diabetes insipidus. *Nature* 357: 336–339, 1992.
518. LOMASNEY JW, COTECCHIA S, LEFKOWITZ RJ, AND CARON MG. Molecular biology of α-adrenergic receptors: implications for receptor classification and for structure-function relationships. *Biochim Biophys Acta* 1095: 127–139, 1991.
519. LOMBÈS M, FARMAN N, OBLIN ME, BAULIEU P, BONVALET EE, ERLANGER BF, AND GASC M. Immunohistochemical localization of renal mineralocorticoid receptor by using an anti-idiotypic antibody that is an internal image of aldosterone. *Proc Natl Acad Sci USA* 87: 1086–1088, 1990.
520. LONGO N. Insulin stimulates the Na^+,K^+-ATPase and the $Na^+/K^+/Cl^-$ cotransporter of human fibroblasts. *Biochim Biophys Acta* 1281: 38–44, 1996.
521. LOWENSTEIN EJ, DALY RJ, BATZER AG, LI W, MARGOLIS B, LAMMERS R, ULLRICH A, SKOLNIK EY, BAR-SAGI D, AND SCHLESSINGER J. The SH2 and SH3 domain-containing protein Grb2 links receptor tyrosine kinases to Ras signalling. *Cell* 70: 431–442, 1992.
522. LOWNDES JM, HOKIN-NEAVERSON M, AND BERTICS PJ. Kinetics of phosphorylation of Na^+/K^+-ATPase by protein kinase C. *Biochim Biophys Acta* 1052: 143–151, 1990.
523. LU M, ZHU Y, BALAZY M, REDDY KM, FALCK JR, AND WANG WH. Effect of angiotensin II on the apical K^+ channel in the thick ascending limb of the rat kidney. *J Gen Physiol* 108: 537–547, 1996.
524. LUETSCHER JA JR. Studies of aldosterone in relation to water and electrolyte balance in man. *Rec Prog Horm Res* 12: 175–180, 1956.
525. LUO Y, KOKKONEN GC, WANG X, NEVE KA, AND ROTH GS. D_2 dopamine receptors stimulate mitogenesis through pertussis toxin-sensitive G proteins and Ras-involved ERK and SAP/JNK pathways in rat C6–D2L glioma cells. *J Neurochem* 71: 980–990, 1998.
526. LUTTRELL LM, DAAKA Y, DELLA ROCCA GJ, AND LEFKOWITZ RJ. G protein-coupled receptors mediate two functionally distinct pathways of tyrosine phosphorylation in rat 1a fibroblasts. Shc phosphorylation and receptor endocytosis correlate with the activation of ERK kinases. *J Biol Chem* 272: 31648–31656, 1997.
527. LUTTRELL LM, FERGUSON SSG, DAAKA Y, MILLER WE, MAUDSLEY S, DELLA ROCCA GJ, LIN FT, KAWAKATSU H, OWADA K, LUTTRELL DK, CARON MG, AND LEFKOWITZ RJ. β-Arrestin-dependent formation of β_2-adrenergic receptor-Src protein kinase complexes. *Science* 283: 655–661, 1999.
528. LUTTRELL LM, HAWES BE, VAN BIESEN T, LUTTRELL DK, LANSING TJ, AND LEFKOWITZ RJ. Role of c-Src tyrosine kinase in G protein-coupled receptor and $G\beta\gamma$ subunit-mediated activation of mitogen-activated protein kinases. *J Biol Chem* 271: 19443–19450, 1996.
529. LYNCH CJ, MADER ACL, MCCALL KM, NG YC, AND HAZEN SA. Okadaic acid stimulates ouabain-sensitive $^{86}Rb^+$-uptake and phosphorylation of the Na^+/K^+-ATPase α-subunit in rat hepatocytes. *FEBS Lett* 355: 157–162, 1994.

530. LYNCH CJ, WILSON PB, BLACKMORE PF, AND EXTON JH. The hormone-sensitive hepatic Na-pump. *J Biol Chem* 261: 14551–14556, 1986.
531. LYTTON J. Insulin affects the sodium affinity of the rat adipocyte (Na^+,K^+)-ATPase. *J Biol Chem* 260: 10075–10080, 1985.
532. MAEDA Y, HAN JS, GIBSON CC, AND KNEPPER MA. Vasopressin and oxytocin receptors coupled to Ca^{2+} mobilization in rat inner medullary collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F15–F25, 1993.
533. MAEDA S, WU S, GREEN J, KIM H, BOSCH R, LEE I, ADAMS J, CLEMENS TL, AND KURTZ I. The N-terminal portion of parathyroid hormone-related protein mediates the inhibition of apical Na^+/H^+ exchange in opossum kidney cells. *J Am Soc Nephrol* 9: 175–181, 1998.
534. MANDON B, SIGA E, CHABARDÈS D, FIRSOV D, ROINEL N, AND DE ROUFFIGNAC C. Insulin stimulates Na^+, Cl^-, Ca^{2+}, and Mg^{2+} transports in TAL of mouse nephron: cross potentiation with AVP. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F361–F369, 1993.
535. MANDON B, SIGA E, CHAMPIGNEULLE A, IMBERT-TEBOUL M, AND ELALOUF JM. Molecular analysis of β-adrenergic receptor subtypes in rat collecting duct: effect on cell cAMP and Ca^{2+} levels. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F1070–F1080, 1995.
536. MARRERO MB, SCHIEFFER B, PAXTON WG, HEERDT L, BERK BC, DELAFONTAINE P, AND BERNSTEIN KE. Direct stimulation of Jak/STAT pathway by the angiotensin II AT_1 receptor. *Nature* 375: 247–250, 1995.
537. MARSEN TA, SCHRAMEK H, AND DUNN MJ. Renal actions of endothelin: linking cellular signaling pathways to kidney diseases. *Kidney Int* 45: 336–344, 1994.
538. MARUNAKA Y AND EATON DC. Effects of vasopressin and cAMP on single amiloride-blockable Na channels. *Am J Physiol Cell Physiol* 260: C1071–C1084, 1991.
539. MARUNAKA Y, HAGIWARA N, AND TOHDA H. Insulin activates single amiloride-blockable Na channels in a distal nephron cell line (A6). *Am J Physiol Renal Fluid Electrolyte Physiol* 263: F392–F400, 1992.
540. MARVER D AND KOKKO JP. Renal target sites and the mechanism of action of aldosterone. *Miner Electrolyte Metab* 9: 1–18, 1983.
541. MARVER D, STEWART J, FUNDER JW, FELDMAN D, AND EDELMAN IS. Renal aldosterone receptors: studies with ^{3}H aldosterone and antimineralocorticoid ^{3}H spironolactone (SC-26304). *Proc Natl Acad Sci USA* 71: 1431–1435, 1974.
542. MASILAMANI S, KIM GH, MITCHELL C, WADE WB, AND KNEPPER MA. Aldosterone-mediated regulation of ENaC α, β and γ subunits in rat kidney. *J Clin Invest* 104: R19–R23, 1999.
543. MASTROBERARDINO L, SPINDLER B, FORSTER I, LOFFING J, ASSANDRI R, MAY A, AND VERREY F. Ras pathway activates epithelial Na^+ channel and decreases its surface expression in *Xenopus* oocyte. *Mol Cell Biol* 9: 3417–3427, 1998.
544. MATSUDA T, MURATA Y, TANAKA KI, HOSOI R, HAYASHI M, TAMADA K, TAKUMA K, AND BABA A. Involvement of Na^+,K^+-ATPase in the mitogenic effect of insulin-like growth factor-I on cultured rat astrocytes. *J Neurochem* 66: 511–516, 1996.
545. MATSUSHIMA Y, AKABANE S, AND ITO K. Characterization of α_1- and α_2-adrenoceptors directly associated with basolateral membranes from rat kidney proximal tubules. *Biochem Pharmacol* 35: 2593–2600, 1986.
546. MAY A, PUOTI A, GAEGGELER HP, HORISBERGER JD, AND ROSSIER BC. Early effect of aldosterone on the rate of synthesis of the epithelial sodium channel α subunit in A6 renal cells. *J Am Soc Nephrol* 8: 1813–1822, 1997.
547. MCDONOUGH AA, MAGYAR CE, AND KOMATSU Y. Expression of Na^+-K^+-ATPase α- and β-subunits along rat nephron: isoform specificity and response to hypokalemia. *Am J Physiol Cell Physiol* 267: C901–C908, 1994.
548. MCEWAN IJ, WRIGHT APH, AND GUSTAFSSON JA. Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. *Bioessays* 19: 153–160, 1997.
549. MCKINNEY TD AND MYERS P. Bicarbonate transport by proximal tubules: effect of parathyroid hormone and dibutyryl cyclic AMP. *Am J Physiol Renal Fluid Electrolyte Physiol* 238: F166–F174, 1980.
550. MEISTER B, DAGERLIND A, NICHOLAS AP, AND HOKFELT T. Patterns of messenger RNA expression for adrenergic receptor subtypes in the rat kidney. *J Pharmacol Exp Ther* 268: 1605–1611, 1994.
551. MEISTER B, FRYCKSTEDT J, SCHALLING M, CORTÉS R, HÖKFELT T, APERIA A, HEMMINGS HC JR, NAIRN AC, EHRLICH M, AND GREENGARD P. Dopamine- and cAMP-regulated phosphoprotein (DARPP-32) and dopamine DA1 agonist-sensitive Na,K-ATPase in renal tubule cells. *Proc Natl Acad Sci USA* 86: 8068–8072, 1989.
552. MELLAS J AND HAMMERMAN MR. Phorbol ester-induced alkalinization of canine renal proximal tubular cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 250: F451–F459, 1986.
553. MELLOR H AND PARKER PJ. The extended protein kinase C superfamily. *Biochem J* 332: 281–292, 1998.
554. MERCER RW, BIEMESDERFER D, BLISS DP JR, COLLINS JH, AND FORBUSH B III. Molecular cloning and immunological characterization of the γ polypeptide, a small protein associated with Na,K-ATPase. *J Cell Biol* 121: 579–586, 1993.
555. MIDDLETON JP, KHAN WA, COLLINSWORTH G, HANNUN YA, AND MEDFORD RM. Heterogeneity of protein kinase C-mediated rapid regulation of Na/K-ATPase in kidney epithelial cells. *J Biol Chem* 268: 15958–15964, 1993.
556. MISSALE C, NASH SR, ROBINSON SW, JABER M, AND CARON MG. Dopamine receptors: from structure to function. *Physiol Rev* 78: 189–225, 1998.
557. MIYATA N, PARK F, LI XF, AND COWLEY AW JR. Distribution of angiotensin AT_1 and AT_2 receptor subtypes in the rat kidney. *Am J Physiol Renal Physiol* 277: F437–F446, 1999.
558. MOCHLY-ROSEN D AND GORDON AS. Anchoring proteins for protein kinase C: a means for isozyme selectivity. *FASEB J* 12: 35–42, 1998.
559. MOE OW, AMEMIYA M, AND YAMAJI Y. Activation of protein kinase A acutely inhibits and phosphorylates Na/H exchanger NHE3. *J Clin Invest* 96: 2187–2194, 1995.
560. MOE OW, UJIIE K, STAR RA, MILLER RT, WIDELL J, ALPERN RJ, AND HENRICH WL. Renin expression in renal proximal tubule. *J Clin Invest* 91: 774–779, 1993.
561. MOHUCZY-DOMINIAK D AND GARG LC. α_2 Adrenoceptors in medullary thick ascending limbs of the rabbit kidney. *J Pharmacol Exp Ther* 266: 279–287, 1993.
562. MOLLOY CJ, TAYLOR DS, AND WEBER H. Angiotensin II stimulation of rapid protein tyrosine phosphorylation and protein kinase activation in rat aortic smooth muscle cells. *J Biol Chem* 268: 7338–7345, 1993.
563. MOLONY DA, REEVES WB, HEBERT SC, AND ANDREOLI TE. ADH increases apical Na,K,2Cl entry in mouse medullary thick ascending limbs of Henle. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F177–F187, 1987.
564. MORDUCHOWICZ GA, SHEIKH-HAMAD D, DWYER BE, STERN N, JO OD, AND YANAGAWA N. Angiotensin II directly increases rabbit renal brush-border membrane sodium transport: presence of local signal transduction system. *J Membr Biol* 122: 43–53, 1991.
565. MOREL A, O'CARROLL AM, BROWNSTEIN MJ, AND LOLAIT SJ. Molecular cloning and expression of a rat Vla arginine vasopressin receptor. *Nature* 356: 523–526, 1992.
566. MOREL F AND DOUCET A. Hormonal control of kidney functions at the cell level. *Physiol Rev* 66: 377–468, 1986.
567. MORGUNOV NS, YOU YD, AND HIRSCH DJ. Response of mouse proximal straight tubule and medullary thick ascending limb to β-agonist. *J Am Soc Nephrol* 4: 1151–1158, 1993.
568. MORIYA S, KAZLAUSLAS A, AKIMOTO K, HIRAI SI, MIZUNO K, TANEKAWA T, FUKUI Y, WANATABE Y, OZAKI S, AND OHNO S. Platelet-derived growth factor activates protein kinase Cϵ through redundant and independent signalling pathways involving phospholipase Cγ or phosphatidylinositol 3-kinase. *Proc Natl Acad Sci USA* 93: 151–155, 1996.
569. MORRISON P, TAKISHIMA K, AND ROSNER MR. Role of threonine residues in regulation of epidermal growth factor receptor by protein kinase C and mitogen-activated protein kinase. *J Biol Chem* 268: 15536–15543, 1993.
570. MOUNT DB, BAEKGAARD A, HALL AE, PLATA C, XU J, BEIER DR, GAMBA G, AND HEBERT SC. Isoforms of the Na-K-2Cl cotransporter in murine TAL. I. Molecular characterization and intrarenal localization. *Am J Physiol Renal Physiol* 276: F347–F358, 1999.
571. MRKIC B, TSE CM, FORGO J, HELMLE-KOLB C, DONOWITZ M, AND MURER H. Identification of PTH-responsive Na/H-exchanger isoforms in a rabbit proximal tubule cell line (RKPC-2). *Pflügers Arch* 424: 377–384, 1993.
572. MUJAIS SK, KAUFFMAN S, AND KATZ AI. Angiotensin II binding sites in

individual segments of the rat nephron. *J Clin Invest* 77: 315–318, 1986.
573. MUKOYAMA M, NAKAJIMA M, HORIUCHI M, SASAMURA H, PRATT RE, AND DZAU VJ. Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven-transmembrane receptors. *J Biol Chem* 268: 24539–24542, 1993.
574. MUNTZ KH, GARCIA C, AND HAGLER HK. α_1-Receptor localization in rat heart and kidney using autoradiography. *Am J Physiol Heart Circ Physiol* 249: H512–H519, 1985.
575. MUNTZ KH, MEYER L, GADOL S, AND CALIANOS TA. α_2 Adrenergic receptor localization in the rat heart and kidney using autoradiography and tritiated rauwolscine. *J Pharmacol Exp Ther* 236: 542–547, 1986.
576. MURASAWA S, MORI Y, NOZAWA Y, GOTOH N, SHIBUYA M, MASAKI H, MARUYAMA K, TSUTSUMI Y, MORIGUCHI Y, SHIBAZAKI Y, TANAKA Y, IWASAKA T, INADA M, AND MATSUBARA H. Angiotensin II type 1 receptor-induced extracellular signal-regulated protein kinase activation is mediated by Ca^{2+}/calmodulin-dependent transactivation of epidermal growth factor receptor. *Circ Res* 82: 1338–1348, 1998.
577. MURAYAMA N, RUGGLES BT, GAPSTUR SM, WERNESS JL, AND DOUSA TP. Evidence for β adrenoceptors in proximal tubules. Isoproterenol-sensitive adenylate cyclase in pars recta of canine nephron. *J Clin Invest* 76: 474–481, 1985.
578. MURAYAMA Y, SUZUKI A, TADOKORO M, AND SAKAI F. Microperfusion of Henle's loop in the kidney of adrenalectomized rat. *Jpn J Pharmacol* 18: 518–519, 1968.
579. MURER H, LOTSCHER M, KAISSLING B, LEVI M, KEMPSON SA, AND BIBER J. Renal brush border membrane Na/P_i-cotransport: molecular aspects in PTH-dependent and dietary regulation. *Kidney Int* 49: 1769–1773, 1996.
580. MURPHY TJ, ALEXANDER RW, GRIENDLING KK, RUNGE MS, AND BERNSTEIN KE. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature* 351: 233–236, 1991.
581. MUZZIN P, REVELLI JP, KUHNE F, GOCAYNE JD, MCCOMBIE WR, VENTER JC, GIACOBINO JP, AND FRASER CM. An adipose tissue-specific β-adrenergic receptor. *J Biol Chem* 266: 24053–24058, 1991.
582. MYERS MG, WANG LM, SUN XJ, ZHANG Y, YENUSH L, SCHLESSINGER J, PIERCE JH, AND WHITE MF. Role of IRS1-GRB2 complexes in insulin signaling. *Mol Cell Biol* 14: 3577–3587, 1994.
583. NAGEL W AND CRABBÉ J. Mechanism of action of aldosterone on active sodium transport across toad skin. *Pflügers Arch* 385: 181–187, 1980.
584. NAHMIAS C, CAZAUBON SM, BRIEND-SUTREN MM, LAZARD D, VILLAGEOIS P, AND STROSBERG AD. Angiotensin II AT_2 receptors are functionally coupled to protein tyrosine dephosphorylation in N1E-115 neuroblastoma cells. *Biochem J* 306: 87–92, 1995.
585. NARAY-FEJES-TOTH A, COLOMBOWALA IK, AND FEJES-TOTH G. The role of 11β-hydroxysteroid dehydrogenase in steroid hormone specificity. *J Steroid Biochem Mol Biol* 65: 311–316, 1998.
586. NARAY-FEJES-TOTH A AND FEJES-TOTH G. Sgk, an aldosterone-induced early-response gene in mineralocorticoid target cells, regulates epithelial sodium channel. *Kidney Int.* In press.
587. NARUSE M, UCHIDA S, OGATA E, AND KUROKAWA K. Endothelin 1 increases cell calcium in mouse collecting tubule cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 261: F720–F725, 1991.
588. NARUSE M, YOSHITOMI K, HANAOKA K, IMAI M, AND KUROKAWA K. Electrophysiological study of luminal and basolateral vasopressin in rabbit cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F20–F29, 1995.
589. NASH SR, GODINOT N, AND CARON MG. Cloning and characterization of the opossum kidney cell D_1 dopamine receptor: expression of identical D_{1A} and D_{1B} dopamine receptor mRNAs in opossum kidney and brain. *Mol Pharmacol* 44: 918–925, 1993.
590. NAVAR LG, HARRISON-BERNARD LM, IMIG JD, WANG CT, CERVENKA L, AND MITCHELL KD. Intrarenal angiotensin II generation and renal effects of AT_1 receptor blockade. *J Am Soc Nephrol* 10: S266–S272, 1999.
591. NAVAR LG, LEWIS L, HYMEL A, BRAAM B, AND MITCHELL KD. Tubular fluid concentrations and kidney contents of angiotensins I and II in anesthetized rats. *J Am Soc Nephrol* 5: 1153–1158, 1994.
592. NÉANT F AND BAILLY C. Luminal and intracellular cGMP inhibits the mTAL reabsorptive capacity through different pathways. *Kidney Int* 44: 741–746, 1993.
593. NÉANT F, IMBERT-TEBOUL M, AND BAILLY C. Cyclic guanosine monophosphate is the mediator of platelet-activating factor inhibition on transport by the mouse thick ascending limb. *J Clin Invest* 94: 1156–1162, 1994.
594. NIELSEN S, KWON TH, CHRISTENSEN BM, PROMENEUR D, FROKIAER J, AND MARPLES D. Physiology and pathophysiology of renal aquaporins. *J Am Soc Nephrol* 10: 647–663, 1999.
595. NITSCHKE R, FROBE U, AND GREGER R. Antidiuretic hormone acts via V_1 receptors on intracellular calcium in the isolated perfused rabbit cortical thick ascending limb. *Pflügers Arch* 417: 622–632, 1991.
596. NOËL J AND POUYSSEGUR J. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na^+/H^+ exchanger isoforms. *Am J Physiol Cell Physiol* 268: C283–C296, 1995.
597. NONOGUCHI H, OWADA A, KOBAYASHI N, TAKAYAMA M, TERADA Y, KOIKE J, UJIIE K, MARUMO F, SAKAI T, AND TOMITA K. Immunohistochemical localization of V2 receptor along the nephron and functional role of luminal V2 receptor in terminal inner medullary collecting ducts. *J Clin Invest* 96: 1768–1778, 1995.
598. NONOGUCHI H, TOMITA K, AND MARUMO F. Effects of atrial natriuretic peptide and vasopressin on chloride transport in long- and short-looped medullary thick ascending limbs. *J Clin Invest* 90: 349–357, 1992.
599. NORD EP, HOWARD MJ, HAFEZI A, MORADESHAGI P, VAYSTUB S, AND INSEL PA. α_2 Adrenergic agonists stimulate Na-H antiport activity in the rabbit renal proximal tubule. *J Clin Invest* 80: 1755–1762, 1987.
600. OBERLEITHNER H. Aldosterone-regulated ion transporters in the kidney. *Klin Wochenschr* 68: 1087–1090, 1990.
601. OBERLEITHNER H, WEIGT M, WESTPHALE HJ, AND WANG W. Aldosterone activates Na^+/H^+ exchange and raises cytoplasmic pH in target cells of the amphibian kidney. *Proc Natl Acad Sci USA* 84: 1464–1468, 1987.
602. O'BRIEN WJ, LINGREL JB, AND WALLICK ET. Ouabain binding kinetics of the rat α_2 and α_3 isoforms of sodium-potassium adenosine triphosphatase. *Arch Biochem Biophys* 310: 29–32, 1994.
603. O'CONNELL DP, AHERNE AM, LANE E, FELDER RA, AND CAREY RM. Detection of dopamine receptor D_{1A} subtype-specific mRNA in rat kidney by in situ amplification. *Am J Physiol Renal Physiol* 274: F232–F241, 1998.
604. O'CONNELL DP, BOTKIN SJ, RAMOS SI, SIBLEY DR, ARIANO MA, FELDER RA, AND CAREY RM. Localization of dopamine D_{1A} receptor protein in rat kidneys. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F1185–F1197, 1995.
605. O'CONNELL DP, VAUGHAN CJ, AHERNE AM, BOTKIN SJ, WANG ZQ, FELDER RA, AND CAREY RM. Expression of the dopamine D_3 receptor protein in the rat kidney. *Hypertension* 32: 886–895, 1998.
606. OHBU K AND FELDER RA. Nephron specificity of dopamine receptor-adenylyl cyclase defect in spontaneous hypertension. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F274–F279, 1993.
607. OLIVERIO MI, KIM HS, ITO M, LE T, AUDOLY L, BEST CF, HILLER S, KLUCKMAN K, MAEDA N, SMITHIES O, AND COFFMAN TM. Reduced growth, abnormal kidney structure, and type 2 (AT_2) angiotensin receptor-mediated blood pressure regulation in mice lacking both AT_{1A} and AT_{1B} receptors for angiotensin II. *Proc Natl Acad Sci USA* 95: 15496–15501, 1998.
608. OMINATO M, SATOH T, AND KATZ AI. Regulation of Na-K-ATPase activity in the proximal tubule: role of the protein kinase C pathway and eicosanoids. *J Membr Biol* 152: 235–243, 1996.
609. O'NEIL RG AND HELMAN SI. Transport characteristics of renal collecting tubules: influences of DOCA and diet. *Am J Physiol Renal Fluid Electrolyte Physiol* 232: F544–F558, 1977.
610. ORCHINIK M AND MCEWEN BS. Rapid steroid actions in the brain: a critique of genomic and non genomic mechanisms. In: *Genomic and Non-genomic Effects of Aldosterone*, edited by Wehling M. Boca Raton, FL: CRC, 1994, p. 77–108.
611. OSBORN JL AND HARLAND RW. α_1-Adrenoceptor mediation of urinary bicarbonate excretion. *Am J Physiol Renal Fluid Electrolyte Physiol* 255: F1116–F1121, 1988.
612. OSTLUND E, MENDEZ CF, JACOBSSON G, FRYCKSTEDT J, MEISTER B, AND APERIA A. Expression of protein kinase C isoforms in renal tissue. *Kidney Int* 47: 766–773, 1995.
613. OSTROWSKI NL, YOUNG WS III, KNEPPER MA, AND LOLAIT SJ. Expression of vasopressin V_{1a} and V_2 receptor messenger ribonucleic acid

in the liver and kidney of embryonic, developing, and adult rats. *Endocrinology* 133: 1849–1859, 1993.
614. OUDE WEERNINCK PA AND RIJKSEN G. Activation and translocation of c-Src to the cytoskeleton by both platelet-derived growth factor and epidermal growth factor. *J Biol Chem* 270: 2264–2267, 1995.
615. OVCHINNIKOV YA, MODYANOV NN, BROUDE NE, PETRUKHIN KE, GRISHIN AV, ARZAMAZOVA NH, ALDANOVA NA, MONASTYRSKAYA GS, AND SVERDLOV ED. Pig kidney Na^+,K^+-ATPase. Primary structure and spatial organisation. *FEBS Lett* 201: 237–245, 1986.
616. OWADA S, LARSSON O, ARKHAMMAR P, KATZ AI, CHIBALIN AV, BERGGREN PO, AND BERTORELLO AM. Glucose decreases Na^+,K^+-ATPase activity in pancreatic β-cells. An effect mediated via Ca^{2+}-independent phospholipase A_2 and protein kinase C-dependent phosphorylation of the α-subunit. *J Biol Chem* 274: 2000–2008, 1999.
617. OZONO R, O'CONNELL DP, WANG ZQ, MOORE AF, SANADA H, FELDER RA, AND CAREY RM. Localization of the dopamine D_1 receptor protein in the human heart and kidney. *Hypertension* 30: 725–729, 1997.
618. PACHA J, FRINDT G, ANTONIAN L, SILVER RB, AND PALMER LG. Regulation of Na channels of the rat cortical collecting tubule by aldosterone. *J Gen Physiol* 102: 25–42, 1993.
619. PACHA J, FRINDT G, SACKIN H, AND PALMER LG. Apical maxi K channels in intercalated cells of CCT. *Am J Physiol Renal Fluid Electrolyte Physiol* 261: F696–F705, 1991.
620. PAILLARD M. Na^+/H^+ exchanger subtypes in the renal tubule: function and regulation in physiology and disease. *Exp Nephrol* 5: 277–284, 1997.
621. PAILLARD M. H^+ and HCO_3^- transporters in the medullary thick ascending limb of the kidney: molecular mechanisms, function and regulation. *Kidney Int* 65 *Suppl*: S36–S41, 1998.
622. PALMER LG AND FRINDT G. Amiloride-sensitive Na channels from the apical membrane of the rat cortical collecting tubule. *Proc Natl Acad Sci USA* 83: 2767–2770, 1986.
623. PALMER LG, LI JHY, LINDEMAN B, AND EDELMAN IS. Aldosterone control of the density of sodium channels in the toad urinary bladder. *J Membr Biol* 64: 91–102, 1982.
624. PASTORIZA-MUNOZ E, HARRINGTON RM, AND GRABER ML. Parathyroid hormone decreases HCO_3 reabsorption in the rat proximal tubule by stimulating phosphatidylinositol metabolism and inhibiting base exit. *J Clin Invest* 89: 1485–1495, 1992.
625. PAXTON WG, RUNGE M, HORAIST C, COHEN C, ALEXANDER RW, AND BERNSTEIN KE. Immunohistochemical localization of the rat angiotensin II AT_1 receptor. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F989–F995, 1993.
626. PAZ K, HEMI R, LEROITH D, KARASIK A, ELHANANY E, KANER H, AND ZICK Y. A molecular basis for insulin resistance. Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. *J Biol Chem* 272: 29911–29918, 1997.
627. PEARCE D AND YAMAMOTO KR. Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science* 259: 1161–1165, 1993.
628. PEDEMONTE CH, PRESSLEY TA, LOKHANDWALA MF, AND CINELLI AR. Regulation of Na,K-ATPase transport activity by protein kinase C. *J Membr Biol* 155: 219–227, 1997.
629. PEREZ DM, DEYOUNG MB, AND GRAHAM RM. Coupling of expressed α_{1B}- and α_{1D}-adrenergic receptor to multiple signaling pathways is both G protein and cell type specific. *Mol Pharmacol* 44: 784–795, 1993.
630. PERKINS FM AND HANDLER JS. Transport properties of toad kidney epithelia in culture. *Am J Physiol Cell Physiol* 241: C154–C159, 1981.
631. PETERSON DR, OPARIL S, FLOURET G, AND CARONE FA. Handling of angiotensin II and oxytocin by renal tubular segments perfused in vitro. *Am J Physiol Renal Fluid Electrolyte Physiol* 232: F319–F324, 1977.
632. PETERSON LN AND WRIGHT FS. Effect of sodium intake on renal potassium excretion. *Am J Physiol Renal Fluid Electrolyte Physiol* 233: F225–F234, 1977.
633. PETTY KJ, KOKKO JP, AND MARVER D. Secondary effect of aldosterone on Na-K-ATPase activity in the rabbit cortical collecting tubule. *J Clin Invest* 68: 1514–1521, 1981.
634. PFAHL M. Nuclear receptor/AP-1 interaction. *Endocrinol Rev* 14: 651–658, 1993.
635. PFISTER MF, RUF I, STANGE G, ZIEGLER U, LEDERER E, BIBER J, AND MURER H. Parathyroid hormone leads to the lysosomal degradation of the renal type II Na/P_i cotransporter. *Proc Natl Acad Sci USA* 95: 1909–1914, 1998.
636. PICARD D, DHURSHEED B, GARABEDIAN MJ, FORTIN MG, LINDQUIST S, AND YAMAMOTO KR. Signal transduction by steroid receptors: reduced levels of HSP90 compromise receptor action in vitro. *Nature* 348: 166–168, 1990.
637. PINTO-DO-O PC, CHIBALIN AV, KATZ AI, SOARES-DA-SILVA P, AND BERTORELLO AM. Short-term vs sustained inhibition of proximal tubule Na,K-ATPase activity by dopamine: cellular mechanisms. *Clin Exp Hypertens* 19: 73–86, 1997.
638. PIPPARD C AND BAYLIS PH. The stimulation of Na^+,K^+-ATPase activity in the medulla of the rat kidney by [arginine] vasopressin and its analogs. *Clin Sci* 66: 561–567, 1984.
639. PLATA C, MEADE P, MOUNT DB, HEBERT SC, AND GAMBA G. Alternatively spliced isoforms of mBSC1 encode the murine mTAL furosemide-sensitive Na:Cl cotransporter (Abstract). *J Am Soc Nephrol* 10: 41A, 1999.
640. PLATA C, MOUNT DB, HEBERT SC, AND GAMBA G. Isoforms of the Na-K-2Cl cotransporter in murine TAL. I. Functional characterization and activation by cAMP. *Am J Physiol Renal Physiol* 276: F359–F366, 1999.
641. POGGIOLI J, LAZAR G, HOUILLIER P, GARDIN JP, ACHARD JM, AND PAILLARD M. Effects of angiotensin II and nonpeptide receptor antagonists on transduction pathways in rat proximal tubule. *Am J Physiol Cell Physiol* 263: C750–C758, 1992.
642. POGGIOLI J, LAZAR G, HOUILLIER P, GARDIN JP, AND PAILLARD M. Acute variations in extracellular pH modulate transduction pathways of PTH in rat proximal tubule. *Am J Physiol Cell Physiol* 263: C941–C947, 1992.
643. POLLOCK AS, WARNOCK DG, AND STREWLER GJ. Parathyroid hormone inhibition of Na^+-H^+ antiporter activity in a cultured renal cell line. *Am J Physiol Renal Fluid Electrolyte Physiol* 250: F217–F225, 1986.
644. PORTER GA, BOGOROCH R, AND EDELMAN IS. On the mechanism of action of aldosterone on sodium transport: the role of RNA synthesis. *Proc Natl Acad Sci USA* 52: 1326–1333, 1964.
645. PORTILLA D, MANDEL LJ, BAR-SAGI D, AND MILLINGTON DS. Anoxia induces phospholipase A_2 activation in rabbit renal proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F354–F360, 1992.
646. PRAT AG, AUSIELLO DA, AND CANTIELLO HF. Vasopressin and protein kinase A activate G protein-sensitive epithelial Na channels. *Am J Physiol Cell Physiol* 265: C218–C223, 1993.
647. PRAT AG, BERTORELLO AM, AUSIELLO DA, AND CANTIELLO HF. Activation of epithelial Na channels by protein kinase A requires actin filaments. *Am J Physiol Cell Physiol* 265: C224–C233, 1993.
648. PRICE EM AND LINGREL JB. Structure-function relationships in the Na,K-ATPase a subunit: site-directed mutagenesis of glutamine-111 to arginine and asparagine-122 to aspartic acid generates a ouabain-resistant enzyme. *Biochemistry* 27: 8400–8408, 1988.
649. PRICE EM, RICE DA, AND LINGREL JB. Structure function studies of Na,K-ATPase. Site-directed mutagenesis of the border residues from the H1–H2 extracellular domain of the α subunit. *J Biol Chem* 265: 6638–6641, 1990.
650. PRONK GJ, DE VRIES-SMITS AM, BUDAY L, DOWNWARD J, MAASSEN JA, MEDEMA RH, AND BOS JL. Involvement of Shc in insulin- and epidermal growth factor-induced activation of $p21^{ras}$. *Mol Cell Biol* 14: 1575–1581, 1994.
651. PULLMAN TN, OPARIL S, AND CARONE FA. Fate of labeled angiotensin II microinfused into individual nephrons in the rat. *Am J Physiol* 228: 747–751, 1975.
652. PUSCHETT JB, ZURBACH P, AND SYLK D. Acute effects of parathyroid hormone on proximal bicarbonate transport in the dog. *Kidney Int* 9: 501–510, 1976.
653. QUAN A AND BAUM M. Endogenous production of angiotensin II modulates rat proximal tubule transport. *J Clin Invest* 97: 2878–2882, 1996.
654. QUAN A AND BAUM M. Endogenous angiotensin II modulates rat

proximal tubule transport with acute changes in extracellular volume. *Am J Physiol Renal Physiol* 275: F74–F78, 1998.
655. QUAN A AND BAUM M. Effect of luminal angiotensin II receptor antagonists on proximal tubule transport. *Am J Hypertens* 12: 499–503, 1999.
656. RABINOWITZ L. Aldosterone and renal potassium excretion. *Renal Physiol* 2: 229–243, 1979.
657. RAFESTIN-OBLIN ME, COUETTE B, BARLET-BAS C, CHEVAL L, VIGER A, AND DOUCET A. Renal action of progesterone and 18-substituted derivatives. *Am J Physiol* 260: F828–F832, 1991.
658. RAFESTIN-OBLIN ME, COUETTE B, RADANYI CD, LOMBÈS M, AND BAULIEU EE. Mineralocorticosteroid receptor of the chick intestine: oligomeric structure and transformation. *J Biol Chem* 264: 9304–9309, 1989.
659. RAJERISON RM, MARCHETTI J, ROY C, BOCKAERT J, AND JARD S. The vasopressin-sensitive adenylate cyclase of the rat kidney. Effect of adrenalectomy and corticosteroids on hormonal receptor-enzyme coupling. *J Biol Chem* 249: 6390–6400, 1974.
660. RAKOWSKI RF, GADSBY DC, AND DE WEER P. Stoichiometry and voltage dependence of the sodium pump in voltage-clamped, internally dialyzed squid giant axon. *J Gen Physiol* 93: 903–941, 1989.
661. RAMEH LE AND CANTLEY LC. The role of phosphoinositide 3-kinase lipid products in cell function. *J Biol Chem* 274: 8347–8350, 1999.
662. RAYSON BM AND LOWTHER SO. Steroid regulation of Na^+-K^+-ATPase: differential sensitivities along the rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 246: F656–F662, 1984.
663. REEVES WB, MCDONALD GA, MEHTA P, AND ANDREOLI TE. Activation of K^+ channels in renal medullary vesicles by cAMP-dependent protein kinase. *J Membr Biol* 109: 65–72, 1989.
664. REEVES WB, WINTERS CJ, FILIPOVIC DM, AND ANDREOLI TE. Cl^- channels in basolateral renal medullary vesicles. IX. Channels from mouse MTAL cell patches and medullary vesicles. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F621–F627, 1995.
665. REICHARDT HM, KAESTNER KH, TUCKERMANN J, KRETZ O, WESSELY O, BOCK R, GASS P, SCMID W, HERRLICH P, ANGEL P, AND SCHÜTZ G. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* 93: 531–541, 1998.
666. REIF K, BUDAY L, DOWNWARD J, AND CANTRELL DA. Sh3 domains of the adaptor molecule Grb2 complex with two proteins in T cells: the guanine nucleotide exchange protein Sos and a 75-kDa protein that is a substrate for T cell antigen receptor-activated tyrosine kinases. *J Biol Chem* 269: 14081–14087, 1994.
667. REIF MC, TROUTMAN SL, AND SCHAFER JA. Sustained response to vasopressin in isolated rat cortical collecting tubule. *Kidney Int* 26: 725–732, 1984.
668. REIF MC, TROUTMAN SL, AND SCHAFER JA. Sodium transport by rat cortical collecting tubule. Effects of vasopressin and desoxycorticosterone. *J Clin Invest* 77: 1291–1298, 1986.
669. REILLY AM, HARRIS PJ, AND WILLIAMS DA. Biphasic effect of angiotensin II on intracellular sodium concentration in rat proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F374–F380, 1995.
670. RENARD S, LINGUEGLIA E, VOILLEY N, LAZDUNSKI M, AND BARBRY P. Biochemical analysis of the membrane topology of the amiloride-sensitive Na^+ channel. *J Biol Chem* 269: 12981–12986, 1994.
671. RESH MD, NEMENOFF RA, AND GUIDOTTI G. Insulin stimulation of (Na^+,K^+)-adenosine triphosphatase-dependent $^{86}Rb^+$ uptake in rat adipocytes. *J Biol Chem* 255: 10938–10945, 1980.
672. RIBEIRO CMP, DUBAY GR, FALCK JR, AND MANDEL LJ. Parathyroid hormone inhibits Na-K-ATPase through a cytochrome *P*-450 pathway. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F497–F505, 1994.
673. RIBEIRO CP AND MANDEL LJ. Parathyroid hormone inhibits proximal tubule Na^+-K^+-ATPase activity. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F209–F216, 1992.
674. RICCARDI D, LEE WS, LEE K, SEGRE GV, BROWN EM, AND HEBERT SC. Localization of the extracellular Ca^{2+}-sensing receptor and PTH/PTHrP receptor in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F951–F956, 1996.
675. RICCI A, ESCAF S, VEGA JA, AND AMENTA F. Autoradiographic localization of dopamine D_1 receptors in the human kidney. *J Pharmacol Exp Ther* 264: 431–437, 1993.
676. ROMERO MF AND BORON WF. Electrogenic Na^+/HCO_3^- cotransporters: cloning and physiology. *Annu Rev Physiol* 61: 699–723, 1999.
677. RON D, NAPOLITANO EW, VORONOVA A, VASQUEZ NJ, ROBERTS DN, CALIO BL, CAOTHIEN RH, PETTIFORD SM, WELLIK S, MANDAC JB, AND KAUVAR LM. Direct interaction in T-cells between θPKC and the tyrosine kinase p59fyn. *J Biol Chem* 274: 19003–19010, 1999.
678. ROSSIER BC AND PALMER L. Mechanisms of aldosterone action on sodium and potassium transport. In: *The Kidney: Physiology and Physiopathology*, edited by Seldin DW and Giebisch G. New York: Raven, 1991, p. 1373–1409.
679. ROSSIER BC, VERREY F, AND KRAEHENBÜHL JP. Transepithelial sodium transport and its control by aldosterone: a molecular approach. *Curr Top Membr Transp* 34: 167–183, 1989.
680. ROTH MG AND STERNWEIS PC. The role of lipids signaling in constitutive membrane traffic. *Curr Opin Cell Biol* 9: 519–526, 1997.
681. ROUFFIGNAC DE C, CORMAN B, AND ROINEL N. Stimulation by antidiuretic hormone of electrolyte tubular reabsorption in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 244: F156–F164, 1983.
682. ROULEAU MF, WARSHAWSKY H, AND GOLTZMAN D. Parathyroid hormone binding in vivo to renal, hepatic, and skeletal tissues of the rat using a radioautographic approach. *Endocrinology* 118: 919–931, 1986.
683. ROUSE D, WILLIAMS S, AND SUKI WN. Clonidine inhibits fluid absorption in the rabbit proximal convoluted renal tubule. *Kidney Int* 38: 80–85, 1990.
684. RUGGLES BT, MURAYAMA N, WERNESS JL, GAPSTUR SM, BENTLEY MD, AND DOUSA TP. The vasopressin-sensitive adenylate cyclase in collecting tubules and in thick ascending limb of Henle's loop of human and canine kidney. *J Clin Endocrinol Metab* 60: 914–921, 1985.
685. RUIZ OS AND ARRUDA JAL. Regulation of the renal Na-HCO_3 cotransporter by cAMP and Ca-dependent protein kinases. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F560–F565, 1992.
686. RUIZ OS, QIU YY, WANG LJ, AND ARRUDA JAL. Regulation of the renal Na-HCO_3 cotransporter. IV. Mechanisms of the stimulatory effect of angiotensin II. *J Am Soc Nephrol* 6: 1202–1208, 1995.
687. RUIZ OS, QIU YY, WANG LJ, AND ARRUDA JAL. Regulation of the renal Na-HCO_3 cotransporter. V. Mechanism of the inhibitory effect of parathyroid hormone. *Kidney Int* 49: 396–402, 1996.
688. RUNDLE SE, FUNDER JW, LAKSHMI V, AND MONDER C. The intrarenal localization of mineralocorticoid receptors and 11β-dehydrogenase: immunocytochemical studies. *Endocrinology* 125: 1700–1704, 1989.
689. SABRI A, GOVINDARAJAN G, GRIFFIN TM, BYRON KL, SAMAREL AM, AND LUCCHESI PA. Calcium- and protein kinase C-dependent activation of the tyrosine kinase PYK2 by angiotensin II in vascular smooth muscle. *Circ Res* 83: 841–851, 1998.
690. SACCOMANI G, MITCHELL KD, AND NAVAR LG. Angiotensin II stimulation of Na-H exchange in proximal tubule cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F1188–F1195, 1990.
691. SADOSHIMA J AND IZUMO S. The heterotrimeric G_q protein-coupled angiotensin II receptor activates p21 ras via the tyrosine kinase-Shc-Grb2-Sos pathway in cardiac myocytes. *EMBO J* 15: 775–787, 1996.
692. SANDBERG K, JI H, CLARK AJ, SHAPIRA H, AND CATT KJ. Cloning and expression of a novel angiotensin II receptor subtype. *J Biol Chem* 267: 9455–9458, 1992.
693. SANSOM S, MUTO S, AND GIEBISCH G. Na-dependent effects of DOCA on cellular transport properties of CCDs from ADX rabbits. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F753–F759, 1987.
694. SANSOM SC AND O'NEIL RG. Mineralocorticoid regulation of apical membrane Na and K transport of the cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 248: F858–F868, 1985.
695. SANSOM SC AND O'NEIL RG. Effects of mineralocorticoids on transport properties of cortical collecting duct basolateral membrane. *Am J Physiol Renal Fluid Electrolyte Physiol* 251: F743–F757, 1986.
696. SARIBAN-SOHRABY S, BURG M, WIESMANN WP, CHIANG PK, AND JOHNSON JP. Methylation increases sodium transport into A6 apical membrane vesicles: possible mode of aldosterone action. *Science* 225: 745–746, 1984.
697. SARIBAN-SOHRABY S AND FISHER RS. Guanine-nucleotide-dependent

carboxymethylation: a pathway for aldosterone modulation of apical Na^+ permeability in epithelia. *Kidney Int* 48: 965–969, 1995.
698. SASAKI S AND IMAI M. Effects of vasopressin on water and NaCl transport across the in vitro perfused medullary thick ascending limb of Henle's loop of mouse, rat, and rabbit kidneys. *Pflügers Arch* 383: 215–221, 1980.
699. SASAKI S AND MARUMO F. Mechanisms of inhibition of proximal acidification by PTH. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F833–F838, 1991.
700. SATOH T, COHEN HT, AND KATZ AI. Intracellular signaling in the regulation of renal Na-K-ATPase. I. Role of cyclic AMP and phospholipase A_2. *J Clin Invest* 89: 1496–1500, 1992.
701. SATOH T, COHEN HT, AND KATZ AI. Intracellular signaling in the regulation of renal Na-K-ATPase. II. Role of eicosanoids. *J Clin Invest* 91: 409–415, 1993.
702. SATOH T, COHEN HT, AND KATZ AI. Different mechanisms of renal Na-K-ATPase regulation by protein kinases in proximal and distal nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F399–F405, 1993.
703. SATOH T, OMINATO M, AND KATZ AI. Different mechanisms of renal Na-K-ATPase regulation by dopamine in the proximal and distal nephron. *Hypertens Res* 18 *Suppl*: S137–S140, 1995.
704. SAYEGH R, AUERBACH SD, LI X, RANDY RW, HUSTED RF, STOKES JB, AND THOMAS CP. Glucocorticoid induction of epithelial sodium channel expression in lung and renal epithelia occurs via *trans*-activation of a hormone response element in the 5′-flanking region of the human epithelial sodium channel α subunit gene. *J Biol Chem* 274: 12431–12437, 1999.
705. SAYESKI PP, ALI MS, HAWKS K, FRANK SJ, AND BERNSTEIN KE. The angiotensin II-dependent association of Jak2 and c-Src requires the N-terminus of Jak2 and the SH2 domain of c-Src. *Circ Res* 84: 1332–1338, 1999.
706. SCHAFER JA AND TROUTMAN SL. Effect of ADH on rubidium transport in isolated perfused rat cortical collecting tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 250: F1063–F1072, 1986.
707. SCHAFER JA AND TROUTMAN SL. Potassium transport in cortical collecting tubules from mineralocorticoid-treated rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F76–F88, 1987.
708. SCHAFER JA AND TROUTMAN SL. cAMP mediates the increase in apical membrane Na^+ conductance produced in rat CCD by vasopressin. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F823–F831, 1990.
709. SCHAFER JA, TROUTMAN SL, AND SCHLATTER E. Vasopressin and mineralocorticoid increase apical membrane driving force for K^+ secretion in rat CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F199–F210, 1990.
710. SCHATZMANN HJ. Herzglykoside als Hemmstoffe für den aktiven Kaliumund Natriumtransport durch die Erythrocytenmembran. *Helv Physiol Pharmacol Acta* 11: 346–354, 1953.
711. SCHELLING JR, DELUCA DJ, KONIECZKOWSKI M, MARZEC R, SEDOR JR, DUBYAK GR, AND LINAS SL. Glucocorticoid uncoupling of angiotensin II-dependent phospholipase C activation in rat vascular smooth muscle cells. *Kidney Int* 46: 675–682, 1994.
712. SCHELLING JR, SINGH H, MARZEC R, AND LINAS SL. Angiotensin II-dependent proximal tubule sodium transport is mediated by cAMP modulation of phospholipase C. *Am J Physiol Cell Physiol* 267: C1239–C1245, 1994.
713. SCHIEFFER B, PAXTON WG, CHAI Q, MARRERO MB, AND BERNSTEIN KE. Angiotensin II controls p21ras activity via pp60c-src. *J Biol Chem* 271: 10329–10333, 1996.
714. SCHLATTER E AND GREGER R. cAMP increases the basolateral Cl^- conductance in the isolated perfused medullary thick ascending limb of Henle's loop of the mouse. *Pflügers Arch* 405: 367–376, 1985.
715. SCHLATTER E, HAXELMANS S, ANKORINA I, AND KLETA R. Regulation of Na^+/H^+ exchange by diadenosine polyphosphates, angiotensin II, and vasopressin in rat cortical collecting duct. *J Am Soc Nephrol* 6: 1223–1229, 1995.
716. SCHLATTER E AND SCHAFER JA. Electrophysiological studies in principal cells of rat cortical collecting tubules. ADH increases the apical membrane Na^+-conductance. *Pflügers Arch* 409: 81–92, 1987.
717. SCHLONDORFF D, ZANGER R, SATRIANO JA, FOLKERT VW, AND EVELOFF J. Prostaglandin synthesis by isolated collecting tubules from adult and neonatal rabbits. *Am J Physiol Renal Fluid Electrolyte Physiol* 248: F134–F144, 1985.
718. SCHMIDT U AND DUBACH UC. Activity of (Na^+ K^+)-stimulated adenosine-triphosphatase in rat nephron. *Pflügers Arch* 306: 219–226, 1969.
719. SCHORB W, PEELER TC, MADIGAN NN, CONRAD KM, AND BAKER KM. Angiotensin II-induced protein tyrosine phosphorylation in neonatal rat cardiac fibroblasts. *J Biol Chem* 269: 19626–19632, 1994.
720. SCHUSTER VL, KOKKO JP, AND JACOBSON HR. Angiotensin II directly stimulates sodium transport in rabbit proximal convoluted tubules. *J Clin Invest* 73: 507–515, 1984.
721. SCHUSTER VL, KOKKO JP, AND JACOBSON HR. Interaction of lysyl-bradykinin and antidiuretic hormone in the rabbit cortical collecting tubule. *J Clin Invest* 73: 1659–1667, 1984.
722. SCHWARTZ GJ AND BURG MB. Mineralocorticoid effects on cation transport by cortical collecting tubules in vitro. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F576–F586, 1978.
723. SCHWARTZ IL, SCHLATZ LJ, KINNE-SAFFRAN E, AND KINNE R. Target cell polarity and membrane phosphorylation in relation to the mechanism of action of antidiuretic hormone. *Proc Natl Acad Sci USA* 71: 2595–2599, 1974.
724. SCHWINDINGER WF, FREDERICKS J, WATKINS L, ROBINSON H, BATHON JM, PINES M, SUVA LJ, AND LEVINE MA. Coupling of the PTH/PTHrP receptor to multiple G-proteins. Direct demonstration of receptor activation of G_s, $G_{q/11}$, and $G_{i\text{-}1}$ by [α-^{32}P]GTP-gamma-azidoanilide photoaffinity labeling. *Endocrine* 8: 201–209, 1998.
725. SEKI G, TANIGUCHI S, UWATOKO S, SUZUKI K, AND KUROKAWA K. Effect of parathyroid hormone on acid/base transport in rabbit renal proximal tubule S3 segment. *Pflügers Arch* 423: 7–13, 1993.
726. SEKI G, TANIGUCHI S, UWATOKO S, SUZUKI K, AND KUROKAWA K. Activation of the basolateral Cl^- conductance by cAMP in rabbit renal proximal tubule S3 segments. *Pflügers Arch* 430: 88–95, 1995.
727. SERI I, KONE BC, GULLANS SR, APERIA A, BRENNER BM, AND BALLERMANN BJ. Locally formed dopamine inhibits Na-K-ATPase activity in rat renal cortical tubule cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 255: F666–F673, 1988.
728. SERI I, KONE BC, GULLANS SR, APERIA A, BRENNER BM, AND BALLERMANN BJ. Influence of Na intake on dopamine-induced inhibition of renal cortical Na-K-ATPase. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F52–F60, 1990.
729. SEYBOLD J, NEWTON R, WRIGHT L, FINNEY PA, SUTTORP N, BARNES PJ, ADCOCK IM, AND GIEMBYCZ MA. Induction of phosphodiesterases 3B, 4A4, 4D1, 4D2, and 4D3 in Jurkat T-cells and in human peripheral blood T-lymphocytes by 8-bromo-cAMP and G_s-coupled receptor agonists. Potential role in β_2-adrenoreceptor desensitization. *J Biol Chem* 273: 20575–20588, 1998.
730. SHAMRAJ OI AND LINGREL JB. A putative fourth Na,K-ATPase α subunit gene is expressed in testis. *Proc Natl Acad Sci USA* 91: 12952–12956, 1994.
731. SHARP GW AND LEAF A. Mechanism of action of aldosterone. *Physiol Rev* 46: 593–633, 1966.
732. SHEIKH-HAMAD D, WANG YP, JO OD, AND YANAGAWA N. Dopamine antagonizes the actions of angiotensin II in renal brush-border membrane. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F737–F743, 1993.
733. SHIMKETS RA, LIFTON R, AND CANESSA CM. In vivo phosphorylation of the epithelial sodium channel. *Proc Natl Acd Sci USA* 95: 3301–3305, 1998.
734. SHULL GE, GREEB J, AND LINGREL JB. Molecular cloning of three distinct forms of the Na,K-ATPase α-subunit from rat brain. *Biochemistry* 25: 8125–8132, 1986.
735. SHULL GE, LANE LK, AND LINGREL JB. Amino-acid sequence of the β-subunit of the (Na^+ + K^+)-ATPase deduced from a cDNA. *Nature* 321: 429–431, 1986.
736. SHULL GE, SCHWARTZ A, AND LINGREL JB. Amino-acid sequence of the catalytic subunit of the (Na^+ + K^+)-ATPase deduced from a complementary DNA. *Nature* 316: 691–695, 1985.
737. SIGA E, CHAMPIGNEULLE A, AND IMBERT-TEBOUL M. cAMP-dependent effects of vasopressin and calcitonin on cytosolic calcium in rat CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F354–F365, 1994.
738. SIMON DB, BINDRA RS, MANSFIELD TA, NELSON-WILLIAMS C, MENDONCA

E, STONE R, SCHURMAN S, NAYIR A, ALPAY H, BAKKALOGLU A, RODRIGUEZ-SORIANO J, MORALES JM, SANJAD SA, TAYLOR CM, PILZ D, BREM A, TRACHMAN H, GRISWOLD W, RICHARD GA, JOHN E, AND LIFTON RP. Mutations in the chloride channel gene, CLCNKB, cause Bartter's syndrome type III. *Nat Genet* 17: 171–178, 1997.

739. SIMON H, GAO Y, FRANKI N, AND HAYS RM. Vasopressin depolymerizes F-actin in rat inner medullary collecting duct. *Am J Physiol Cell Physiol* 265: C757–C762, 1993.
740. SIMON DB, KARET FE, HAMDAN JH, DIPIETRO A, SANJAD SA, AND LIFTON RP. Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-2Cl cotransporter NKCC2. *Nat Genet* 13: 183–188, 1996.
741. SIMON DB, KARET FE, RODRIGUEZ-SORIANO J, HAMDANA JH. DIPIETROH TRACHTMAN H, SANJAD SA, AND LIFTON RP. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K^+ channel, ROMK. *Nat Genet* 14: 152–156, 1996.
742. SIMON DB, LU Y, CHOATE KA, VELAZQUEZ H, EL-SABBAN E, PRAGA M, CASARI G, BETTINELLI A, COLUSSI G, RODRIGUEZ-SORIANO J, MCCREDIE D, MILFORD D, SANJAD S, AND LIFTON RP. Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. *Science* 285: 103–106, 1999.
743. SINGH H AND LINAS S. β_2-Adrenergic function in cultured rat proximal tubule epithelial cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F71–F77, 1996.
744. SINGH H AND LINAS SL. Role of protein kinase C in β_2-adrenoceptor function in cultured rat proximal tubule epithelial cells. *Am J Physiol Renal Physiol* 273: F193–F199, 1997.
745. SIRAGY HM AND CAREY RM. The subtype-2 (AT2) angiotensin receptor regulates renal cyclic guanosine 3′,5′-monophosphate and AT1 receptor-mediated protaglandin E_2 production in conscious rats. *J Clin Invest* 97: 1978–1982, 1996.
746. SIRAGY HM, INAGAMI T, ICHIKI T, AND CAREY RM. Sustained hypersensitivity to angiotensin II and its mechanism in mice lacking the subtype-2 (AT2) angiotensin receptor. *Proc Natl Acad Sci USA* 96: 6506–6510, 1999.
747. SKOU JC. The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim Biophys Acta* 23: 394–401, 1957.
748. SKOU JC. Nobel lecture. The identification of the sodium-potassium pump. *Biosci Rep* 18: 155–169, 1998.
749. SLIVKA SR AND INSEL PA. α_1-Adrenergic receptor-mediated phosphoinositide hydrolysis and prostaglandin E_2 formation in Madin-Darby canine kidney cells. Possible parallel activation of phospholipase C and phospholipase A_2. *J Biol Chem* 262: 4200–4207, 1987.
750. SLIVKA SR, MEIER KE, AND INSEL PA. α_1-Adrenergic receptors promote phosphatidylcholine hydrolysis in MDCK-D1 cells. A mechanism for rapid activation of protein kinase C. *J Biol Chem* 263: 12242–12246, 1988.
751. SLOBODYANSKY E, AOKI Y, GAZNABI AKM, AVILES DH, FILDES RD, AND JOSE PA. Dopamine and protein phosphatase activity in renal proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F279–F284, 1995.
752. SMART ML, HIRANYACHATTADA S, AND HARRIS PJ. Effects of angiotensin II receptor blockade on proximal fluid uptake in the rat kidney. *Br J Pharmacol* 126: 697–700, 1999.
753. SMIGEL MD. Purification of the catalyst of adenylate cyclase. *J Biol Chem* 261: 1976–1982, 1986.
754. SMITH RM, SEELY BL, SHAH N, OLEFSKY JM, AND JARRETT L. Tyrosine kinase-defective insulin receptors undergo insulin-induced microaggregation but do not concentrate in coated pits. *J Biol Chem* 266: 17522–17530, 1991.
755. SNYDER PM, MCDONALD FJ, STOKES JB, AND WELSH MJ. Membrane topology of the amiloride-sensitive epithelial sodium channel. *J Biol Chem* 269: 24379–24383, 1994.
756. SOARES-DA-SILVA P AND FERNANDES MH. Sodium-dependence and ouabain-sensitivity of the synthesis of dopamine in renal tissues of the rat. *Br J Pharmacol* 105: 811–816, 1992.
757. SOARES-DA-SILVA P, PESTANA M, AND FERNANDES MH. Involvement of tubular sodium in the formation of dopamine in the human renal cortex. *J Am Soc Nephrol* 3: 1591–1599, 1993.
758. SOARES-DA-SILVA P, SERRAO MP, AND VIEIRA-COELHO MA. A comparative study on the synthesis of the natriuretic hormone dopamine in OK and LLC-PK_1 cells. *Cell Biol Int* 20: 539–544, 1996.
759. SOARES-DA-SILVA P, SERRAO MP, AND VIEIRA-COELHO MA. Apical and basolateral uptake and intracellular fate of dopamine precursor L-dopa in LLC-PK_1 cells. *Am J Physiol Renal Physiol* 274: F243–F251, 1998.
760. SOKOLOFF P, GIROS B, MARTRES MP, BOUTHENET ML, AND SCHWARTZ JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* 347: 146–151, 1990.
761. SONGYANG Z, CARRAWAY KLI, ECK MJ, HARRISON SC, FELDMAN RA, MOHAMMADI M, SCHLESSINGER J, HUBBARD SR, SMITH DP, ENG C, LORENZO MJ, PONDER BAJ, MAYER BJ, AND CANTLEY LC. Catalytic specificity of protein tyrosine kinases is critical for selective signalling. *Nature* 373: 536–539, 1995.
762. SONNENBERG H, HONRATH U, AND WILSON DR. Effect of vasopressin analog (dDAVP) on potassium transport in medullary collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F986–F991, 1987.
763. SORKIN A AND CARPENTER G. Interaction of activated EGF receptors with coated pits adaptins. *Science* 261: 612–615, 1993.
764. SPINDLER B, MASTROBERARDINO L, CUSTER M, AND VERREY F. Characterization of early aldosterone-induced RNAs identified in A6 kidney epithelia. *Pflügers Arch* 434: 323–331, 1997.
765. SPOONER PM AND EDELMAN IS. Further studies on the effect of aldosterone on electrical resistance of toad bladder. *Biochim Biophys Acta* 406: 304–314, 1975.
766. STANLEY PR, ZHANG F, ZAYAS RM, MUNIYAPPA R, WALSH MF, CRAGOE E, AND SOWERS JR. IGF-I regulation of Na^+-K^+-ATPase in rat arterial smooth muscle. *Am J Physiol Endocrinol Metab* 273: E113–E121, 1997.
767. STANTON BA. Regulation by adrenal corticosteroids of sodium and potassium transport in loop of Henle and distal tubule of rat kidney. *J Clin Invest* 78: 1612–1620, 1986.
768. STANTON B, PUGLISI E, AND GELLAI M. Localization of α_2-adrenoceptor-mediated increase in renal Na^+, K^+, and water excretion. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F1016–F1021, 1987.
769. STAR RA, NONOGUCHI H, BALABAN R, AND KNEPPER MA. Calcium and cyclic adenosine monophosphate as second messengers for vasopressin in the rat inner medullary collecting duct. *J Clin Invest* 81: 1879–1888, 1988.
770. STOCKAND JD, AL-BALDAWI NF, AL-KHALILI OK, WORRELL RT, AND EATON DC. *S*-adenosyl-L-homocysteine hydrolase regulated aldosterone-induced Na^+ transport. *J Biol Chem* 274: 3842–3850, 1999.
771. STOCKAND JD, EDINGER RS, AL-BALDAWI NF, SARIBAN-SOHRABY S, AL-KHALILI OK, EATON DC, AND JOHNSON JP. Isoprenylcysteine-*O*-carboxyl methyltransferase regulates aldosterone-sensitive Na^+ reabsorption. *J Biol Chem* 274: 26912–26916, 1999.
772. STOKES JB. Effect of prostaglandin E_2 on chloride transport across the rabbit thick ascending limb of Henle. Selective inhibition of the medullary portion. *J Clin Invest* 64: 495–502, 1979.
773. STOKES JB. Potassium secretion by cortical collecting tubule: relation to sodium absorption, luminal Na^+ concentration, and transepithelial voltage. *Am J Physiol Renal Fluid Electrolyte Physiol* 241: F395–F402, 1981.
774. STOKES JB. Sodium and potassium transport by the collecting duct. *Kidney Int* 38: 679–686, 1990.
775. STOKES JB, INGRAM MJ, WILLIAMS AD, AND INGRAM D. Heterogeneity of the rabbit collecting tubule: localization of mineralocorticoid hormone action to the cortical portion. *Kidney Int* 20: 340–347, 1981.
776. STOKES JB AND KOKKO JP. Inhibition of sodium transport by protaglandins E_2 across the isolated, perfused rabbit collecting duct. *J Clin Invest* 59: 1099–1104, 1977.
777. STREULI M. Protein tyrosine phosphatases in signaling. *Curr Opin Cell Biol* 8: 182–188, 1996.
778. SUGAYA T, NISHIMATSU SI, TANIMOTO K, TAKIMOTO E, YAMAGISHI T, IMAMURA K, GOTO S, IMAIZUMI K, HISADA Y, OTSUKA A, UCHIDA H, SUGIURA M, FUKUTA K, FUKAMIZU A, AND MURAKAMI K. Angiotensin II type 1a receptor-deficient mice with hypotension and hyperreninemia. *J Biol Chem* 270: 18719–18722, 1995.
779. SUN A, GROSSMAN EB, LOMBARDI M, AND HEBERT SC. Vasopressin alters the mechanism of apical Na^+:Cl^- to Na^+:K^+:$2Cl^-$ cotransport in mouse medullary thick ascending limb. *J Membr Biol* 120: 83–94, 1991.
780. SUN AM, LIU Y, DWORKIN LD, TSE CM, DONOWITZ M, AND YIP KP.

Na^+/H^+ exchanger isoform 2 (NHE2) is expressed in the apical membrane of the medullary thick ascending limb. *J Membr Biol* 160: 85–90, 1997.
781. SUN D AND SCHAFER JA. Dopamine inhibits AVP-dependent Na^+ transport and water permeability in rat CCD via a D_4-like receptor. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F391–F400, 1996.
782. SUN D, WILBORN TW, AND SCHAFER JA. Dopamine D_4 receptor isoform mRNA and protein are expressed in the rat cortical collecting duct. *Am J Physiol Renal Physiol* 275: F742–F751, 1998.
783. SUN XJ, PONS S, ASANO T, MYERS MG JR, GLASHEEN E, AND WHITE MF. The Fyn tyrosine kinase binds Irs-1 and forms a distinct signaling complex during insulin stimulation. *J Biol Chem* 271: 10583–10587, 1996.
784. SUNDARESAN PR, FORTIN TL, AND KELVIE SL. α- and β-adrenergic receptors in proximal tubules of rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F848–F856, 1987.
785. SUZUKI M, MORITA T, HANAOKA K, KAWAGUCHI Y, AND SAKAI O. A Cl channel activated by parathyroid hormone in rabbit renal proximal tubule cells. *J Clin Invest* 88: 735–742, 1991.
786. SWEADNER KJ. Isozymes of the Na^+/K^+-ATPase. *Biochim Biophys Acta* 988: 185–220, 1989.
787. TAKAHASHI T, KAWAHARA Y, OKUDA M, UENO H, TAKESHITA A, AND YOKOYAMA M. Angiotensin II stimulates mitogen-activated protein kinases and protein synthesis by a Ras-independent pathway in vascular smooth muscle cells. *J Biol Chem* 272: 16018–16022, 1997.
788. TAKAHASHI N, KONDO Y, ITO O, IGARASHI Y, OMATA K, AND ABE K. Vasopressin stimulates Cl transport in ascending thin limb of Henle's loop in hamster. *J Clin Invest* 95: 1623–1627, 1995.
789. TAKAICHI K AND KUROKAWA K. Inhibitory guanosine triphosphate-binding protein-mediated regulation of vasopressin action in isolated single medullary tubules of mouse kidney. *J Clin Invest* 82: 1437–1444, 1988.
790. TAKEMOTO F, COHEN HT, SATOH T, AND KATZ AI. Dopamine inhibits Na-K-ATPase in single tubules and cultured cells from distal nephron. *Pflügers Arch* 421: 302–306, 1992.
791. TAKEMOTO F, SATOH T, COHEN HT, AND KATZ AI. Localization of dopamine-1 receptors along the microdissected rat nephron. *Pflügers Arch* 419: 243–248, 1991.
792. TANAKA H, SMOGORZEWSKI M, KOSS M, AND MASSRY SG. Pathways involved in PTH-induced rise in cytosolic Ca concentration of rat renal proximal tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F330–F337, 1995.
793. TANG WJ AND GILMAN AG. Type-specific regulation of adenylyl cyclase by G protein $\beta\gamma$ subunits. *Science* 254: 1500–1503, 1991.
794. TANTI JF, GRÉMEAUX T, VAN OBBERGHEN E, AND MARCHAND-BRUSTEL Y. Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J Biol Chem* 269: 6051–6057, 1994.
795. TAUSSIG R AND GILMAN AG. Mammalian membrane-bound adenylyl cyclases. *J Biol Chem* 270: 1–4, 1995.
796. TAUSSIG R, QUARMBY LM, AND GILMAN AG. Regulation of purified type I and type II adenylyl cyclases by G protein $\beta\gamma$ subunits. *J Biol Chem* 268: 9–12, 1993.
797. TAYLOR SS, BUECHLER JA, AND YONEMOTO W. cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annu Rev Biochem* 59: 971–1005, 1990.
798. TEITELBAUM I. Vasopressin-stimulated phosphoinositide hydrolysis in cultured rat inner medullary collecting duct cells is mediated by the oxytocin receptor. *J Clin Invest* 87: 2122–2126, 1991.
799. TEJEDOR A, NOËL J, VINAY P, BOULANGER Y, AND GOUGOUX A. Characterization and metabolism of canine proximal tubules, thick ascending limbs, and collecting ducts in suspension. *Can J Physiol Pharmacol* 66: 997–1009, 1988.
800. TERADA Y AND KNEPPER MA. Thiazide-sensitive NaCl absorption in rat cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F519–F528, 1990.
801. TERADA Y, TOMITA K, HOMMA MK, NONOGUCHI H, YANG T, YAMADA T, YUASA Y, KREBS EG, AND MARUMO F. Sequential activation of MAP kinase cascade by angiotensin II in opossum kidney cells. *Kidney Int* 48: 1801–1809, 1995.
802. TERADA Y, TOMITA K, NONOGUCHI H, AND MARUMO F. PCR localization of angiotensin II receptor and angiotensinogen mRNAs in rat kidney. *Kidney Int* 43: 1251–1259, 1993.
803. TERADA Y, TOMITA K, NONOGUCHI H, YANG T, AND MARUMO F. Different localization and regulation of two types of vasopressin receptor messenger RNA in microdissected rat nephron segments using reverse transcription polymerase chain reaction. *J Clin Invest* 92: 2339–2345, 1993.
804. THEKKUMKARA TJ, COOKSON R, AND LINAS SL. Angiotensin (AT_{1A}) receptor-mediated increases transcellular sodium transport in proximal tubule cells. *Am J Physiol Renal Physiol* 274: F897–F905, 1998.
805. THERIEN AG, GOLDSHLEGER R, KARLISH SJD, AND BLOSTEIN R. Tissue-specific distribution and modulatory role of the γ subunit of the Na,K-ATPase. *J Biol Chem* 272: 32628–32634, 1997.
806. THERIEN AG, KARLISH SJD, AND BLOSTEIN R. Expression and functional role of the γ subunit of the Na,K-ATPase in mammalian cells. *J Biol Chem* 274: 12252–12256, 1999.
807. THEROUX SJ, LATOUR DA, STANLEY K, RADEN DL, AND DAVIS RJ. Signal transduction by the epidermal growth factor receptor is attenuated by a COOH-terminal domain serine phosphorylation site. *J Biol Chem* 267: 16620–16626, 1992.
808. TODD-TURLA KM, SCHNERMANN J, FEJES-TOTH G, NARAY-FEJES-TOTH A, SMART A, KILLEN PD, AND BRIGGS JP. Distribution of mineralocorticoid and glucocorticoid receptor mRNA along the nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F781–F791, 1993.
809. TOGAWA K, ISHIGURO T, KAYA S, SHIMADA A, IMAGAWA T, AND TANIGUSHI K. Reversible phosphorylation of both Tyr^7 and Tyr^{10} in the α-chain of pig stomach H,K-ATPase by a membrane bound kinase and a phosphatase. *J Biol Chem* 270: 15475–15478, 1995.
810. TOMITA K, NONOGUCHI H, AND MARUMO F. Effects of endothelin on peptide-dependent cyclic adenosine monophosphate accumulation along the nephron segments of the rat. *J Clin Invest* 85: 2014–2018, 1990.
811. TOMITA K, NONOGUCHI H, TERADA Y, AND MARUMO F. Effects of ET-1 on water and chloride transport in cortical collecting ducts of the rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F690–F696, 1993.
812. TOMITA K, OWADA A, IINO Y, YOSHIYAMA N, AND SHIIGAI T. Effect of vasopressin on Na-K-ATPase activity in rat cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F874–F879, 1987.
813. TOMITA K, PISANO JJ, AND KNEPPER MA. Control of sodium and potassium transport in the cortical collecting duct of the rat. Effects of bradykinin, vasopressin, and deoxycorticosterone. *J Clin Invest* 76: 132–136, 1985.
814. TORIKAI S AND KUROKAWA K. Effect of PGE_2 on vasopressin-dependent cell cAMP in isolated single nephron segments. *Am J Physiol Renal Fluid Electrolyte Physiol* 245: F58–F66, 1983.
815. TORIKAI S, WANG MS, KLEIN KL, AND KUROKAWA K. Adenylate cyclase and cell cyclic AMP of rat cortical thick ascending limb of Henle. *Kidney Int* 20: 649–654, 1981.
816. TSE CM, LEVINE SA, YUN CHC, BRANT SR, POUYSSEGUR J, MONTROSE MH, AND DONOWITZ M. Functional characteristics of a cloned epithelial Na/H exchanger (NHE3): resistance to amiloride and inhibition by protein kinase C. *Proc Natl Acad Sci USA* 90: 9110–9114, 1993.
817. TSUCHIDA S, MATSUSAKA T, CHEN X, OKUBO S, NIIMURA F, NISHIMURA H, FOGO A, UTSUNOMIYA H, INAGAMI T, AND ICHIKAWA I. Murine double nullizygotes of the angiotensin type 1A and 1B receptor genes duplicate severe abnormal phenotypes of angiotensinogen nullizygotes. *J Clin Invest* 101: 755–760, 1998.
818. TSUCHIYA K, GIEBISCH G, AND WELLING PA. Aldosterone-dependent regulation of Na-K-ATPase subunit mRNA in the rat CCD: competitive PCR analysis. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F7–F15, 1996.
819. TSUGANEZAWA H, PREISIG PA, AND ALPERN RJ. Dominant negative c-Src inhibits angiotensin II induced activation of NHE3 in OKP cells. *Kidney Int* 54: 394–398, 1998.
820. TUMA PL, FINNEGAN CM, YI JH, AND HUBBARD AL. Evidence for apical endocytosis in polarized hepatic cells: phosphatidylinositide 3-kinase inhibitors lead to the lysosomal accumulation of resident apical plasma membrane proteins. *J Cell Biol* 145: 1089–1102, 1999.
821. TUMLIN JA, HOBAN CA, MEDFORD RM, AND SANDS JM. Expression of

Na-K-ATPase α- and β-subunit mRNA and protein isoforms in the rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F240–F245, 1994.
822. TUMLIN JA, LEA JP, SWANSON CE, SMITH CL, EDGE SS, AND SOMEREN JS. Aldosterone and dexamethasone stimulate calcineurin activity through a transcription-independent mechanism involving steroid receptor-associated heat shock proteins. *J Clin Invest* 99: 1217–1223, 1997.
823. TUMLIN JA AND SANDS JM. Nephron segment-specific inhibition of Na^+/K^+-ATPase activity by cyclosporine A. *Kidney Int* 43: 246–251, 1993.
824. TWAMLEY-STEIN GM, PEPPERKOK R, ANSORGE W, AND COURTNEIDGE SA. The Src family tyrosine kinases are required for platelet-derived growth factor-mediated signal transduction in NIH 3T3 cells. *Proc Natl Acad Sci USA* 90: 7696–7700, 1993.
825. UBEROI NK, HENDRY LB, MULDOON TG, MYERS RB, SEGALOFF A, BRANSOME ED, AND MAHESH VB. Structure-activity relationships of some unique estrogens related to estradiol are predicted by fit into DNA. *Steroids* 45: 325–340, 1985.
826. UCHIDA S AND ENDOU H. Substrate specificity to maintain cellular ATP along the mouse nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 255: F977–F983, 1988.
827. UEDA K, OKAMURA N, HIRAI M, TANIGAWARA Y, SAEKI T, KIOKA N, KOMANO T, AND HORI R. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem* 267: 24248–24252, 1992.
828. UENO S, TAKEDA K, NOGUCHI S, AND KAWAMURA M. Significance of the β-subunit in the biogenesis of Na^+/K^+-ATPase. *Biosci Rep* 17: 173–188, 1997.
829. UMEMURA S, MARVER D, SMYTH DD, AND PETTINGER WA. α_2-Adrenoceptors and cellular cAMP levels in single nephron segments from the rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 249: F28–F33, 1985.
830. UMEMURA S, YAMAGUCHI S, TAMURA K, HIBI K, NYUI N, ISHIGAMI T, KIHARA M, YABANA M, AND ISHII M. Distribution of α_{1B}-adrenergic receptor mRNA expression along rat nephron segments. *Kidney Int* 51: 1548–1552, 1997.
831. UMEMURA S, YASUDA G, UCHINO K, SHINDO T, ISHIKAWA Y, TOYA Y, AND KANEKO Y. Existence of renal α_1- and α_2-adrenoceptors in the human kidney: radioligand binding study in membranes from the human renal cortex and medulla. *J Hypertens* 4 *Suppl*: S222–S225, 1986.
832. UNWIN R, CAPASSO G, AND GIEBISCH G. Bicarbonate transport along the loop of Henle: effects of adrenal steroids. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F234–F239, 1995.
833. USDIN TBBTI, HARTA G, AND MEZEY E. Distribution of parathyroid hormone-2 receptor messenger ribonucleic acid in rat. *Endocrinology* 137: 4285–4297, 1996.
834. VANDER AJ, MALVIN RL, AND WILDE WS. Effects of adrenalectomy and aldosterone on proximal and distal tubular sodium reabsorption. *Proc Soc Exp Biol Med* 99: 323–325, 1958.
835. VASILETS LA. Diversity of regulatory phosphorylation of the Na^+/K^+-ATPase from mammalian kidneys and *Xenopus* oocytes by protein kinases: characterization of the phosphorylation site for protein kinase C. *Cell Physiol Biochem* 7: 1–18, 1997.
836. VENEMA RC, VENEMA VJ, EATON DC, AND MARRERO MB. Angiotensin II-induced tyrosine phosphorylation of signal transducers and activators of transcription 1 is regulated by Janus-activated kinase 2 and Fyn kinases and mitogen-activated protein kinase phosphatase 1. *J Biol Chem* 273: 30795–30800, 1998.
837. VERHEIJEN MH AND DEFIZE LH. Parathyroid hormone inhibits mitogen-activated protein kinase activation in osteosarcoma cells via a protein kinase A-dependent pathway. *Endocrinology* 136: 3331–3337, 1995.
838. VERHEIJEN MHG AND DEFIZE LHK. Parathyroid hormone activates mitogen-activated protein kinase via a cAMP-mediated pathway independent of Ras. *J Biol Chem* 272: 3423–3429, 1997.
839. VERREY F. Antidiuretic hormone action in A6 cells: effect on apical Cl and Na conductances and synergism with aldosterone for NaCl reabsorption. *J Membr Biol* 138: 65–76, 1994.
840. VERREY F, DIGICAYLIOGLU M, AND BOLLINGER U. Polarized membrane movements in A6 kidney cells are regulated by aldosterone and vasopressin/vasotocin. *J Membr Biol* 133: 213–226, 1993.
841. VERREY F, GROSCURTH P, AND BOLLINGER U. Cytoskeletal disruption in A6 kidney cells: impact on endo/exocytosis and NaCl transport regulation by antidiuretic hormone. *J Membr Biol* 145: 193–204, 1995.
842. VERREY F, KRAEHENBÜHL JP, AND ROSSIER BC. Aldosterone induces a rapid increase in the rate of Na,K-ATPase gene transcription in cultured kidney cells. *Mol Endocrinol* 3: 1369–1376, 1989.
843. VERREY F, SCHAERER E, ZOERKLER P, PACCOLAT MP, GEERING K, KRAEHEN-BÜHL JP, AND ROSSIER BC. Regulation by aldosterone of Na^+,K^+-ATPase mRNAs, protein synthesis, and sodium transport in cultured kidney cells. *J Cell Biol* 104: 1231–1237, 1987.
844. WADE JP, O'NEIL RG, PRYOR JL, AND BOULPAEP EL. Modulation of cell membrane area in renal collecting tubules by corticosteroid hormones. *J Cell Biol* 81: 439–445, 1979.
845. WALD H, GARTY H, PALMER LG, AND POPOVTZER MM. Differential regualtion of ROMK expression in kidney cortex and medulla by aldosterone and potassium. *Am J Physiol Renal Physiol* 275: F239–F245, 1998.
846. WANG HY, UNDIE AS, AND FRIEDMAN E. Evidence for the coupling of G_q protein to D_1-like dopamine sites in rat striatum: possible role in dopamine-mediated inositol phosphate formation. *Mol Pharmacol* 48: 988–994, 1995.
847. WANG T AND CHAN YL. Time- and dose-dependent effect of protein kinase C on proximal bicarbonate transport. *J Membr Biol* 117: 131–139, 1990.
848. WANG T, SEGAL AS, GIEBISCH G, AND ARONSON PS. Stimulation of chloride transport by cAMP in rat proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F204–F210, 1995.
849. WANG W, HEBERT SC, AND GIEBISCH G. Renal K^+ channels: structure and function. *Annu Rev Physiol* 59: 413–436, 1997.
850. WANG W, HENDERSON RM, GEIBEL J, WHITE S, AND GIEBISCH G. Mechanism of aldosterone-induced increase of K^+ conductance in early distal renal tubule cells of the frog. *J Membr Biol* 111: 277–289, 1989.
851. WANG WH. Two types of K channel in thick ascending limb of rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F599–F605, 1994.
852. WANG WH. View of K^+ secretion through the apical K channel of cortical collecting duct. *Kidney Int* 48: 1024–1030, 1995.
853. WANG WH AND HEBERT SC. Cytochrome *P*-450 metabolites mediate extracellular Ca^{2+}-induced inhibition of apical K^+ channels in the TAL. *Am J Physiol Cell Physiol* 271: C103–C111, 1996.
854. WANG X, JAISSER F, AND HORISBERGER D. Role in cation translocation of the N-terminus of the α-subunit of the Na^+-K^+ pump of *Bufo*. *J Physiol (Lond)* 491: 579–594, 1996.
855. WANG ZQ, SIRAGY HM, FELDER RA, AND CAREY RM. Intrarenal dopamine production and distribution in the rat. Physiological control of sodium excretion. *Hypertension* 29: 228–234, 1997.
856. WANG ZQ, SIRAGY HM, FELDER RA, AND CAREY RM. Preferential release of renal dopamine into the tubule lumen: effect of chronic sodium loading. *Clin Exp Hypertens* 19: 107–116, 1997.
857. WATERS SB, CHEN D, KAO AW, OKADA S, HOLT KH, AND PESSIN JE. Insulin and epidermal growth factor receptors regulate distinct pools of Grb2-Sos in the control of Ras activation. *J Biol Chem* 271: 18224–18230, 1996.
858. WAY BA AND MOONEY RA. Activation of phosphatidylinositol-3-kinase by platelet-derived growth factor and insulin-like growth factor-1 is inhibited by a transmembrane phosphotyrosine phosphatase. *J Biol Chem* 268: 26409–26415, 1993.
859. WEBSTER MK, GOYA L, AND FIRESTONE GL. Immediate-early transcriptional regulation and rapid mRNA turnover of a putative serine/threonine protein kinase. *J Biol Chem* 268: 11482–11485, 1993.
860. WEBSTER MK, GOYA L, GE Y, MAIYAR AC, AND FIRESTONE GL. Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. *Mol Cell Biol* 13: 2031–2040, 1993.
861. WEHLING M. Rapid effects of aldosterone: a novel concept of nongenomic steroid action evolves. In: *Genomic and Non-genomic Effects of Aldosterone*, edited by Wehling M. Boca Raton, FL: CRC, 1994, p. 109–149.
862. WEHLING M. Specific, nongenomic actions of steroid hormones. *Annu Rev Physiol* 59: 365–393, 1997.
863. WEI J, ZHAO AZ, CHAN GC, BAKER LP, IMPEY S, BEAVO JA, AND STORM

DR. Phosphorylation and inhibition of olfactory adenylyl cyclase by CaM kinase II in neurons: a mechanism for attenuation of olfactory signals. *Neuron* 21: 495–504, 1998.
864. WEIGT M, DIETL P, SILBERNAGL S, AND OBERLEITHNER H. Activation of luminal Na^+/H^+ exchange in distal nephron of frog kidney. An early response to aldosterone. *Pflügers Arch* 408: 609–614, 1987.
865. WEINMAN EJ, DUBINSKY WP, FISHER K, STEPLOCK D, DINH Q, CHANG L, AND SHENOLIKAR S. Regulation of reconstitued renal Na/H exchanger by calcium-dependent protein kinases. *J Membr Biol* 103: 237–344, 1988.
866. WEINMAN EJ, DUBINSKY WP, AND SHENOLIKAR S. Reconstitution of cAMP-dependent protein kinase regulated renal Na-H exchanger. *J Membr Biol* 101: 11–18, 1988.
867. WEINMAN EJ, SANSOM SC, KNIGHT TF, AND SENEKJIAN HO. α and β adrenergic agonist stimulate water absorption in the rat proximal tubule. *J Membr Biol* 69: 107–111, 1982.
868. WEINMAN EJ AND SHENOLIKAR S. Protein kinase C activates the renal apical membrane Na/H exchanger. *J Membr Biol* 93: 133–139, 1986.
869. WEINMAN EJ, SHENOLIKAR S, AND KAHN AM. cAMP-associated inhibition of Na-H exchanger in rabbit kidney brush-border membranes. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F19–F25, 1987.
870. WEINMAN EJ, STEPLOCK D, BUI G, YUAN N, AND SHENOLIKAR S. Regulation of renal Na^+-H^+ exchanger by cAMP-dependent protein kinase. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F1254–F1258, 1990.
871. WEINMAN EJ, STEPLOCK D, AND SHENOLIKAR S. cAMP-mediated inhibition of the renal brush border membrane Na-H exchanger requires a dissociable phosphoprotein cofactor. *J Clin Invest* 92: 1781–1786, 1993.
872. WEINMAN EJ, STEPLOCK D, WANG Y, AND SHENOLIKAR S. Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane Na-H exchanger. *J Clin Invest* 95: 2143–2149, 1995.
873. WEISS BA AND INSEL PA. Intracellular Ca^{2+} and protein kinase C interact to regulate α_1-adrenergic- and bradykinin receptor-stimulated phospholipase A_2 activation in Madin-Darby canine kidney cells. *J Biol Chem* 266: 2126–2133, 1991.
874. WELSH GI, HALL DA, WARNES A, STRANGE PG, AND PROUD CG. Activation of microtubule-associated protein kinase (Erk) and p70 S6 kinase by D_2 dopamine receptors. *J Neurochem* 70: 2139–2146, 1998.
875. WHITE MF. The IRS1 signaling system. *Curr Opin Gen Dev* 4: 47–54, 1994.
876. WHITE MF AND KAHN CR. The insulin signaling system. *J Biol Chem* 269: 1–4, 1994.
877. WIDMANN C, GIBSON S, JARPE MB, AND JOHNSON GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* 79: 143–180, 1999.
878. WIEDERHOLT M, BEHN C, SCHOORMANS W, AND HANSEN L. Effect of aldosterone on sodium and potassium transport in the kidney. *J Steroid Biochem* 3: 151–159, 1972.
879. WIEDERKEHR MR, ZHAO H, AND MOE OW. Acute regulation of Na/H exchanger NHE3 activity by protein kinase C: role of NHE3 phosphorylation. *Am J Physiol Cell Physiol* 276: C1205–C1217, 1999.
880. WIERZBICKI W AND BLOSTEIN R. The amino-terminal segment of the catalytic subunit of kidney Na,K-ATPase regulates the potassium deocclusion pathway of the reaction cycle. *Proc Natl Acad Sci USA* 90: 70–74, 1993.
881. WILBORN TW, DUO S, AND SCHAFER JA. Expression of multiple α-adrenoreceptors isoforms in rat CCD. *Am J Physiol Renal Physiol* 275: F111–F118, 1998.
882. WILCOX CS, CEMERIKIC DA, AND GIEBISCH G. Differential effects of acute mineralo- and glucocorticoisteroid administration on renal acid elimination. *Kidney Int* 21: 546–556, 1982.
883. WILEY HS, HERBST JJ, WALSH BJ, LAUFFENBURGER DA, ROSENFELD MG, AND GILL GN. The role of tyrosine kinase activity in endocytosis, compartmemtation, and downregulation of the epidermal growth factor receptor. *J Biol Chem* 266: 11083–11094, 1991.
884. WILLIAMSON HE. Mechanism of the antinatriuretic action of aldosterone. *Biochem Pharmacol* 12: 1449–1450, 1963.
885. WILLMANN JK, BLEICH M, RIZZO M, SCHMIDT-HIEBER M, ULLRICH KJ, AND GREGER R. Amiloride-inhibitable Na^+ conductance in rat proximal tubule. *Pflügers Arch* 434: 173–178, 1997.
886. WINGO CS, KOKKO JP, AND JACOBSON HR. Effects of in vitro aldosterone on the rabbit cortical collecting tubule. *Kidney Int* 28: 51–57, 1985.
887. WITTNER M AND DI STEFANO A. Effects of antidiuretic hormone, parathyroid hormone and glucagon on transepithelial voltage and resistance of the cortical and medullary thick ascending limb of Henle's loop of the mouse nephron. *Pflügers Arch* 415: 707–712, 1990.
888. WITTNER M, DI STEFANO A, MANDON B, ROINEL N, AND DE ROUFFIGNAC C. Stimulation of NaCl reabsorption by antidiuretic hormone in the cortical thick ascending limb of Henle's loop of the mouse. *Pflügers Arch* 419: 212–214, 1991.
889. WITTNER M, MANDON B, ROINEL N, DE ROUFFIGNAC C, AND DI STEFANO A. Hormonal stimulation of Ca^{2+} and Mg^{2+} transport in the cortical thick ascending limb of Henle's loop of the mouse: evidence for a change in the paracellular pathway permeability. *Pflügers Arch* 423: 387–396, 1993.
890. WONG KR, BERRY CA, AND COGAN MG. Chloride transport in the rat S1 proximal tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F723–F729, 1995.
891. WONG KR, BERRY CA, AND COGAN MG. α_1-Adrenergic control of chloride transport in the rat S1 proximal tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F1049–F1056, 1996.
892. WONG PS AND JOHNS EJ. The action of angiotensin II on the intracellular sodium content of suspensions of rat proximal tubules. *J Physiol (Lond)* 497: 219–227, 1996.
893. WONG PS AND JOHNS EJ. The receptor subtype mediating the action of angiotensin II on intracellular sodium in rat proximal tubules. *Br J Pharmacol* 124: 41–46, 1998.
894. WONG R, HAESLEY L, AO L, AND BERL T. Expression of GTPase-deficient Ras inhibits vasopressin signalling in cultured cortical collecting duct cells. *J Clin Invest* 96: 597–601, 1995.
895. WOO AL, JAMES PF, AND LINGREL JB. Characterization of the fourth α isoform of the Na,K-ATPase. *J Membr Biol* 169: 39–44, 1999.
896. WOODCOCK EA AND JOHNSTON CI. Inhibition of adenylate cyclase by angiotensin II in rat renal cortex. *Endocrinology* 111: 1687–1691, 1982.
897. WOODCOCK EA AND JOHNSTON CI. Selective inhibition by epinephrine of parathyroid hormone-stimulated adenylate cyclase in rat renal cortex. *Am J Physiol Renal Fluid Electrolyte Physiol* 242: F721–F726, 1982.
898. WOODCOCK EA AND JOHNSTON CI. Renal proximal tubular α-adrenergic receptors oppose urinary 3′,5′-cyclic adenosine monophosphate response to parathyroid hormone in vivo. *Endocrinology* 116: 1085–1089, 1985.
899. WORK J, GALLA JH, BOOKER BB, SCHAFER JA, AND LUKE RG. Effect of ADH on chloride reabsorption in the loop of Henle of the Brattleboro rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 249: F698–F703, 1985.
900. WORK J AND JAMISON RL. Effect of adrenalectomy on transport in the rat medullary thick ascending limb. *J Clin Invest* 80: 1160–1164, 1987.
901. WU MS, BENS M, CLUZEAUD F, AND VANDEWALLE A. Role of F-actin in the activation of Na-K-Cl-cotransport by forskolin and vasopressin in mouse kidney cultured thick ascending limb cells. *J Membr Biol* 142: 323–336, 1994.
902. WU MS, BIEMESDERFER D, GIEBISCH G, AND ARONSON PS. Role of NHE_3 in mediating renal brush border Na^+-H^+ exchange. Adaptation to metabolic acidosis. *J Biol Chem* 271: 32749–32752, 1996.
903. WU D, KATZ A, LEE CH, AND SIMON MI. Activation of phospholipase C by α_1-adrenergic receptors is mediated by the α subunits of G_q family. *J Biol Chem* 267: 25798–25802, 1992.
904. WU D, KATZ A, AND SIMON MI. Activation of phospholipase Cβ_2 by the α and $\alpha\gamma$ subunits of trimeric GTP-binding protein. *Proc Natl Acad Sci USA* 90: 5297–5301, 1993.
905. XIAO S, ROSE DW, SASAOKA T, MAEGAWA H, BURKE TR JR, ROLLER PP, SHOELSON SE, AND OLEFSKY JM. Syp (SH-PTP2) is a positive mediator of growth factor-stimulated mitogenic signal transduction. *J Biol Chem* 269: 21244–21248, 1994.
906. XIE MH, LIU FY, WONG PC, TIMMERMANS PB, AND COGAN MG. Proximal nephron and renal effects of DuP 753, a nonpeptide angiotensin II receptor antagonist. *Kidney Int* 38: 473–479, 1990.
907. XING M AND INSEL PA. Protein kinase C-dependent activation of

cytosolic phospholipase A_2 and mitogen-activated protein kinase by alpha$_1$-adrenergic receptors in Madin-Darby canine kidney cells. *J Clin Invest* 97: 1302–1310, 1996.

908. XU JZ, HALL AE, PETERSON LN, BIENKOWSKI MJ, EESSALU TE, AND HEBERT SC. Localization of the ROMK protein on apical membranes of rat kidney nephron segments. *Am J Physiol Renal Physiol* 273: F739–F748, 1997.
909. YAGAWA Y, KAWAKAMI K, AND NAGANO K. Cloning and analysis of the 5′-flanking region of rat Na^+/K^+-ATPase α_1 subunit gene. *Biochim Biophys Acta* 1049: 286–292, 1990.
910. YAMADA H, SEKI G, TANIGUCHI S, UWATOKO S, NOSAKA K, SUSUKI K, AND KUROKAWA K. Roles of Ca^{2+} and PKC in regulation of acid/base transport in isolated proximal tubules. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F1068–F1076, 1996.
911. YAMADA H, SEXTON PM, CHAI SY, ADAM WR, AND MENDELSOHN FA. Angiotensin II receptors in the kidney. Localization and physiological significance. *Am J Hypertens* 3: 250–255, 1990.
912. YAMAGUCHI I, JOSE PA, MOURADIAN MM, CANESSA LM, MONSMA FJ JR, SIBLEY DR, TAKEYASU K, AND FELDER RA. Expression of dopamine D1A receptor gene in proximal tubule of rat kidneys. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F280–F285, 1993.
913. YANG LY, RHEE SG, AND WILLIAMSON JR. Epidermal growth factor-induced activation and translocation of phospholipase C-γ_1 to the cytoskeleton in rat hepatocytes. *J Biol Chem* 269: 7156–7162, 1994.
914. YANG T, HASSAN S, HUANG YG, SMART AM, BRIGGS JP, AND SCHNERMANN JB. Expression of PTHrP, PTH/PTHrP receptor, and Ca^{2+}-sensing receptor mRNAs along the rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 272: F751–F758, 1997.
915. YAO LP, LI XX, YU PY, ASICO LD, AND JOSE PA. Dopamine D_1 receptor and protein kinase C isoforms in spontaneously hypertensive rats. *Hypertension* 32: 1049–1053, 1999.
916. YE MQ AND HEALY DP. Characterization of an angiotensin type-1 receptor partial cDNA from rat kidney: evidence for a novel AT_{1B} receptor subtype. *Biochem Biophys Res Commun* 185: 204–210, 1992.
917. YIP JW, KO WH, VIBERI GC, HUGANIR RL, DONOWITZ M, AND TSE CM. Regulation of the epithelial brush border Na^+/H^+ exchanger isoform 3 stably expressed in fibroblasts by fibroblast growth factor and phorbol esters is not through changes in phosphorylation of the exchanger. *J Biol Chem* 272: 18473–18480, 1997.
918. YOSHITOMI K, NARUSE M, HANAOKA K, YAMAMURA Y, IMAI M, AND KUROKAWA K. Functional characterization of vasopressin V1 and V2 receptors in the rabbit cortical collecting duct. *Kidney Int* 55: S177–S182, 1996.
919. YOUNG WS III AND KUHAR MJ. α_2-Adrenergic receptors are associated with renal proximal tubules. *Eur J Pharmacol* 67: 493–495, 1980.
920. YU H, LI X, MARCHETTO GS, DY R, HUNTER D, CALVO B, DAWSON TL, WILM M, ANDEREGG RJ, GRAVES LM, AND EARP HS. Activation of a novel calcium-dependent protein-tyrosine kinase. Correlation with c-Jun N-terminal kinase but not mitogen-activated protein kinase activation. *J Biol Chem* 271: 29993–29998, 1996.
921. YU PY, ASICO LD, EISNER GM, AND JOSE PA. Differential regulation of renal phospholipase C isoforms by catecholamines. *J Clin Invest* 95: 304–308, 1995.
922. YU PY, EISNER GM, YAMAGUCHI I, MOURADIAN MM, FELDER RA, AND JOSE PA. Dopamine D_{1A} receptor regulation of phospholipase C isoforms. *J Biol Chem* 271: 19503–19508, 1996.
923. YUN CH, LAMPRECHT G, FORSTER DV, AND SIDOR A. Nhe3 kinase A regulatory protein E3KARP binds the epithelial brush border Na^+/H^+ exchanger NHE3 and the cytoskeletal protein ezrin. *J Biol Chem* 273: 25856–25863, 1998.
924. YUN CHC, OH S, ZIZAK M, STEPLOCK D, TSAO S, TSE CM, WEINMAN EJ, AND DONOWITZ M. cAMP-mediated inhibition of the epithelial brush border Na^+/H^+ exchanger, NHE3, requires an associated regulatory protein. *Proc Natl Acad Sci USA* 94: 3010–3015, 1997.
925. YUN CHC, TSE CM, AND DONOWITZ M. Chimeric Na^+/H^+ exchangers: an epithelial membrane-bound N-terminal domain requires an epithelial cytoplasmic C-terminal domain for regulation with protein kinases. *Proc Natl Acad Sci USA* 92: 10723–10727, 1995.
926. YUN CHC, TSE CM, LEVINE SK, BRANT SR, AND DONOWITZ M. Mammalian Na^+/H^+ exchanger gene family: structure and function studies. *Am J Physiol Gastrointest Liver Physiol* 269: G1–G11, 1995.
927. ZACHARY I, GIL J, LEHMANN W, SINNETT-SMITH J, AND ROZENGURT E. Bombesin, vasopressin, and endothelin rapidly stimulate tyrosine phosphorylation in intact Swiss 3T3-cells. *Proc Natl Acad Sci USA* 88: 4577–4581, 1991.
928. ZHANG Y, NORIAN JM, MAGYAR CE, HOLSTEIN-RATHLOU NH, MIRCHEFF AK, AND MCDONOUGH AA. In vivo PTH provokes apical NHE3 and NaPi2 redistribution and Na-K-ATPase inhibition. *Am J Physiol Renal Physiol* 276: F711–F719, 1999.
929. ZHAO H, WIEDERKER MR, FAN L, COLLAZO RL, CROWDER LA, AND MOE OW. Acute inhibition of Na/H exchanger NHE-3 by cAMP. Role of protein kinase A and NHE-3 phosphoserines 552 and 605. *J Biol Chem* 274: 3978–3987, 1999.
930. ZHEN X, URYU K, WANG HY, AND FRIEDMAN E. D_1 dopamine receptor agonists mediate activation of p38 mitogen-activated protein kinase and c-Jun amino-terminal kinase by a protein kinase A-dependent mechanism in SK-N-MC human neuroblastoma cells. *Mol Pharmacol* 54: 453–458, 1998.
931. ZHUO J, THOMAS D, HARRIS PJ, AND SKINNER SL. The role of endogenous angiotensin II in the regulation of renal haemodynamics and proximal fluid reabsorption in the rat. *J Physiol (Lond)* 453: 1–13, 1992.
932. ZIZAK M, LAMPRECHT G, STEPLOCK D, TARIQ N, SHENOLIKAR S, DONOWITZ M, YUN CHC, AND WEINMAN EJ. cAMP-induced phosphorylation and inhibition of Na^+/H^+ exchanger 3 (NHE3) are dependent on the presence but not the phosphorylation of NHE regulatory factor. *J Biol Chem* 274: 24753–24758, 1999.
933. ZOU Y, KOMURO I, YAMAZAKI T, AIKAWA R, KUDOH S, SHIOJIMA I, HIROI Y, MIZUNO T, AND YASAKI Y. Protein kinase C, but not tyrosine kinases or ras, plays a critical role in angiotensin II-induced activation of Raf-1 kinase and extracellular signal-regulated protein kinases in cardiac myocytes. *J Biol Chem* 271: 33592–33597, 1996.
934. ZUSMAN RM AND KEISER HR. Prostaglandin E_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture: mechanism of stimulation by angiotensin II, bradykinin, and arginine vasopressin. *J Biol Chem* 252: 2069–2071, 1977.