**NA-K ATPASE IS RESTRICTED** to the basolateral membrane domain of renal epithelial cells, where it provides the driving force for the vectorial transport of various solutes and ions (28). In renal proximal tubules, the activity of Na-K ATPase is regulated by several signal transduction pathways, including protein kinase A (PKA), protein kinase C (PKC), and various eicosanoids (4–16, 19–21, 23, 32, 34–37, 41). Dopamine (DA) and parathyroid hormone (PTH) inhibit the activity of Na-K ATPase in renal proximal tubular cells through similar and dissimilar pathways (1–3, 12, 14, 17, 38–40, 45). DA stimulates two classes of receptors, DA₁ and DA₂, which couple to Gₛ and Gᵢ (26), whereas PTH inhibits the activity of Na-K ATPase through stimulation of a single class of PTH/PTHrP receptors that are coupled to Gₛ and Gᵢ/G₁₁ (27). DA inhibits Na-K ATPase activity through activation of at least two parallel pathways, the effects of which on Na-K ATPase vary depending on the duration of treatment (1–3, 9, 17, 38). Short-term inhibition of Na-K ATPase activity by DA is dependent on stimulation of DA₁ and DA₂ receptors through activation of the phospholipase C (PLC)-PKC cascade. However, long-term inhibition of Na-K ATPase activity by DA occurs through a PKA-dependent pathway. PTH also inhibits Na-K ATPase initially through activation of PKC (14, 39–40) but through PKA-dependent pathways in the long term (38). Both DA and PTH inhibit Na-K ATPase activity by the activation of calcium-independent phospholipase A₂ (PLA₂), which releases arachidonic acid (AA). AA is metabolized by cytochrome P-450 to ω-hydroxyeicosatetraenoic acid (20-HETE). 20-HETE regulates rat renal Na-K ATPase via PKC activation (34).

Our laboratory has previously shown that, in opossum kidney (OK) cells, a model of renal proximal tubule, PTH and DA regulation of sodium-dependent phosphate transport differ in magnitude and duration despite activation of many similar signal transduction pathways (29). We have attributed these differences to the fact that PTH and DA also activate some dissimilar pathways. PTH, but not DA, regulates sodium-dependent phosphate transport through activation of the mitogen-activated protein kinase, i.e., extracellular signal-regulated kinase (ERK) (30). DA, but not PTH, inhibits sodium-dependent phosphate uptake by a pertussis toxin-sensitive pathway. In preliminary experiments, we have demonstrated that inhibition of Na-K ATPase by 1 μM ouabain for 30 min inhibits sodium-dependent phosphate uptake (21.83 ± 2.18 vs. 8.80 ± 0.14 nmol Pi·mg protein⁻¹·min⁻¹, n = 2) in OK cells, confirming that Na-K ATPase activity contributes to sodium-dependent phosphate transport. We speculated that differences in the regulation of Na-K ATPase by PTH and DA could partially explain the differences in mechanisms of regulation of phosphate uptake by these agonists. The present study was undertaken to compare regulation of inhibition of Na-K ATPase by PTH and DA in OK cells. We hypothesized that PTH and DA would regulate Na-K ATPase through divergent pathways.
gent signaling mechanisms, similar to differences in regulation of phosphate transport.

**MATERIALS AND METHODS**

**Materials.** Wild-type OK cells were a generous gift from Dr. Steven Scheinman (Health Sciences Center, Syracuse, NY). Antibody against the α-subunit of the OK cell Na-K ATPase was obtained from RBI (Natick, MA). Phosphoserine antibody was purchased from Zymed Laboratories (San Francisco, CA). Antibodies to PKC isoforms were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Bovine parathyroid hormone [PTH(1–34)] was obtained from Bachem (Philadelphia, PA). DA, PD-098059, and calphostin C were purchased from Calbiochem. H89 was purchased from Biomol Research Laboratories (Plymouth, PA). Bromo- nol lactone (BEL) and 20-HETE were obtained from Cayman Chemicals (Ann Arbor, MI). ATP, ouabain, AA, and ferrous sulfate were purchased from Sigma (St. Louis, MO). Other reagents were of the highest quality available.

**Cell culture.** Wild-type OK cells, passages 83–89, were grown in monolayer in 75-cm² plastic flasks (Falcon) or 3 × 10-mm tissue culture plates (Nunc) in Eagle’s medium with Earle’s salts (GIBCO BRL Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum, 4 mM glutamine, 100 μg/ml streptomycin, and 100 IU/ml penicillin in a humidified 5% CO₂-95% air environment at 37°C. They were fed three times a week and split 1:4 once a week by trypsinization and dispersal. Cells were used for experiments at 100% confluence. They were washed the evening before use. For experiments requiring preincubation with specific inhibitor agents, these agents were added for the designated period of time at 37°C, followed by the addition of the signaling pathway activator also for the designated period of time. The inhibitor was present during the entire incubation period. The reaction was stopped by washing with ice-cold Hanks’ basic salt solution (HBSS) followed by homogenization. Phosphatase inhibitors were not present.

**Membrane isolation.** The cells were lysed in 50 mM mannitol-5 mM Tris-HEPES buffer, pH 7.4, and centrifuged at 750 g for 10 min. The pellet was again resuspended in 100 mM mannitol-5 mM Tris-HEPES buffer, pH 7.4, and centrifuged at 750 g for 15 min. The supernatant was removed and centrifuged at 48,000 g in an Ultracentrifuge (Beckman) for 30 min. The pellet derived from centrifugation of the supernatant was resuspended in 50% sucrose, using a dounce homogenizer. The sample was overlaid on a discontinuous sucrose gradient, made by mixing 12 ml 38% sucrose with 5 ml 41% sucrose, and centrifuged at 88,000 g for 60 min, in an appropriate dilution of primary antibody in HBSS and homogenized using a high-speed homogenizer (Powergen 125, Fisher Scientific). MgCl₂ was added in a final concentration of 10 mM to the homogenate and incubated for 20 min on ice with occasional stirring. The homogenate was centrifuged at 2,500 g for 5 min at 4°C. The pellet was resuspended in 100 mM Mannitol-5 mM Tris-HEPES buffer, and MgCl₂ was added to a final concentration of 15 mM. The suspension was incubated for 20 min on ice with occasional stirring and centrifuged at 2,500 g for 5 min. The pellet was again resuspended in 100 mM mannitol-5 mM Tris-HEPES buffer, pH 7.4, and centrifuged at 750 g for 15 min. The supernatant was removed and centrifuged at 48,000 g in an Ultracentrifuge (Beckman) for 3 h. The upper layer was carefully collected and resuspended in 1 mM bicarbonate buffer, pH 7.5, and centrifuged at 48,000 g in a Beckman Ultracentrifuge for 30 min. The pellet was resuspended in 300 mM mannitol-5 mM Tris-HEPES buffer, pH 7.4 (33). The basolateral membranes were characterized by the five- to eightfold enrichment of Na-K ATPase (data not shown).

**Immunoblots.** OK cell membrane proteins were solubilized in Laemmli sample buffer, separated by 10% SDS-PAGE, and transferred electrophoretically to a nitrocellulose membrane. The nitrocellulose sheet was incubated with 5% milk in 20 mM Tris and 50 mM NaCl (TBS) at room temperature for 60 min, in an appropriate dilution of primary antibody in TBS with 0.2% Tween (TTBS) for at least 60 min at room temperature or overnight at 4°C, and an appropriate concentration of horseradish peroxidase-linked goat anti-rabbit IgG (or secondary antibody of appropriate species) for 50 min at room temperature, washed, and developed by chemiluminescence assay (Biolab). The developed blots were scanned and analyzed using a Meridian densitometer and expressed as densitometric units.

**Immunoprecipitation of Na-K ATPase.** The crude membranes were precleared with protein A-Sepharose beads for 2 h at 4°C and were incubated with 1 ng of rabbit polyclonal antibodies against the α-subunit of Na-K ATPase (Research Diagnostics, Flanders, NJ) overnight at 4°C. Antibody A-Sepharose beads were added and incubated for 2 h at 4°C. The beads were washed three times with 1× PBS, and an equal volume of 2× Laemmli sample buffer was added and boiled for 5 min. The beads were centrifuged, and the proteins were separated by 10% SDS-PAGE, transferred to nitrocellulose membranes, and blotted against phosphoserine antibodies (Zymed Laboratories, San Francisco, CA).

**Statistics.** Data are shown as means ± SE. The n values shown represent the number of separate experiments. Each experiment was done in triplicate. The P value was calculated using SigmaStat software utilizing Student’s t-test. A P

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value <0.05 was a priori considered as statistically significant.

RESULTS

Effect of PKA and PKC activation on Na-K ATPase activity in OK cells. Activation of PKC decreases the activity of Na-K ATPase in proximal tubular cells. The effect of activation of PKA in proximal tubule cells is somewhat controversial, with some studies suggesting an increase in activity (10, 23) whereas others suggest a decrease (1, 9). To determine the effect of activation of PKA or PKC on Na-K ATPase activity in OK cells, the cells were treated with \(10^{-6}\) M 8-bromoadenosine 3',5'-cyclic monophosphate (8-BrcAMP), a phosphodiesterase-resistant cAMP analog that directly activates PKA, or \(10^{-4}\) M phorbol 12-myristate 13 acetate (PMA), a phorbol ester that directly activates PKC, for 15 min or 2 h. Activation of PKA by 8-BrcAMP caused a time-dependent decrease in the activity of Na-K ATPase. Activation of PKC by PMA caused maximum inhibition of Na-K ATPase activity after a 15-min incubation, and the degree of inhibition did not change after 2 h of incubation (Fig. 1, A and B).

PTH and DA inhibit Na-K ATPase in OK cells. In renal proximal tubules, PTH and DA regulate Na-K ATPase through biphasic signaling pathways. PTH inhibits Na-K ATPase activity by 15–30% after short-term (15 min) (39, 40) and by 50% after long-term (2 h) incubation (38). DA inhibits Na-K ATPase activity by 35–40% after short- and long-term incubation (1, 3, 9, 17, 38). To confirm that these agonists had similar effects in OK cells, we measured Na-K ATPase activity in OK cell membranes after 15-min and 2-h incubation with \(10^{-7}\) M PTH(1–34) and \(10^{-5}\) M DA. PTH inhibited Na-K ATPase activity by 35% (21.00 ± 1.37 vs. 13.74 ± 0.46 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), \(n = 3, P < 0.005\)) after 15 min and by 57% (22.45 ± 1.82 vs. 9.73 ± 0.99 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), \(n = 3, P < 0.005\)) after 2-h incubation (Fig. 2, A and B). DA resulted in 32% (21.00 ± 1.37 vs. 14.25 ± 0.41 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), \(n = 3, P < 0.005\)) inhibition after 15 min and 59% (22.45 ± 1.82 vs. 9.28 ± 0.70 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), \(n = 3, P < 0.005\)) inhibition of Na-K ATPase activity after 2 h incubation (Fig. 2, A and B).

Signaling pathways involved in regulation of Na-K ATPase. In renal proximal tubules, PTH and DA inhibit Na-K ATPase activity through multiple signaling pathways. To examine the pathways responsible for PTH and DA inhibition of Na-K ATPase in OK cells, we measured Na-K ATPase inhibition in cells treated with specific inhibitors of several signaling pathways. To determine whether DA and PTH regulate Na-K ATPase activity through a PKC-dependent mechanism, the cells were pretreated with \(10^{-6}\) M calphostin C for 30 min in the presence of light at 37°C, required for activation. Calphostin C alone had no significant effect on basal Na-K ATPase activity. Pretreatment with calphostin C completely blocked the ability of both PTH and DA to inhibit Na-K ATPase activity at 15 min (Fig. 3A). Calphostin C caused less (60 and 60%) but still significant inhibition of the effect of both DA and PTH on Na-K ATPase activity after 2 h (Fig. 3B). We confirmed these results in cells preincubated with another PKC inhibitor, Ro 81-322 (Ro; \(10^{-5}\) M) for 1 h before treatment with PTH or DA for 15 min or 2 h. Ro alone had no effect on Na-K ATPase activity after short-term (27.38 vs. 29.6 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), Ro vs. control, \(n = 2\)) and long-term (31.12 vs. 27.12 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), Ro vs. control, \(n = 2\)) incubation. Ro completely blocked PTH and DA inhibition of Na-K ATPase activity at 15 min (Fig. 3B, A and B). PTH and DA inhibited Na-K ATPase activity at 15 min (Fig. 3B, A and B). The results suggest that immediate inhibition

\[\text{Na-K ATPase activity} = \text{activity in control} - \text{activity in agonist-treated cells} \]

\[\text{P_i released} = \text{nmol Pi released} \]

\[\text{P < 0.01, Na-K ATPase activity significantly different from control.} \]

\[\text{Effect of PKA and PKC activation on Na-K ATPase activity in OK cells.} \]

\[\text{Activation of PKC decreases the activity of Na-K ATPase in proximal tubular cells. The effect of activation of PKA in proximal tubule cells is somewhat controversial, with some studies suggesting an increase in activity (10, 23) whereas others suggest a decrease (1, 9).} \]

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\[\text{Signaling pathways involved in regulation of Na-K ATPase. In renal proximal tubules, PTH and DA inhibit Na-K ATPase activity through multiple signaling pathways. To examine the pathways responsible for PTH and DA inhibition of Na-K ATPase in OK cells, we measured Na-K ATPase inhibition in cells treated with specific inhibitors of several signaling pathways. To determine whether DA and PTH regulate Na-K ATPase activity through a PKC-dependent mechanism, the cells were pretreated with} \ 10^{-6}\ M \text{calphostin C for 30 min in the presence of light at 37°C, required for activation. Calphostin C alone had no significant effect on basal Na-K ATPase activity. Pretreatment with calphostin C completely blocked the ability of both PTH and DA to inhibit Na-K ATPase activity at 15 min (Fig. 3A). Calphostin C caused less (60 and 60%) but still significant inhibition of the effect of both DA and PTH on Na-K ATPase activity after 2 h (Fig. 3B). We confirmed these results in cells preincubated with another PKC inhibitor, Ro 81-322 (Ro;} \ 10^{-5}\ M) \text{for 1 h before treatment with PTH or DA for 15 min or 2 h. Ro alone had no effect on Na-K ATPase activity after short-term (27.38 vs. 29.6 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), Ro vs. control,} \ n = 2\) and long-term (31.12 vs. 27.12 nmol P_i released mg protein\(^{-1}\).h\(^{-1}\), Ro vs. control,} \ n = 2\) incubation. Ro completely blocked PTH and DA inhibition of Na-K ATPase activity at 15 min (Fig. 3B, A and B).} \]

\[\text{The results suggest that immediate inhibition} \]

\[\text{of} \ \text{Na-K ATPase} \]

\[\text{activity by} \ 8\text{-bromoadenosine 3',5'-cyclic monophosphate (8-BrcAMP) and phorbol 12-myristate 13 acetate (PMA).} \]

\[\text{Opossum kidney (OK) cell monolayers in} \ 35 \times 10\text{-mm plates were incubated in medium containing vehicle,} \ 10^{-4}\ M \text{8-BrcAMP, or} \ 10^{-6}\ M \text{PMA for either 15 min (A) or 2 h (B). Na-K ATPase activity was measured in the membranes as described in MATERIALS AND METHODS. Each assay was performed in triplicate, averaged, and considered as a single data point. Values are means ± SE expressed as nmol P_i released mg protein\(^{-1}\).h\(^{-1}\).} \]
of Na-K ATPase by PTH and DA is PKC dependent but that long-term inhibition is only partially PKC dependent.

To determine whether PTH or DA inhibited Na-K ATPase by a PKA-dependent mechanism, the cells were treated with 10^{-4} M H-89, a PKA inhibitor, for 30 min before incubation with PTH and DA. H-89 at that concentration completely inhibited the activity of PKA as measured by a Peptag PKA kit (Promega; data not shown). H-89 alone did not significantly change basal Na-K ATPase activity. Pretreatment with H-89 had no effect on the inhibition of Na-K ATPase activity by either PTH or DA after 15-min incubation (Fig. 4A). However, pretreatment with H-89 caused significant attenuation of the effect of both PTH (83%) and DA (76%) on Na-K ATPase activity after 2-h incubation (Fig. 4B). These results were confirmed in cells pretreated with the PKA inhibitor Rp diastereomer of cAMP (data not shown). The results indicate that short-term inhibition of Na-K ATPase by PTH and DA is independent of PKA. However, long-term inhibition of Na-K ATPase by PTH and DA is dependent on PKA activation.

Previous studies have demonstrated that DA and PTH inhibit the activity of Na-K ATPase through a PLA2 pathway in renal proximal tubular cells. To determine whether DA and/or PTH regulates the activity of Na-K ATPase by the PLA2 pathway in OK cells, the cells were treated for 10 min with 1 \mu M/ml of the PLA2 suicide inhibitor BEL before treatment with PTH and DA. BEL alone did not significantly change basal Na-K ATPase activity (data not shown). Pretreatment with BEL significantly attenuated the effect of both PTH (102 and 73%) and DA (104 and 84%) after 15-min and 2-h incubation, respectively (Fig. 5, A and B). The results suggest that, similar to proximal tubular cells, in OK cells both PTH and DA inhibit Na-K ATPase activity through the activation of PLA2.
We have previously demonstrated that PTH, but not DA, inhibits phosphate transport through an ERK-dependent pathway (30). To determine whether similar pathways are activated in OK cells, OK cell monolayers were treated with 10 nM pertussis toxin (an inhibitor of Gi) overnight before treatment with PTH and DA. Pretreatment with pertussis toxin had no effect on basal activity of Na-K ATPase. Pertussis toxin partially but significantly inhibited the effect of PTH (70%) at 15-min incubation (Fig. 7A) but did not inhibit the effect of PTH on the activity of Na-K ATPase after 2-h incubation (Fig. 7B). However, pertussis toxin completely blocked the effect of DA after both 15-min and 2-h incubation (Fig. 7, A and B).

DA regulates Na-K ATPase through both DA1 and DA2 receptors in proximal tubules. To determine the DA receptor(s) responsible for inhibition of Na-K ATPase in

**Fig. 4. Effect of H-89 on the inhibition of Na-K ATPase activity by DA and PTH.** OK cell monolayers in 35 × 10-mm plates were incubated in medium containing vehicle, or 10−4 M H-89 for 30 min before treatment with 10−6 M DA, or 10−7 M PTH for either 15 min (A) or 2 h (B). Na-K ATPase activity was measured in the membranes as described in MATERIALS AND METHODS. Each assay was performed in triplicate, averaged, and considered as a single data point. Values are means ± SE expressed as nmol P i released/mg protein•h−1 (n = 3 separate experiments). *P < 0.01, Na-K ATPase activity significantly different from control.

**Fig. 5. Effect of bromoenol lactone (BEL) on the inhibition of Na-K ATPase activity by DA and PTH.** OK cell monolayers in 35 × 10-mm plates were incubated in medium containing vehicle, or 1 μM BEL for 10 min before treatment with 10−6 M DA, or 10−7 M PTH for either 15 min (A) or 2 h (B). Na-K ATPase activity was measured in the membranes as described in MATERIALS AND METHODS. Each assay was performed in triplicate, averaged, and considered as a single data point. Values are means ± SE expressed as nmol P i released/mg protein•h−1 (n = 3 separate experiments). *P < 0.01, Na-K ATPase activity significantly different from control.
OK cells, the cells were treated with 10^{-5} M SCH-23390 (DA_{1} antagonist) or 10^{-7} M sulpiride (DA_{2} antagonist) for 30 min before treatment with 10^{-5} M DA, or 10^{-7} M PTH for either 15 min (A) or 2 h (B). Na-K ATPase activity was measured in the membranes as described in MATERIALS AND METHODS. Each assay was performed in triplicate, averaged, and considered as a single data point. Values are means ± SE expressed as nmol Pi released·mg protein^{-1}·h^{-1} (n = 3 separate experiments). *P < 0.01, Na-K ATPase activity significantly different from control.

Mapping agonist-stimulated pathways involved in regulation of Na-K ATPase. The previous results implicate several signaling molecules in the regulation of Na-K ATPase: PKA, PKC, and PLA2 in the case of DA and PKA, and PKC, PLA2, and ERK in the case of PTH. The requirement for PKC activation in the short-term regulation of Na-K ATPase suggests that PKC activation may be the initiating step. To map out the remainder of the pathway, OK cells were stimulated with specific activators of PKC or with PLA2 metabolites after pretreatment with specific inhibitors of the other pathways.

Figures 9, A and B, show the effects of inhibitors of PLA2 and ERK on regulation of Na-K ATPase by PKC activated by 10^{-6} M PMA or by 10^{-6} M PTH(3–34) (a PTH analog that activates PKC but not PKA). Both PMA and PTH(3–34) produced the expected inhibition of Na-K ATPase activity after 15-min incubation. The inhibition by both agents was blocked by preincubation with either the PLA2 inhibitor BEL or the ERK inhibitor PD-098059. These results suggest that PKC inhibits Na-K ATPase through PLA2- and ERK-dependent pathways.

To define the PLA2-dependent pathway, OK cells were treated with two metabolites of PLA2 activity, AA or 20-HETE, under control conditions and after pretreatment with a PKC inhibitor, calphostin C, or an ERK inhibitor, PD-098059. Both AA and 20-HETE inhibited Na-K ATPase activity, as seen in Fig. 10, A and B. Pretreatment with PD-098059 had no effect on

Fig. 7. Effect of pertussis toxin on the inhibition of Na-K ATPase activity by DA and PTH. OK cell monolayers in 35 × 10-mm plates were incubated in medium containing vehicle, or 10 mM pertussis toxin overnight before treatment with 10^{-5} M DA, or 10^{-7} M PTH for either 15 min (A) or 2 h (B). Na-K ATPase activity was measured in the membranes as described in MATERIALS AND METHODS. Each assay was performed in triplicate, averaged, and considered as a single data point. Values are means ± SE expressed as nmol Pi released·mg protein^{-1}·h^{-1} (n = 3 separate experiments). *P < 0.01, Na-K ATPase activity significantly different from control.
the inhibitory effect of either metabolite; however, pre-
treatment with calphostin C completely abolished in-
hibition of Na-K ATPase by AA and 20-HETE.

Mechanisms of short- and long-term regulation of
Na-K ATPase. Previous investigators have demonstrated
that short-term regulation of Na-K ATPase by DA in
proximal tubule cells is dependent on phosphorylation
of the α-subunit followed by endocytosis into clathrin-
coated vesicles. These processes ultimately result in
decreased expression of the subunit, producing long-
term inhibition of Na-K ATPase. To determine whether
PTH, like DA, decreased expression of the α-subunit in
OK cells, we immunoblotted membrane proteins from
OK cells for the α-subunit of Na-K ATPase and for phos-
phoserine after treatment with PTH(1-34), PTH(3-34),
DA, 20-HETE, PMA, and 8-BrcAMP. Figure 11A shows
that 15-min incubation with all agonists did not alter the
expression of Na-K ATPase. Immunoblotting with the
phosphoserine antibody in Na-K ATPase-immunopre-
cipitated membranes showed increased phosphorylation,
especially with PTH(1-34), PTH(3-34), DA, 20-HETE,
and AA (Fig. 11B). Figure 11C shows the results of 2-h
incubation, demonstrating a marked decrease in expres-
sion of the α-subunit in response to all agonists. Conse-
sequently, the phosphoserine immunoblot shows no phos-
phorylation (Fig. 11D).

Effect of PTH and DA on basolateral membrane
expression of Na-K ATPase. For the study of the effect
of PTH and DA on basolateral membrane expression of
Na-K ATPase, cells were grown on membrane inserts
placed in six-well plates to optimize cell polarization
and treated with PTH(1-34 or 3-34) and DA. Immu-
noblot analysis of isolated basolateral membranes
showed that DA, but not PTH, decreased the basolat-
eral expression of Na-K ATPase (Fig. 12) after 15 min
of treatment.

DISCUSSION

The function of the renal Na-K ATPase is central to
the regulation of all transport processes in the prox-
imal renal tubule. Although many hormones regulate
transport processes through alterations in specific
membrane transporter expression and function, sev-
eral hormones exert a more global regulation of proximal renal tubule transport through alterations in the activity of the Na-K ATPase. For example, insulin (18) and norepinephrine (35) increase Na-K ATPase activity, thereby increasing proximal renal tubule sodium reabsorption and preserving extracellular fluid volume. In contrast, DA (2) and PTH (43) inhibit Na-K ATPase activity, resulting in decreased proximal renal tubule sodium reabsorption and decreased extracellular fluid volume. Not surprisingly, the pathways regulating Na-K ATPase activity are numerous, often redundant, and often antagonistic. Furthermore, regulatory mechanisms activated by several of the hormones can differ depending on the length of time of exposure to specific hormonal influences.

Previous publications investigating the regulation of Na-K ATPase in several models of proximal renal tubule, including OK cells, have yielded conflicting results. Some investigators have demonstrated an increase in Na-K ATPase activity after activation of PKA or PKC, whereas others have demonstrated a decrease. Our studies concur with the latter result. The explanation for these conflicting results is not immediately apparent, but they may be due to differences in the technique for measuring Na-K ATPase activity or differences in the several models of proximal tubule. Many of the reports showing an increase in Na-K ATPase measure activity as rubidium uptake, whereas we have measured enzyme activity directly in membrane preparations. Discrepancies in methodology, however, cannot fully explain this controversy, as the opposing effects on Na-K ATPase by PKA and PKC have been confirmed by demonstrating concurrent appropriate increases or decreases in epithelial transport. In some instances, different time points have been chosen for measurement of Na-K ATPase. We have chosen 15 min and 2 h on the basis of previous reports demonstrating the time course for phosphorylation of subunits (short-term regulation) and decreased expression of subunits (long-term regulation).

It is of interest to compare the mechanisms of DA and PTH on the regulation of the proximal renal tubule Na-K ATPase, as both agonists inhibit the activity of the Na-K ATPase, inhibit sodium-dependent hydrogen exchange, and inhibit sodium-dependent phosphate uptake. Because the characteristics of their inhibition of phosphate transport differ significantly, we considered the possibility that differences in the regulation of Na-K ATPase by DA and PTH could account for some of these differences (3, 29). In fact, we did find significant similarities and differences in the mechanisms activated by PTH and by DA to inhibit Na-K ATPase in OK cells. PTH and DA inhibited Na-K ATPase similarly after 15 min and 2 h. Our findings agree with previous reports showing a biphasic inhibition of Na-K ATPase activity by PTH and DA. The initial, short-term inhibition is mediated by PKC, whereas the long-term inhibition is mediated predominantly by PKA. For both PTH and DA, short-term inhibition is associated with an increase in phosphoserine labeling of the Na-K ATPase α-subunit, whereas long-term inhibition is accompanied by downregulation of the subunit expression. Our findings confirm previous reports that short-term regulation by PTH is quantitatively less than that seen with long-term regulation (38–40). However, previous reports have failed to show as we have here that short-term regulation by DA is also quantitatively less than long-term regulation (38). The reasons for this discrepancy are not clear. One possibility is that the concentration of DA used activates α-adrenergic receptors as well as DA receptors, which might have the opposite effects on Na-K ATPase activity (29). This would not explain why the inhibition of Na-K ATPase activity by DA differs at the two time points, unless the effect of α-adrenergic stimulation on Na-K ATPase is transient. Our studies also confirm that short-term and long-term regulation of Na-K ATPase activity by PTH and DA were dependent on PLA2 activity (14, 34, 36) in OK cells, a situation similar to renal tubular cells. We have demonstrated that inhibition of Na-K ATPase by DA involves both DA1 and DA2 receptors, as has been shown in proximal renal tubule cells (38).

**Fig. 10.** Effect of inhibitors of PKC and ERK on regulation of Na-K ATPase by PLA2 metabolites. OK cell monolayers in 35 x 10-mm plates were incubated in medium containing vehicle, 10^{-6} M calphostin C for 30 min, or 10^{-6} M PD-098059 for 30 min, before treatment with 5 x 10^{-6} M arachidonic acid (AA; A) or 10^{-5} M α-hydroxyeicosatetraenoic acid (20-HETE; B) for 15 min. Na-K ATPase activity was measured in the membranes as described in MATERIALS AND METHODS. Each assay was performed in triplicate, averaged, and considered as a single data point. Values are means ± SE expressed as nmol P, released·mg protein⁻¹·h⁻¹ (n = 3 separate experiments). *P < 0.01, Na-K ATPase activity significantly different than control.

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**Table 1.** Comparison of Na-K ATPase activity with vehicle, calphostin C, and PD-098059. OK cell monolayers in 35 x 10-mm plates were incubated in medium containing vehicle, 10^{-6} M calphostin C for 30 min, or 10^{-6} M PD-098059 for 30 min, before treatment with 5 x 10^{-6} M arachidonic acid (AA; A) or 10^{-5} M α-hydroxyeicosatetraenoic acid (20-HETE; B) for 15 min. Na-K ATPase activity was measured in the membranes as described in MATERIALS AND METHODS. Each assay was performed in triplicate, averaged, and considered as a single data point. Values are means ± SE expressed as nmol P, released·mg protein⁻¹·h⁻¹ (n = 3 separate experiments). *P < 0.01, Na-K ATPase activity significantly different than control.
the well-established coupling of the PTH/PTHrP receptor predominantly to Gs and Gq, with little coupling to Gi (25, 27). DA receptors couple with pertussis toxin-sensitive G proteins (26). Another significant difference between PTH and DA regulation of Na-K ATPase activity is that PTH, but not DA, regulation was dependent on ERK activity. We have previously reported that PTH inhibition of phosphate uptake is dependent, in part, on ERK activation, whereas inhibition of phosphate uptake by DA is not (30). These data suggest that PTH inhibits sodium-dependent phosphate uptake, in part, by inhibition of Na-K ATPase through an ERK-dependent pathway. ERK regulates Na-K ATPase in other cell types (22). ERK regulation of Na-K ATPase in renal cells has not been reported until now.

How PTH- but not DA-stimulated ERK could play a role in the regulation of Na-K ATPase is not apparent. Previous studies from other laboratories suggest that PKC activation is the final step in the pathway stimulated by DA and PTH, directly phosphorylating the α-subunit, leading to its endocytosis. In this scenario, PTH or DA activates phospholipase C, resulting in activation of PLA2 (31). The products of PLA2 stimulation activate PKC (34, 36). We demonstrated that inhibition of Na-K ATPase by PLA2 metabolites is blocked by PKC inhibition. Our data, however, also suggest the possibility of a more upstream site of action for PKC. PTH-stimulated ERK is blocked by inhibition of PKC, suggesting that PKC activation occurs before ERK activation (30). In this study we have shown that inhibition of Na-K ATPase activity by PKC activation through PMA or PTH(3−34) can be blocked by inhibitors of PLA2 and ERK, suggesting also that PKC is an upstream event. PTH-stimulated phospholipase C could activate PKC (4, 11−13, 19−20), leading to sequential activation of PLA2 and/or ERK. Such a mechanism for PLA2 activation has been reported in other cell types (reviewed in Ref. 31). Activated PLA2 may then stimulate a second PKC activation, activating either the same or a different PKC (15, 32). Alternatively, simultaneous PKC and ERK activation may be required for full inhibition of Na-K ATPase through parallel additive pathways. Further analysis of the signal pathways will be required to answer these questions.

We demonstrated that 15-min treatment with DA, but not PTH, diminishes basolateral membrane expression of the α-subunit of Na-K ATPase. Total cellular expression remains unchanged after short-term treatment with both agonists. After 2 h, PTH and DA both decrease expression of the α-subunit. Previous investigators have demonstrated that DA stimulates endocytosis of Na-K ATPase proteins into clathrin-
coated vesicles. This has not been shown for PTH. Our findings suggest that, in all likelihood, PTH down-regulates α-subunit expression through a different pathway.

In summary, we have demonstrated that PTH and DA inhibit Na-K ATPase by some similar and some different mechanisms in proximal renal tubule cells, possibly accounting for some of the differences in PTH and DA regulation of tubular transport.

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