In vivo PTH provokes apical NHE3 and NaPi2 redistribution and Na-K-ATPase inhibition

YIBIN ZHANG, JOHNN M. NORIAN, CLARA E. MAGYAR, NIELS-H. HOLSTEIN-RATHLOU, AUSTIN K. MIRCHEFF, AND ALICIA A. MCDONOUGH. In vivo PTH provokes apical NHE3 and NaPi2 redistribution and Na-K-ATPase inhibition. Am. J. Physiol. 276 (Renal Physiol. 45): F711–F719, 1999.—The aim of this study was to test the hypothesis that in vivo administration of parathyroid hormone (PTH) provokes diuresis/natriuresis through redistribution of proximal tubule apical sodium cotransporters (NHE3 and NaPi2) to internal stores and inhibition of basolateral Na-K-ATPase activity and to determine whether the same cellular signals drive the changes in apical and basolateral transporters. PTH-(1–34) (20 U), which couples to adenylate cyclase (AC), phospholipase C (PLC), and phospholipase A2 (PLA2), or [Nle8,18,Tyr34]PTH-(3–34) (10 U), which couples to PLC and PLA2 but not AC, were given to anesthetized rats as an intravenous bolus followed by low-dose infusion (1 U·kg⁻¹·min⁻¹ for 1 h). Renal cortex membranes were fractionated on sorbitol density gradients. PTH-(1–34) increased urinary cAMP excretion 3-fold, urine output (V) 2.0 ± 0.1-fold, and lithium clearance (C Li) 2.8 ± 0.3-fold. With this diuresis/natriuresis, 25% of NHE3 and 18% of NaPi2 immunoreactivity redistributed from apical membranes to higher density fractions containing intracellular membrane markers, and basolateral Na-K-ATPase activity decreased 25%. [Nle8,18,Tyr34]PTH-(3–34) failed to increase V or C Li or to provoke redistribution of NHE3 or NaPi2, but it did inhibit Na-K-ATPase activity 25%. We conclude that in vivo PTH stimulates natriuresis/diuresis associated with internalization of apical NHE3 and NaPi2 and inhibition of Na-K-ATPase activity, that cAMP-protein kinase A stimulation is necessary for the natriuresis/diuresis and NHE3 and NaPi2 internalization, and that Na-K-ATPase inhibition is not secondary to depressed apical Na⁺ transport.

Sodium-potassium-adenosinetriphosphatase; NHE3; NaPi2; parathyroid hormone-(1–34); membrane trafficking.

Parathyroid hormone (PTH) has multiple effects on renal ion transport: the classic stimulation of calcium transport in the distal nephron and inhibition of both sodium-phosphate (Na-Pi)–coupled transport and sodium/hydrogen exchange in the proximal tubule. In fact, PTH treatment is associated with not only phosphaturia but also natriuresis and diuresis (1, 31). The rapid decrease in proximal tubule sodium transport activity by PTH may result from 1) decreased activity of transporters in the surface membranes, 2) trafficking of transporters from plasma membranes to endosomal stores, or 3) rapid degradation of transporters. There is evidence for all three types of regulation with in vitro PTH treatment of isolated proximal tubule cells or cultured renal cell lines: 1) PTH inhibits Na/H exchanger and Na-Pi, transporter activity in brush-border membrane vesicles (16, 17, 26) (the NaPi2 isofrom of the cotransporter and the NHE3 isofrom of the exchanger are located in the apical brush border; see Refs. 4 and 7) and sodium pump (Na-K-ATPase) activity in basolateral membranes (8, 28, 30); 2) immunocytochemical evidence indicates the transit of NaPi2 from brush border to subapical vesicles (7), and transport assays indicate a transient increase in Na/H exchanger activity in endosomal membranes after PTH treatment (13); 3) Na-Pi transporters are rapidly degraded in response to PTH, and the reappearance/recovery of the transporter protein and function requires de novo protein synthesis, implicating PTH-dependent degradation (7, 25).

How does PTH effect this array of changes? PTH coupling to its receptor(s) can stimulate multiple signaling pathways, including cAMP-protein kinase A (cAMP-PKA) (27), phospholipase C-protein kinase C (PLC-PKC) (10, 27), and phospholipase A2 (PLA2) (23). This complexity of signaling pathways suggests the possibility of independent regulation of apical and basolateral sodium transporters by PTH. For example, there is evidence from in vitro studies that PTH inhibits NHE and NaPi2 transport activity by cAMP-PKA and/or PKC pathway (2, 3, 19), and there is evidence that PTH inhibits Na-K-ATPase activity independent of cAMP generation via stimulation of production of 20-hydroxyeicosatetraenoic acid (20-HETE), a metabolite of arachidonic acid through PLA2-cytochrome P-450 pathway (8, 28).

Despite the body of evidence collected in vitro for PTH inhibition of sodium transporter activity, there are no studies, to our knowledge, that have linked the cellular effects observed in vitro to in vivo physiological responses to PTH treatment. We test the hypothesis that in vivo PTH treatment provokes proximal tubule natriuresis and diuresis associated with parallel redistribution of apical sodium transporters (NHE3 and NaPi2) from apical membranes to internal stores and with inhibition of basolateral Na-K-ATPase activity. In addition, we dissect the signaling pathways involved by using PTH analogs that activate distinct signaling pathways.
Experimental Procedures

Animal protocols. Experiments were performed with male Sprague-Dawley rats (290–310 g body wt) that had free access to food and water. Rats were anesthetized intramuscularly with ketamine (Fort Dodge Laboratories) and xylazine (Miles) (1:1, vol/vol), then placed on a thermostatically controlled warming table to maintain body temperature at 37°C. Polyethylene catheters were placed into the carotid artery for monitoring blood pressure, into the right jugular vein for infusion of 0.9% NaCl ± PTH (50 µl/min during entire experimental period), and in the ureter for urine collection.

Two sets of experiments were conducted: 1) bPTH-(1–34), a synthetic bovine PTH-(1–34) fragment (Sigma Chemicals, St. Louis, MO), was infused in a bolus dose of 20 U (66 U/kg) followed by a continuous infusion at 1 U · kg⁻¹ · min⁻¹ for 1 h. 2) [Nle⁸,¹⁸,Tyr³⁴]PTH-(3–34) (Peninsula Lab, Belmont, CA), a competitive inhibitor of PTH stimulation of adenyl cyclase (AC) (15), was infused in a bolus dose of 10 U (33 U/kg) followed by an infusion at 1 U · kg⁻¹ · min⁻¹ for 1 h. The specific doses of the two PTH analogs were chosen because at higher doses of either there was a fall in blood pressure in a subset of the animals. Each PTH-treated rat was paired to a sham-operated control rat that received a matched volume of saline by bolus and continuous infusion. After a 30-min equilibration period after surgery, urine was collected from the ureter during 15-min intervals before and during PTH treatment.

Urinary production rate, endogenous lithium clearance, and urinary cAMP excretion. Rate of urinary production was assessed by collecting urine from the ureter, and volume was determined gravimetrically. A blood sample was collected at the end of each experiment, before death. The concentration of endogenous lithium in blood and urine samples was measured by flameless atomic absorption spectrophotometry (Perkin-Elmer model 5100PC) as described previously (33).

Endogenous lithium clearance, an inverse measure of proximal Na⁺ reabsorption, was calculated in the usual manner as urinary [Li⁻] × urine output/plasma [Li⁺]. Urinary cAMP concentration was measured by radioimmunoassay (Amerham Life Science, Arlington Heights, IL).

Subcellular fractionation. Kidneys were cooled in situ before excision by flushing the abdominal cavity with ice-cold PBS solution to block further membrane trafficking. After excision, the renal cortices were rapidly dissected in isolation buffer (5% sorbitol, 0.5 mM disodium EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 9 µg/ml aprotinin, and 5 mM histidine-imidazole buffer, pH 7.5). The procedure for subcellular fractionation of the renal cortex membranes has been described in detail previously (13, 35, 36). Briefly, each cortex was homogenized in two rounds with a Tissuemizer (Tekmar Instruments) for 10 min at a thyrinator setting of 45 and centrifuged at 2,000 g for 10 min. The resultant low-speed supernatants (S₂₀) were pooled (aliquot taken and stored for later assay), and PTH-treated and control samples were loaded onto separate hyperbolic sorbitol gradients (35–70% sorbitol) and centrifuged in a swinging bucket rotor (100,000 g, 5 h). Twelve fractions were collected from the top of each gradient with a Buchler AutoDensi Flow, diluted with isolation buffer, pelleted by centrifugation (250,000 g, 1.5 h), resuspended in 1 ml isolation buffer, and stored at −80°C pending assays.

Na-K-ATPase and enzymatic marker measurements. All assays were conducted in parallel on the PTH-treated and paired control samples. Na-K-ATPase (sodium pump) activity was measured by the K⁺-dependent p-nitrophenyl phosphate (K-pNPPase) reaction as reported before (35), since our previous analysis demonstrated indistinguishable distribution patterns for K-pNPPase activity and ouabain-sensitive ATPase activity in kidney cortex (35). Standard assay was used for protein (18).

Immunoblot analysis and antibodies. A constant volume of sample from each gradient fraction was prepared in SDS-PAGE sample buffer (final concentration: 2% SDS, 1% β-mercaptoethanol, 0.25 mM disodium EDTA, and 2.5 mM H₃PO₄/HPO₄ buffer, pH 7.0), denatured for 30 min at 37°C, resolved on 7.5% SDS-PAGE gels, and transferred to polyvinylidene difluoride membranes according to standard methods. Samples for Na-P, cotransporter detection were prepared in the sample buffer without β-mercaptoethanol. The antibody incubation protocol has been detailed previously (35). For detection of Na-K-ATPase α1-subunit, the monoclonal antibody α464.6 was generously provided by M. Kashgarian (Yale) and used at 1:200 dilution. The β1-subunit was detected with a polyclonal anti-β1 fusion protein antibody generated in the laboratory of A. A. McDonough according to a previously described procedure (32) and used at 1:500 dilution; it had been used in many studies from this laboratory (see Refs. 35 and 36). Polyclonal antibody to the Na-P, cotransporter isoform 2 (NaPi2) was provided by H. Murer (7) and used at 1:4,000 dilution. The monoclonal antibody to villin was obtained from Immunotech (Westbrook, Maine) and used at 1:1,000. All the above antigen-antibody complexes were detected with 125I-labeled Protein A. For detection of the Na/H exchanger NHE3 isoform, monoclonal cell culture supernatant (19F5), generously provided by D. Biemesderfer and P. Aronson (Yale) (4), was used without dilution on blots and detected with an ECL enhanced chemiluminescence kit from Amersham. For all blots, autoradiographic signals were quantified with a Bio-Rad Imaging Densitometer with Molecular Analyst software. Multiple exposures of autoradiograms were analyzed to ensure that signals were within the linear range of the film.

Quantitation and statistical analysis. Data are expressed as means ± SE. ANOVA was applied to determine whether there was a significant effect of treatment on the overall fractionation pattern of a given parameter. Treatment was one repeated factor, and fraction was another repeated factor. If the interaction between treatment and fraction was found to be significant (P < 0.05), then it was concluded that the treatment had a significant effect. If so, the location of the difference in the pattern was assessed by one-tailed Student's t-test for paired samples, and differences were regarded as significant at P < 0.05, after applying an adjustment for multiple comparisons. A one-tailed t-test was applied in all cases, because we were testing the hypotheses that there was an increase in urine production and lithium clearance and a decrease in sodium transporter activity and/or abundance in surface membranes with PTH treatment.

Results

Effect of PTH analogs on urinary cAMP. PTH receptors couple to AC, PLCl, and PLAl, and there is evidence for coupling to all three pathways in the proximal tubule (8, 10, 23, 27). Two PTH analogs, PTH-(1–34) and [Nle⁸,¹⁸,Tyr³⁴]PTH-(3–34), were chosen for distinct, albeit overlapping, coupling to signaling paths. Both analogs stimulate PLC and PLA2 (5, 8, 27). Whereas PTH-(1–34) stimulates AC, [Nle⁸,¹⁸,Tyr³⁴]PTH-(3–34) is a potent competitive antagonist of native PTH AC stimulation assayed in renal cortical membranes (29). However, when infused in vivo in a canine model, this analog is reported to have weak agonist activity as it increases urinary cAMP (15). To evaluate the effect of these analogs in our in vivo protocols, urinary cAMP...
Urinary cAMP excretion was measured between 45 and 60 min after starting PTH infusion (Fig. 1). PTH-(1–34) (20 U bolus followed by 1 U·kg⁻¹·min⁻¹ for 1-h infusion) increased urinary cAMP excretion from 18.3 ± 2.7 to 61.0 ± 9.0 pmol/min, as predicted, whereas [Nle⁸,¹⁸,Tyr³⁴]PTH-(3–34) (10 U bolus followed by 1 U·kg⁻¹·min⁻¹ for 1-h infusion) did not change cAMP excretion rate (24.4 ± 2.4 vs. 22.9 ± 2.5 pmol/min). These findings are consistent with the conclusion that PTH-(1–34) but not [Nle⁸,¹⁸,Tyr³⁴]PTH-(3–34) stimulates the cAMP-PKA pathway with these experimental protocols.

Effect of PTH analogs on urine output and endogenous lithium clearance. One-hour PTH-(1–34) treatment increased urine output 2.0-fold and endogenous lithium clearance 2.8-fold over the basal period (Fig. 2A). Since endogenous lithium clearance provides an inverse measurement of Na⁺ reabsorption in the proximal tubule (30), this result indicates that PTH-(1–34) treatment decreases sodium reabsorption in proximal tubule. That is, at this dose, it is both diuretic and natriuretic. In comparison, [Nle⁸,¹⁸,Tyr³⁴]PTH-(3–34) did not increase urine output or endogenous lithium clearance (Fig. 2B), consistent with the conclusion that the diuretic and natriuretic effects of PTH require stimulation of the AC-cAMP pathway in the proximal tubule. Blood pressure, measured in every experimental animal, did not change with the PTH dose given.

Regulation of NHE3 and NaPi2 by PTH analogs. The apical Na/H exchanger is the key transporter driving hydrogen ion secretion and sodium bicarbonate and sodium chloride reabsorption in proximal tubule; the apical isoform is NHE3 (4). About 80% of the filtered phosphate is reabsorbed in the proximal tubule by the apical Na-Pi cotransporter; the apical isoform is NaPi2 (7, 20). PTH treatment decreases activity of both NHE3 and NaPi2 (2, 9, 13, 16, 20). We tested the hypothesis that the decrease in proximal tubule sodium reabsorption after in vivo PTH is, at least in part, accounted for by a relocation of NHE3 and NaPi2 from apical membranes to putative internal stores. The comparison of the effects of the two PTH analogs allows us to determine whether the relocation response requires stimulation of the AC-cAMP pathway; the design does not rule out the possibility that there may also be changes in Na/H exchanger and/or Na-Pi cotransport activity in the apical membrane.

Redistribution of apical sodium transporters was assessed by subcellular fractionation of renal cortex by sorbitol density gradient centrifugation. Figure 3 contains typical immunoblots of density gradient fractions probed with antibodies to NHE3 or NaPi2. NHE3 is detected at 80–83 kDa, and NaPi2 is detected as a doublet of bands between 80–90 kDa. Both NHE3 and NaPi2 transit to higher density membranes after PTH-(1–34) treatment. Figures 4 and 5 summarize the densitometric analysis of NHE3 and NaPi2 as percent of total transporter immunoreactivity in the four experiments conducted. Fractions 4–6 are empirically designated apical membranes because they contain the major peaks of the apical markers alkaline phosphatase and dipeptidyl peptidase IV (not shown) as previously reported (35, 36). These fractions contain 67% of the total NHE3 and 54% of the total NaPi2 immunoreactivity. Thus NaPi2 has a broader distribution than NHE3 in the sorbitol gradient: 30% of the NaPi2 is found in fractions 7–8 where only 17% of the NHE3 is located. This may indicate that a larger fraction of NaPi2 is localized to subapical vesicles (7). One-hour PTH-(1–34) treatment provokes a shift of 27% of the total NHE3 and 18% of the total Na-Pi immunoreactiv-
From the apical peak (fractions 4–6) to higher density fractions 7–11. These higher density regions were shown previously to contain markers of intermicrovillar membranes (megalin) and endosomes (rab 4 and 5a) (36). Unlike PTH-(1–34), [Nle8,18,Tyr34]PTH-(3–34) does not stimulate redistribution of NHE3 or Na-Pi to higher density membranes (Figs. 4B and 5B). These results are consistent with the conclusion that generation of cAMP is required to trigger redistribution of apical sodium transporters by PTH. [Nle8,18,Tyr34]PTH-(3–34) fails to trigger both apical sodium redistribution and the natriuresis/diuresis response consistent with the conclusion that the decrease in proximal sodium transport during PTH treatment is dependent on the cAMP-dependent sequestration of apical sodium transporters.

PTH treatment of cultured renal cells has been reported to cause reversible internalization of a significant fraction of the brush border microvilli (12). To determine whether the apical transporter redistribution reflected retraction of microvilli, the effect of PTH on the density distribution of the microvillar protein villin was determined. Immunoreactive villin is broadly distributed between fraction 4–10 (Fig. 6), overlapping the peaks of NHE3 and NaPi2, and does not change in response to PTH-(1–34) treatment. That is, the 18–27% shift of total NHE3 and NaPi2 out of fractions 4–6 is not associated with any shift of the villin out of these fractions, supporting the hypothesis that there is a relocation of transporters out of the microvillar surface to the intramicrovillar cleft, subapical domains, or endocytic vacuoles, rather than internalization of the...
microvilli as observed by Goligorsky and colleagues (12).

Effect of PTH analogs on Na-K-ATPase. The basolateral sodium pump (Na-K-ATPase) ultimately drives active transepithelial sodium reabsorption. In vitro studies in isolated proximal tubules demonstrate that PTH decreases Na-K-ATPase activity by PLA$_2$-mediated generation of 20-HETE (8, 28). Chibalin et al. (6) have recently reported that PKC inhibition of proximal tubule Na-K-ATPase activity can be explained by endocytosis of the sodium pump α- and β-subunits from basolateral membranes to early endosomes. In this study, we test the hypothesis that in vivo PTH treatment inhibits Na-K-ATPase activity in parallel with the redistribution of apical sodium transporters, examined in isolation, and the in vivo response depends on the stimulation of CAMP production. Figure 7 summarizes the density gradient distribution pattern of Na-K-ATPase activity in membranes from control renal cortex prepared either with the PTH analogs (Fig. 8) or PTH-(1–34), Na,K-ATPase activity after PTH treatment. With PTH-(1–34), Na,K-ATPase activity decreased 27% in fractions 3–5 and did not decrease in fractions 6–12.

The PTH-driven decrease in basolateral Na-K-ATPase activity was not accompanied by an increase in activity in any other fraction, indicating that either sodium pumps resident in the basolateral membranes are inhibited (i.e., without internalization) or that sodium pumps are both inactivated and internalized and possibly degraded. The second possibility was investigated by determining the density distribution of sodium pump α- and β-subunits after PTH treatment. With use of the same fractionation scheme and renal cortex preparation, markers of endosomes such as rab 4 and 5a were previously detected in fractions 8–11 (36), overlapping with the density of internalized NHE3 and NaPi2. This is where we would expect to see Na-K-ATPase subunits translocated to early endosomes. The summarized data (Fig. 8) indicates that PTH treatment does not significantly change α1- or β1-subunit distribution in the sorbitol density gradient. Although there was minor animal-to-animal variation in the fraction α-subunit detected in fractions 2 and 9–11, evidenced by the wider standard error, the direction of change was not consistent. We conclude that if PTH treatment increases sodium pump subcellular distribution, then it occurs without a detectable change in density of membranes containing α- and β-subunits. The notable difference in overall distribution pattern between α1 and β1, and the coincidence between distribution of Na-K-ATPase activity and β1, has been observed previously (35, 36).

Effect of PTH on abundance of sodium transporters. To test whether the drop in Na-K-ATPase activity or internalization of NHE3 and NaPi2 are associated with increased degradation, the relative abundance of sodium transporters in total starting membranes (i.e., $S_0$ fraction before density gradient analysis) was determined by immunoblot. As summarized in Table 1,
PTH-(1–34) treatment did not significantly change the total pool of immunoreactive Na-K-ATPase α- or β-subunits, NHE3, or NaPi2 in starting membranes. Thus significant rapid degradation of sodium transporters is not implicated in the natriuretic/diuretic response to 1-h PTH treatment.

**DISCUSSION**

This study describes molecular mechanisms underlying decreased proximal tubule sodium reabsorption by PTH observed in vivo. PTH-(1–34), an analog that stimulates cAMP generation as well as PKC and PLA2, provokes natriuresis and diuresis (Fig. 2), apical sodium transporter redistribution (Figs. 3–5), and Na-K-ATPase inhibition (Fig. 7). [Nle8,18,Tyr34]PTH-(3–34), an analog that also stimulates PKC and PLA2, but not cAMP generation, does not provoke natriuresis, diuresis, or apical sodium transporter redistribution, but does cause Na-K-ATPase inhibition. We conclude that the PTH-stimulated natriuresis and diuresis observed in the whole animal requires cAMP generation, which is required for the redistribution of sodium transporters out of the apical membrane.

NHE3 is located in both apical membranes and subapical vesicles (4, 34), and we have provided evidence for rapid regulation of NHE3 by trafficking between surface and internal membrane domains (34, 35, 36). By high-resolution immunocytochemistry, Biemesderfer and colleagues (4) detected NHE3 in subapical vesicles in the proximal tubule, providing the morphological potential for regulation of NHE3 by membrane recycling. In response to acute hypertension, NHE3 rapidly transits from the brush border to subapical vesicles coincident with decreased proximal tubule sodium reabsorption, demonstrated by subcellular fractionation in this laboratory (35) and immunocytochemistry by Yip and colleagues (34). Before molecular probes for NHE3 became available, Hensley et al. (13) demonstrated that PTH provoked a redistribution of Na/H exchanger activity from apical to internal membranes, but the trafficking route and isoform were not identified. Our present study, consistent with this report, demonstrates that in vivo PTH treatment provokes a redistribution of NHE3 from apical surface membranes to heavier density membranes established in a recent report to contain markers of intermicrovilli cleft and endosomes (35). We postulate that the trafficking route is similar to that previously established for insulin receptor internalization (21) and for NHE3 internalization during acute hypertension (35, 36), i.e., lateral migration from apical microvilli to intermicrovilli cleft region and subsequent internalization to endocytic vesicles.

The pattern of NHE3 redistribution precisely mimics that reported for NaPi2 in response to PTH treatment (17, 19, 20); thus stimulation with this hormone leads to internalization of at least two proximal tubule brush-border membrane proteins to subapical vesicles. Our arbitrary delimiting of apical membranes to fractions 4–6 includes 67% of the NHE3 compared with 54% of the NaPi immunoreactivity in control membranes. The percentage of brush border NHE3 corresponds exactly with the observation of Hensley et al. (14) that two-thirds of the Na/H exchanger activity was in the brush border, and no more than one-third was in cytoplasmic membranes. The broader distribution of NaPi2 in the sorbitol gradient might reflect a larger cytoplasmic pool of NaPi2 in the control steady state. Indeed, Custer et al. (7) observed significant NaPi immunoreactivity in subapical vesicles in unstimulated renal cortex. Although two main sodium transporters were translocated after in vivo PTH treatment, we saw no evidence for the retraction of whole microvilli to the cytoplasm previously reported in cultured renal cells (12). Specifically, we did not detect any change in the distribution of the brush border cytoskeleton component villin. We conclude that there is selective internalization of at least two apical membrane proteins from the villi, without retraction of the villi themselves.
In addition to internalization, there is evidence for PTH-dependent increase in the rate of degradation of NaPi. In OK cells, 4-h PTH treatment led to an internalization of NaPi and a decrease in its immunoactive pool size (25). Further evidence for degradation was that restoration of NaPi to the surface after PTH removal required de novo protein synthesis (25). However, in a study from the same investigators of parathyroidectomy rats given PTH for 2 h, no direct evidence for degradation was provided (17). Interestingly, the dose of PTH given to the parathyroidectomy rats (two injections of 7.5 µg/100 g rat = 45 µg/300 g) is about 10 times higher than the dose given over the duration of this study (assuming 10 U/1 µg, 38 U/300 g rat is equivalent to 3.8 µg/300 g rat). In our hands, this level of PTH caused blood pressure to fall, a stimulus that could affect sodium transporter distribution and activity independent of PTH (35, 36). It is possible that treatment with the lower level of PTH does not provoke NaPi degradation after internalization.

PTH receptor coupling activates multiple signaling pathways including: 1) stimulation of AC increasing intracellular cAMP and activation of PKA (27); 2) stimulation of PLC and subsequent activation of PKC (10, 27); and 3) activation of PL2 and release of arachidonic acid from membrane phospholipids, which are further metabolized in the proximal tubule to bioactive eicosanoids such as 20-HETE by the cytochrome P-450-dependent pathway (8, 23, 28).

Previous studies in the proximal tubule concluded that inhibition of apical Na/H exchanger activity by PTH involved activation of cAMP-PKA and/or PLC-PKC pathways (2, 3). In isolated rabbit proximal tubule cells, PTH inhibits oxygen consumption secondary to decreased sodium entry, an effect mimicked by cAMP addition (9), and, in brush-border membranes, PTH or cAMP inhibit Na/H exchange transport activity to the same extent (25–30%) (16). Recently, Azarani et al. (3) established that the PTH analogs PTH-(1–34), PTH-(3–34), PTH-(28–42), and PTH-(28–48) all inhibited NHE3 transport activity in cells transfected with the PTH/PTH-related peptide type I receptor and NHE3 and that inhibition of PKC abolished the effects of PTH-(3–34), -(28–42), and -(28–48), whereas inhibition of both PKA and PKC is required to abolish the effect of PTH-(1–34). This study concludes that activation of either PKA or PKC can independently mediate the regulation of NHE3 by PTH. Likewise, inhibition of either PKA or PKC prevents PTH action on NaPi (19).

In this current report, we demonstrate that redistribution of NHE3 and NaPi2 by PTH occurs only with the analog that activates cAMP-PKA; the analog that activates only PLC-PKC and PL2 failed to provoke the redistribution response, although it did inhibit Na-K-ATPase activity. We cannot conclude that cAMP stimulation is sufficient to stimulate redistribution; it may function in concert with other signaling pathways stimulated by PTH. Redistribution of NHE3 and NaPi to higher densities also occurs during acute hypertension, again associated with a decrease in proximal tubule sodium and volume reabsorption. In a recent study of that response, we assessed the importance of PL2 activation by inhibiting cytochrome P-450-dependent arachidonic acid metabolism with cobalt treatment. After cobalt, the natriuretic and diuretic responses to acute hypertension were blunted and there was no apical NHE3 redistribution or basolateral Na-K-ATPase activity inhibition (37). Thus it is possible that the redistribution of apical transporters also depends on intact signaling through the PL2 pathway.

In contrast to effects on apical sodium transporters, the inhibition of basolateral Na-K-ATPase activity by in vivo PTH does not apparently depend on stimulation of intracellular cAMP generation, since both PTH-(1–34) and [Nle8,18,Tyr34]PTH-(3–34) decreased basolateral Na-K-ATPase activity to the same extent. This indicates that PLC-PKC and/or PL2 regulate Na-K-ATPase activity, and there is evidence for both. The major arachidonic acid metabolite generated in the proximal tubule through PL2 stimulation is 20-HETE (23). In studies in isolated proximal tubules, Mandel and colleagues (8, 28) have provided evidence that PTH stimulates 20-HETE production and that 20-HETE is responsible for the PTH-mediated inhibition of Na-K-ATPase (28). In cultured proximal tubule cells, Friedman and colleagues (11) demonstrated that PTH-(1–34) inhibits Na-K-ATPase activity by 45%, whereas PTH-(1–31), an analog that does not activate PLC-PKC, inhibits Na-K-ATPase activity by only 25%, suggesting that PLC-PKC activation does play a role in PTH-mediated Na-K-ATPase inhibition. In other studies in isolated tubules, Aperia and colleagues (22) have determined that PKC activation leads to an inhibition of Na-K-ATPase activity. There is also evidence that PLC-PKC and PL2 pathways interact to regulate Na-K-ATPase, since the inhibition of PT Na-K-ATPase activity by PKC is abolished with a cytochrome P-450 monoxygenase pathway blocker (24) or enhanced by 20-HETE (20).

Since acute regulation of Na-K-ATPase transport activity in tubules may involve modification of the transporter itself or an associated regulator, or involve trafficking to intracellular pools, it is possible that the PLC-PKC and PL2 pathways have distinct or interacting effects on sodium pump transport activity.

The use of the two PTH analogs permitted the dissociation of inhibition of the Na-K-ATPase from regulation of apical transporters. Our current data support the concept of independent parallel direct regulation of apical and basolateral sodium transport by PTH, since inhibition of basolateral Na-K-ATPase activity is not apparently secondary to inhibition of apical transporters: [Nle8,18,Tyr34]PTH-(3–34) decreases Na-K-ATPase activity to the same extent as PTH-(1–34), even though it has no effect on apical transporter distribution or proximal tubule sodium transport estimated with endogenous lithium transport. Also supporting independent parallel regulation are our previous in vivo results demonstrating that inhibition of proximal Na+ entry and Na+ reabsorption with the carbonic anhydrase inhibitor benzolamide did not provoke inhi-
bition of basolateral Na-K-ATPase activity measured in isolated membranes (35). We conclude that the [Ne$^{6,18}$,Tyr$^{34}$]PTH-(3–34)-induced 20–25% inhibition of Na-K-ATPase, measured in vitro with optimal substrate levels, is not sufficient to trigger a decrease in proximal tubule sodium transport, and we postulate that in vivo the decrease in sodium pump maximal activity may be counteracted by an increase in cytoplasmic Na$^+$, a strong activator of sodium pump activity, leading to a new steady state where the balance between Na$^+$ entry and exit are maintained. Finally, we conclude that the PTH-stimulated alterations in proximal tubule apical sodium transporters are critical for the natriuretic and diuretic responses to this hormone and that changes in basolateral sodium pumps are not merely secondary to PTH-induced changes in transport across the apical membrane.

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REFERENCES


