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# Dual Mechanisms of Regulation of Na/H Exchanger NHE-3 by Parathyroid Hormone in Rat Kidney*

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**Parathyroid hormone (PTH) is a potent inhibitor of mammalian renal proximal tubule sodium absorption via suppression of the apical membrane Na/H exchanger (NHE-3). We examined the mechanisms by which PTH inhibits NHE-3 activity by giving an acute intravenous PTH bolus to parathyroidectomized rats. Parathyroidectomy *per se* increased apical membrane NHE-3 activity and antigen. Acute infusion of PTH caused a time-dependent decrease in NHE-3 activity as early as 30 min. Decrease in NHE-3 activity at 30 and 60 min was accompanied by increased NHE-3 phosphorylation. In contrast to the rapid changes in NHE-3 activity and phosphorylation, decrease in apical membrane NHE-3 antigen was not detectable until 4–12 h after the PTH bolus. The decrease in apical membrane NHE-3 occurred in the absence of changes in total renal cortical NHE-3 antigen. Pretreatment of the animals with the microtubule-disrupting agent colchicine blocked the PTH-induced decrease in apical NHE-3 antigen. We propose that PTH acutely cause a decrease in NHE-3 intrinsic transport activity possibly via a phosphorylation-dependent mechanism followed by a decrease in apical membrane NHE-3 antigen via changes in protein trafficking.**

PTH[1] plays a paramount role in mammalian calcium homeostasis. The calcitropic actions of PTH include direct stimulation of bone turnover (1) and renal Ca absorption (2) and indirect enhancement of intestinal Ca absorption via its action on 1,25-vitamin $D_3$ (3). In the kidney, PTH exerts direct action on the proximal tubule, thick ascending limb, distal convoluted tubule, and connecting tubule (4, 5). In the proximal tubule, PTH is a potent inhibitor of $NaHCO_3$ absorption (6–12). Because proximal tubule calcium and sodium absorption are tightly coupled (6, 13), the potent inhibitory action of PTH on proximal $NaHCO_3$ transport appears to be counterproductive for an anticalciuric hormone. Moreover, the acute inhibition of proximal tubule $NaHCO_3$ absorption seems to serve little purpose because the $HCO_3$ exiting the proximal tubule is largely reclaimed in the distal nephron as evident by the fact that acute PTH only cause modest reductions in plasma $HCO_3$ concentration (14–16). However, in the distal convoluted tubule, luminal $HCO_3$ is an important stimulus for transcellular calcium absorption (17–19). Thus the shift of $NaHCO_3$ absorption from proximal to distal nephron results in minimal net change in acid-base balance but serves as a key enhancer of the anticalciuric effect of PTH.

In the mammalian proximal tubule, two-thirds of the transcellular $NaHCO_3$ absorption is mediated by apical membrane Na/H exchange (20). Immunohistochemical (21–23), pharmacokinetic (24), and genetic (25) data all indicate that the NHE-3 isoform is predominantly responsible for proximal tubule apical membrane Na/H exchange. Direct inhibition of proximal tubule $HCO_3$ absorption by PTH (6–12) is effected at least in part by inhibition of apical membrane Na/H exchange activity, which has been demonstrated in the suspended tubules (26), isolated perfused tubule (27), *in vivo* perfused tubule (28), apical membrane vesicles (29), cultured renal cells (30–35), and nonepithelial cells transfected with the PTH receptor and NHE-3 gene (36, 37). The mechanisms by which PTH inhibits NHE-3 activity has not been examined to date. Phosphorylation of NHE-3 has been shown to play a role in its acute regulation by protein kinase A (PKA) (38–40), and part of the effect of PTH on NHE-3 is PKA-dependent (32–36). One study showed that the PTH-induced decrease in apical membrane Na/H exchange activity was accompanied by commensurate increase in Na/H exchange activity in a different membrane fraction on a density gradient (41). This suggests redistribution of NHE-3 protein as a mechanism of its acute regulation, although there were no antigenic data in this study. NHE-3 protein has been shown to exist both in the apical membrane and in subapical vesicles (23). In a model of pressure natriuresis, the acute inhibition of apical membrane Na/H exchange was associated with redistribution of NHE-3 antigen from the apical to subapical region. (42, 43).

In this study, we present evidence in intact animals that PTH inhibits renal cortical apical membrane Na/H exchange by dual mechanisms: immediate inhibition of the intrinsic transport activity of NHE-3 associated with an increase in NHE-3 phosphorylation followed by redistribution of NHE-3 transporter away from the apical membrane to a nonapical pool.

## EXPERIMENTAL PROCEDURES

*Animal Model and Membrane Preparations*—Parathyroidectomized or sham-operated Sprague-Dawley rats (150–200 g, Charles River, Wilmington, MA) were given free access to food supplement with 4% (w/v) calcium gluconate drinking water until 2 days prior to experiments when they were switched to tap water. Animals were anesthetized by thiopental (100 mg/kg body weight intraperitoneal; Abbot Laboratories, Abbot Park, IL), the femoral vein was exposed, and either PTH (100 $\mu$g/kg body weight; 0.5 mg/ml in 10 mM acetic acid, 0.4 mM

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[1] The abbreviations used are: PTH, parathyroid hormone(s); PKA, protein kinase A; PTX, parathyroidectomy; MES, 4-morpholineethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.

dithiothreitol, 1% bovine serum albumin; Peninsula, San Carlos, CA) or the vehicle was injected intravenously. For the 30–60-min time points, animals were kept under anesthesia on a heating pad until bilateral nephrectomy performed through an anterior abdominal incision. For longer time points, the inguinal incision was sutured, and animals were allowed to recover. At the appropriate time points, anesthesia was again induced and kidneys were harvested. Renal cortex was dissected and homogenized on ice in membrane buffer (150 mM NaCl, 80 mM NaF, 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1 mM EGTA, 25 mM sodium pyrophosphate, 1 mM sodium vanadate, 100 $\mu$g/ml phenylmethylsulfonyl fluoride, 2 $\mu$g/ml leupeptin, 2 $\mu$g/ml aprotinin, and 2 $\mu$g/ml pepstatin A) (Brinkman polytron, Westbury, NY), and apical membrane vesicles were prepared from the cortical homogenate by exposing cortical membranes to three consecutive precipitations by 15 mM $MgCl_2$, and the final apical membrane-enriched vesicles were pelleted from the supernatant (Beckman J2–21 M, JA-20 rotor, 20,000 rpm, 40 min 4 °C; Beckman, Fullerton, CA). For experiments with colchicine, parathyroidectomy (PTX) animals were given colchicine (10 $\mu$g/kg body weight) 3 h prior to infusion of PTH or vehicle.

*Na/H Exchange Activity and Antigen on Membrane Vesicles*—For measurement of Na/H exchange activity in rat renal apical membranes, 200 $\mu$g of vesicles were acid loaded (300 mM mannitol, 20 mM MES, pH 5.5, at 4 °C for 2 h) and exposed to 10× volume uptake solution (300 mM mannitol, 20 mM Tris, pH 7.5, 0.1 mM $^{22}$NaCl) to initiate transport at 20 °C for 10 s. Uptake was stopped by dilution with stop solution (150 mM NaCl, 20 mM Tris, pH 7.5) at 4 °C, and $^{22}$Na uptake was quantified by rapid filtration on 0.65 $\mu$M Millipore filters (Millipore, Bedford, MA) and scintillation counting. For determination of NHE-3 antigen, either 20 $\mu$g of rat renal cortical apical membranes or 50 $\mu$g of cortical membranes were solubilized in SDS buffer, fractionated by SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose filters. Anti-rat NHE-3 antisera were used: 1568 (against epitope DSFLQADGPEEQLQ at 1:1000) (20) to quantified NHE-3 antigen. Controls with the anti-Type II sodium phosphate co-transporter NaPi-2 antisera (1:1000 dilution, gift from Drs. Biber and Murer, Zürich, Switzerland) were performed as described previously (44). After incubation with horseradish peroxidase-coupled mouse anti-rabbit secondary antibody, signals were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech) and quantified by densitometry.

*OK Cells*—OK cells expressing native NHE-3 (47) were used as a control experiment to validate the mobility shift assay for NHE-3 phosphorylation. OK cells were maintained in Dulbecco's modified Eagle medium (Life Technologies, Inc.) supplemented with 4.5 mg/ml glucose, 100 units/ml penicillin, 100 $\mu$g/ml streptomycin, and 10% fetal bovine serum (removed 48 h prior to study). Phosphorylation experiments were done as described previously (40). In brief, confluent monolayers were incubated in phosphate-free Dulbecco's modified Eagle medium and pulsed with [$^{32}$P]orthophosphate (500 $\mu$Ci/ml; 120 min). After addition of, 200 $\mu$M 8Br-cAMP, cells were lysed with RIPA buffer (300 mM NaCl, 80 mM NaF, 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 10 mM EGTA, 25 mM sodium pyrophosphate, 1 mM activated sodium orthovanadate, 50 mM $\beta$-glycerophosphate, 0.5 mM dithiothreitol, Triton X-100 1% (v/v), deoxycholate 0.5% (w/v), SDS 0.1% (w/v), 100 $\mu$g/ml phenylmethylsulfonyl fluoride, 4 $\mu$g/ml leupeptin, 4 $\mu$g/ml aprotinin, 10 $\mu$g/ml pepstatin), centrifuged (109,000 × *g* at $r_{max}$, 50,000 rpm, 25 min, 2 °C, Beckman TLX ultracentrifuge, TLA 100.3 rotor, Fullerton, CA), and NHE-3 was immunoprecipitated (antiserum 5683 against a fusion protein of maltose binding protein and OK NHE-3 amino acid 484–839) (1:250 dilution, v/v) from the supernatant. Immunoblots were performed with antiserum 5683) (1:1000) and goat anti-rabbit antibody (1:5000) using ECL (Amersham Pharmacia Biotech). The $^{32}$P content of NHE-3 was visualized by autoradiography on the same filters after decay of ECL.

*NHE-3 Mobility Shift Assay and in Vitro Treatment with Alkaline Phosphatase*—Mobility shift was detected by immunoblot after SDS-PAGE (6% gel with 80-kDa marker ran to the edge of the gel). For treatment with alkaline phosphatase, we modified a protocol described by Jou *et al.* (48). 20 $\mu$g of apical membrane was washed with reaction buffer (50 mM Tris-HCl, pH 8.5, 2 mM phenylmethylsulfonyl fluoride, 8 mM $MgCl_2$, 0.1% $\beta$-mercaptoethanol (v/v)) three times and resuspended in 45 $\mu$l of reaction buffer containing 10 units of calf-intestine alkaline phosphatase. After incubation at 37 °C for 90 min, the reaction was terminated by adding polyacrylamide gel loading buffer.

## RESULTS

*Effect of PTX on NHE-3*—Parathyroidectomy was complete as plasma PTH levels were undetectable in PTX animals (data not shown). Compared with sham operation, parathyroidectomy *per se* increased apical NHE-3 activity (35 ± 8%, $n = 4$, $p < 0.05$) and antigen (75 ± 8%, $n = 4$, $p < 0.05$) and total renal cortical NHE-3 antigen (55 ± 8%, $n = 4$, $p < 0.05$) (Fig. 1). This suggests that PTH has a tonic suppressive effect on NHE-3 protein expression.

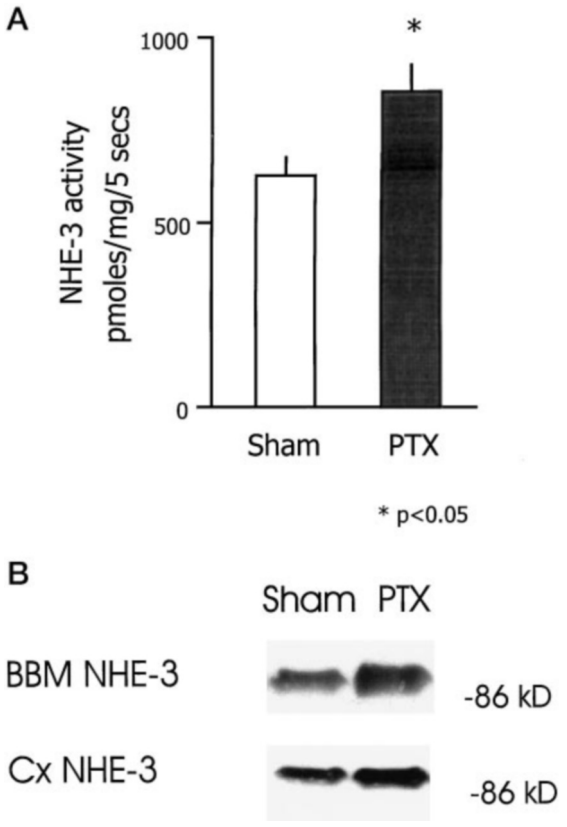


FIG. 1. **Effect of parathyroidectomy on renal NHE-3.** Sham-operated rats (*Sham*; $n = 4$) were compared with parathyroidectomized (*PTX*; $n = 4$) rats. *A*, NHE activity was measured in apical membrane vesicles as ΔpH-driven $^{22}$Na flux. The *asterisk* indicates $p < 0.05$ (*t* test). *B*, NHE-3 antigen was measured in apical brush border membrane (*BBM*) and total cortical membranes (*Cx*) by immunoblot. Mobility on SDS-PAGE is shown in kDa.

*Effect of PTH on Renal Apical Membrane NHE-3 Activity*—Fig. 2 shows a typical experiment where acute intravenous infusion of PTH decreased apical membrane NHE-3 activity in PTX rats in a time-dependent fashion, whereas vehicle injection had no effect. Fig. 3 summarizes all the experiments. Significant inhibition was detected as early as 30 min after the bolus of PTH and persisted up to 20 h (percentage of decrease compared with vehicle time control: 30 min, 14 ± 4%; 1 h, 21 ± 6%, 2 h; 28 ± 7%; 4 h, 38 ± 6; 8 h, 35 ± 7%; 12 h, 33 ± 10%; 20 h, 14 ± 9%; 20 h, 15 ± 9%; 24 h, 7 ± 7%; all $p < 0.05$ except 24 h).

*Effect of PTH on Renal Apical Membrane NHE-3 Phosphorylation*—Acute regulation of NHE-3 by activation of kinases is associated with changes in NHE-3 phosphorylation in cultured cells (38–40). To examine whether PTH acutely modifies NHE-3 phosphorylation in the intact animal, we utilized a mobility shift assay. Fig. 4*A* illustrates data validating this assay. We have shown that protein kinase A activation increases NHE-3 phosphorylation in OK cells (40). Fig. 4*A* shows that the cAMP-induced increase in NHE-3 phosphorylation was associated with deceased NHE-3 mobility on SDS-PAGE (Fig. 4*A*). Treatment of the same samples with alkaline phosphatase removed the $^{32}$P label, increased the mobility of NHE-3, and eliminated the cAMP-induced NHE-3 mobility shift (Fig. 4*A*). Fig. 4*B* shows a similar experiment performed

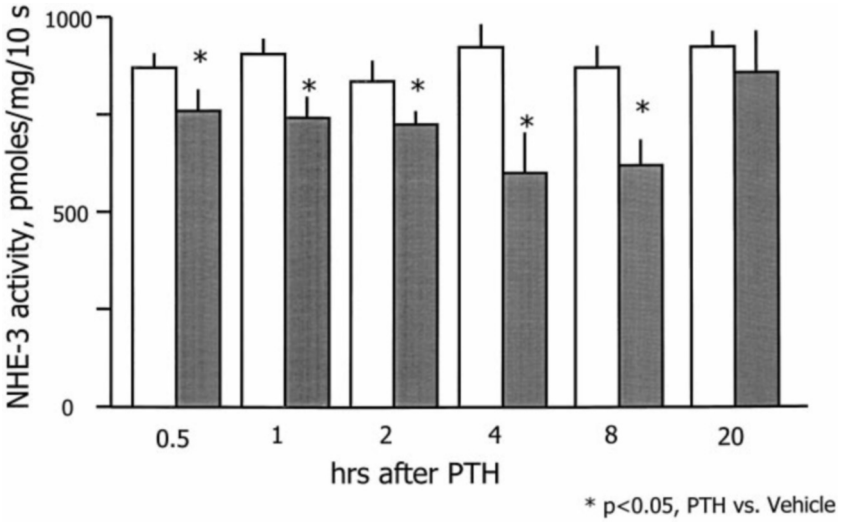


FIG. 2. **Effect of acute PTH on apical membrane NHE activity: typical experiment.** PTH or vehicle was given intravenously into parathyroidectomized (*PTX*) animals, renal cortical apical membranes were harvested at the indicated time points, and NHE activity was measured by ΔpH-driven $^{22}$Na flux. Data from one typical experiment are shown with *bars* and *error bars* depicting the means ± S.E. from three animals/time point. The *asterisks* indicate $p < 0.05$ (*t* test; PTH *versus* vehicle).

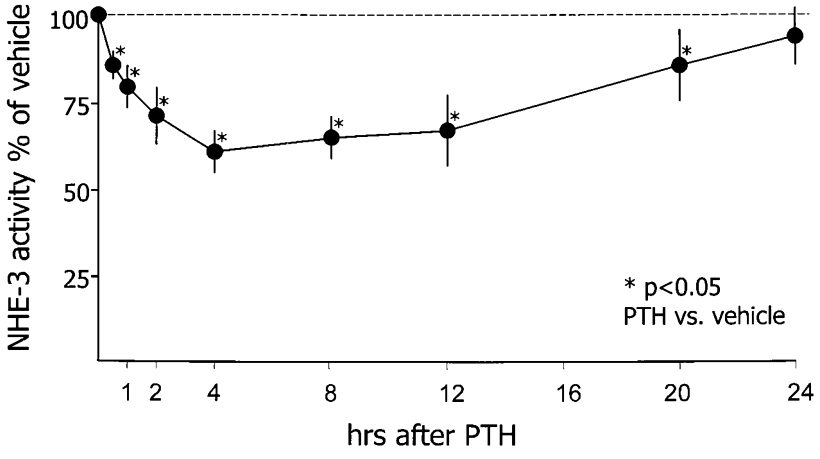


FIG. 3. **Effect of acute PTH on apical membrane NHE activity: summary of all experiments.** PTH or vehicle was given intravenously into parathyroidectomized (*PTX*) animals, renal cortical apical membranes were harvested at the indicated time points, and NHE activity was measured by ΔpH-driven $^{22}$Na flux. NHE-3 activity of PTH-treated animals are expressed as percentages of the vehicle-injected animals for each time point. Number of animals (vehicle/PTH): 30 min, 8/10; 1 h, 8/10; 2 h, 8/8; 4 h, 8/12; 8 h, 10/10; 29 h, 6/8; 24 h, 8/12). *Bars* and *error bars* depict the means ± S.E. The *asterisks* indicate $p < 0.05$ (*t* test; PTH *versus* vehicle).

in rat renal cortical apical membranes. An intravenous bolus of 8Br-cAMP (250 μl of 0.43 mg/ml 8Br-cAMP in saline injected into the suprarenal abdominal aorta with transient infrarenal aortic clamping) induced a mobility shift of NHE-3 that was completely abolished by *in vitro* treatment with alkaline phosphatase. Fig. 4*C* shows a typical experiment of the effect of PTH on NHE-3 phosphorylation in the intact animal. Acute PTH infusion increased NHE-3 phosphorylation as early as 30 min.

*Effect of PTH on NHE-3 Protein Distribution*—Next, we examined whether the decrease in NHE-3 activity is associated with changes in NHE-3 protein abundance in apical and total cortical membranes. Fig. 5*A* shows a typical experiment. Vehicle injection did not alter NHE-3 protein abundance in the apical or total cortical membranes from 30 min throughout to 16 h, indicating that the anesthesia and intravenous catheterization did not lead to redistribution of NHE-3. From 30 min to 1 h after PTH infusion, although NHE-3 activity was clearly decreased (Fig. 3), there was no detectable change in apical membrane NHE-3 antigen (Fig. 5*A*). PTH acutely shifts the type IIa sodium phosphate cotransporter NaPi-2 from an apical to a subapical location (44, 45).[2] As a positive control for our negative finding in NHE-3 trafficking after 30 min and 1 h of PTH, we probed the same apical membrane samples after 30 min and 1 h of PTH with an antiserum against NaPi-2. Fig. 5*B* shows that although there was no change in NHE-3 antigen, NaPi-2 underwent dramatic endocytosis after 30 min of PTH as previously shown (44, 45).[2] Although the early decrease in apical NHE-3 activity was not accompanied by decreased NHE-3 antigen, after 2 h of PTH treatment, NHE-3 antigen was significantly decreased compared with vehicle-treated controls (Fig. 5*A*). Fig. 6 summarizes all the experiments. No significant decrease of NHE-3 was detected in apical membrane in 30–60 min of PTH, and a trend toward decrease was noted at 2 h (12 ± 7%, $p = 0.07$). From 4 to 12 h, PTH significantly decreased apical membrane NHE-3 antigen with recovery by 24 h (percentage of decrease, PTH compared with vehicle time control: 30 min, 6 ± 8%; 1 h, 1 ± 4%; 2 h, 12 ± 7%; 4 h, 33 ± 7; 8 h, 38 ± 10%; 12 h, 36 ± 8%; 24 h, 16 ± 12%; all $p < 0.05$ except 30 min and 1, 2, and 24 h). No change in total cortical NHE-3 antigen was noted in response to acute PTH, although a statistically insignificant decrease occurred after 12 h (15 ± 10% decrease, $p = 0.07$). In concert, the data suggest that the decrease in apical membrane NHE-3 protein is due to redistribution to a nonapical compartment rather than protein degradation.

*Effect of Colchicine on the PTH-induced Shift in NHE-3 Antigen*—To examine whether this redistribution of NHE-3 involves microtubule-dependent protein trafficking, we pretreated the rats with the microtubule disrupting agent colchicine prior to infusion of PTH. Fig. 7 shows one experiment. Colchicine *per se* appeared to increase the baseline apical membrane NHE-3 abundance. In the background of colchicine treatment. PTH failed to induce redistribution of NHE-3 (percentage of decrease compared with vehicle time control: 2 h, 3 ± 7%; 4 h, 8 ± 7; 8 h, 5 ± 14%; 24 h, 5 ± 10%; all not significant),

[2] M. Lötscher, Y. Scarpetta, M. Levi, H. Wang, H. K. Zajicek, J. Biber, H. Murer, and B. Kaissling, submitted for publication.

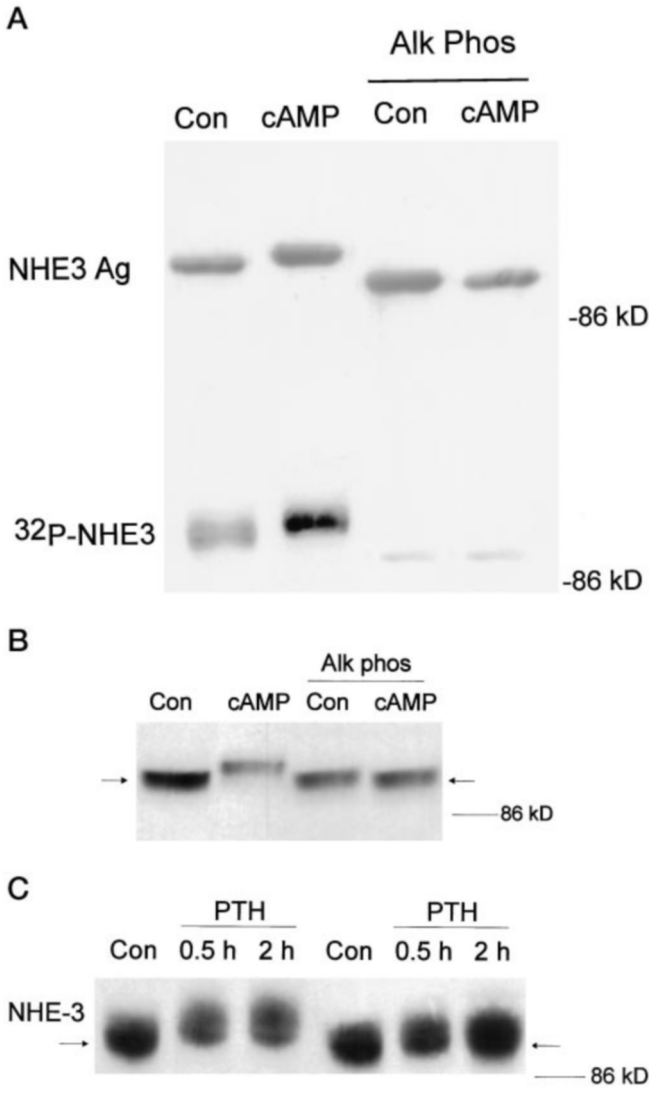


FIG. 4. **Mobility shift assay for phosphorylation and the effect of acute PTH on NHE-3 phosphorylation in intact animals.** *A*, OK cells were pulsed with $^{32}P$ and treated with 500 $\mu$M 8Br-cAMP or vehicle, and $^{32}P$-NHE-3 was immunoprecipitated and resolved by SDS-PAGE. Phosphorylation (*$^{32}P$-NHE3*) and antigenic (*NHE3 Ag*) signals were determined in the same filters by autoradiography and immunoblot, respectively. The *right lanes* show the same samples after dephosphorylation with alkaline phosphatase (*Alk Phos*) *in vitro*. Mobility in kDa are indicated on the *left margin*. The *arrow* marks the mobility of dephosphorylated NHE-3. *Con*, control. *B*, rats were given either 8Br-cAMP or vehicle via the aorta, and renal cortical apical membranes were isolated and analyzed by SDS-PAGE and immunoblot. The *right two lanes* shows the same samples after dephosphorylation with alkaline phosphatase *in vitro*. The *arrow* marks the mobility of the control and phosphatased sample. *C*, apical membranes were prepared from rats after an acute infusion of PTH and subjected to immunoblotting by anti-NHE-3. Mobility in kDa is shown on the *right margin*. The *arrow* marks the mobility of the control sample. Results from two experiments are shown. Total number of experiments = 4.

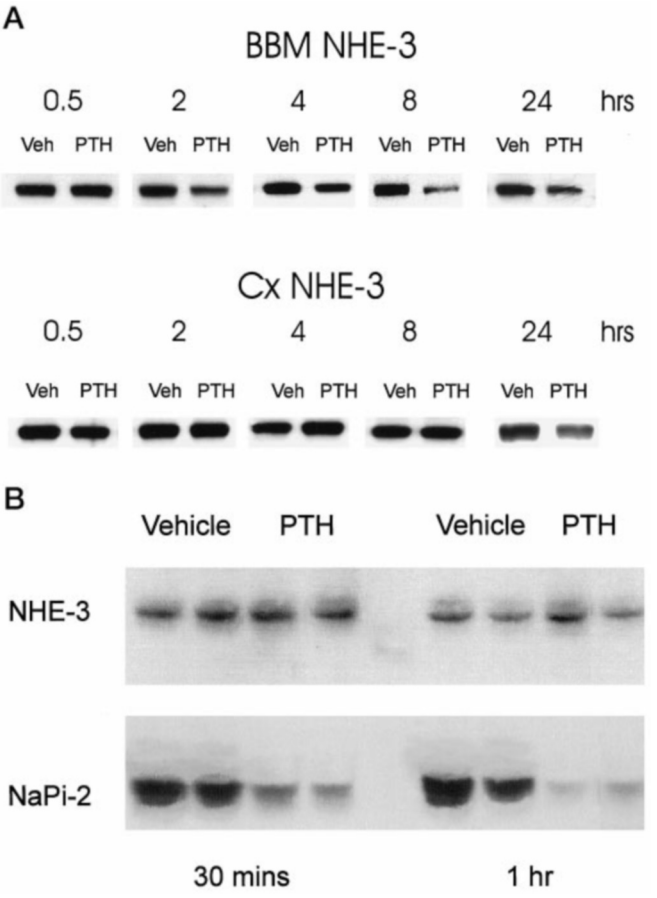


FIG. 5. **Effect of PTH on NHE-3 antigen: typical immunoblots.** PTH or vehicle was given intravenously to PTX animals, renal total cortical (*Cx*) (50 $\mu$g) apical brush border (*BBM*) membranes (20 $\mu$g) were harvested at the indicated time points, resolved by SDS-PAGE, and studied by immunoblot for NHE-3 protein abundance for all the time points (A) and NHE-3 and NaPi-2 protein abundance for 30 min and 1 h (B).

suggesting that the decrease in apical membrane NHE-3 is microtubule-dependent (Fig. 7*B*).

## DISCUSSION

The acute inhibition of renal proximal apical membrane Na/H exchange activity by PTH has been shown in renal tubules, membrane vesicles, and cultured cells (26–37), but the mechanisms for this decrease has not been defined. We found that PTX *per se* increased apical membrane NHE-3 activity as described previously (49), and PTX increased NHE-3 antigen in total renal cortical as well as apical membrane vesicles. This suggests that PTH has a chronic suppressive effect on NHE-3 protein expression. The increase in renal net acid excretion seen in chronic hyperparathyroidism (14–16) is likely due to heightened distal rather proximal tubule acidification. With a single PTH bolus, we did not observed a statistically significant decrease in total cortical NHE-3 antigen in the PTX animals, although a slight trend was observed at 20 h. It is possible that more sustained or repeated doses of PTH are required to inhibit total cortical NHE-3 protein expression.

Both protein kinase C and PKA pathways have been implicated in mediating the effects on PTH on the proximal tubule (27–29, 32–37). Acute regulation of NHE-3 activity by PKA activation has been shown to be associated with NHE-3 phosphorylation (38–40), and mutation of specific phosphorylated serines aborted functional regulation by PKA activation (39, 40). The requirement of the Na/H exchanger regulatory factors NHERF and E3KARP as putative functional A kinase anchoring proteins to effect NHE-3 inhibition and phosphorylation further consolidate the importance of NHE-3 phosphorylation in its functional regulation (50–53). All of these studies were done either in fibroblasts transfected with NHE-3 (38–40, 51, 52) or in cultured epithelial cells expressing native NHE-3 (40, 51, 53). This study is the first demonstration that NHE-3 is indeed phosphorylated as a native protein in an intact animal and that NHE-3 phosphorylation is regulated acutely by administration of a hormone. The association of increased NHE-3 phosphorylation with decreased NHE-3 activity in the intact

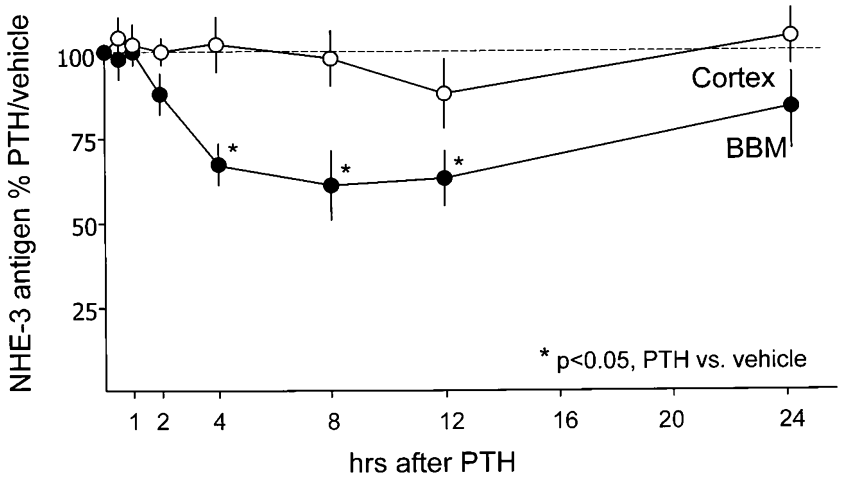


FIG. 6. **Effect of PTH on NHE-3 antigen: summary of all experiments.** NHE-3 Ag in PTH-treated animals was expressed as a percentage of NHE-3 Ag in vehicle-treated animals for each time points. *Open circles* represent renal cortical membranes (*Cortex*). *Closed circles* represent brush border membranes (*BBM*). *Bars* and *error bars* depict the means ± S.E. Number of animals (vehicle/PTH): 30 min, 6/8; 1 h, 8/8; 2 h, 8/8; 4 h, 8/8; 8 h, 10/10; 12 h, 10/10; 24 h, 10/10. *Bars* and *error bars* represent the means ± S.E. The *asterisks* indicate $p < 0.05$, PTH *versus* vehicle.

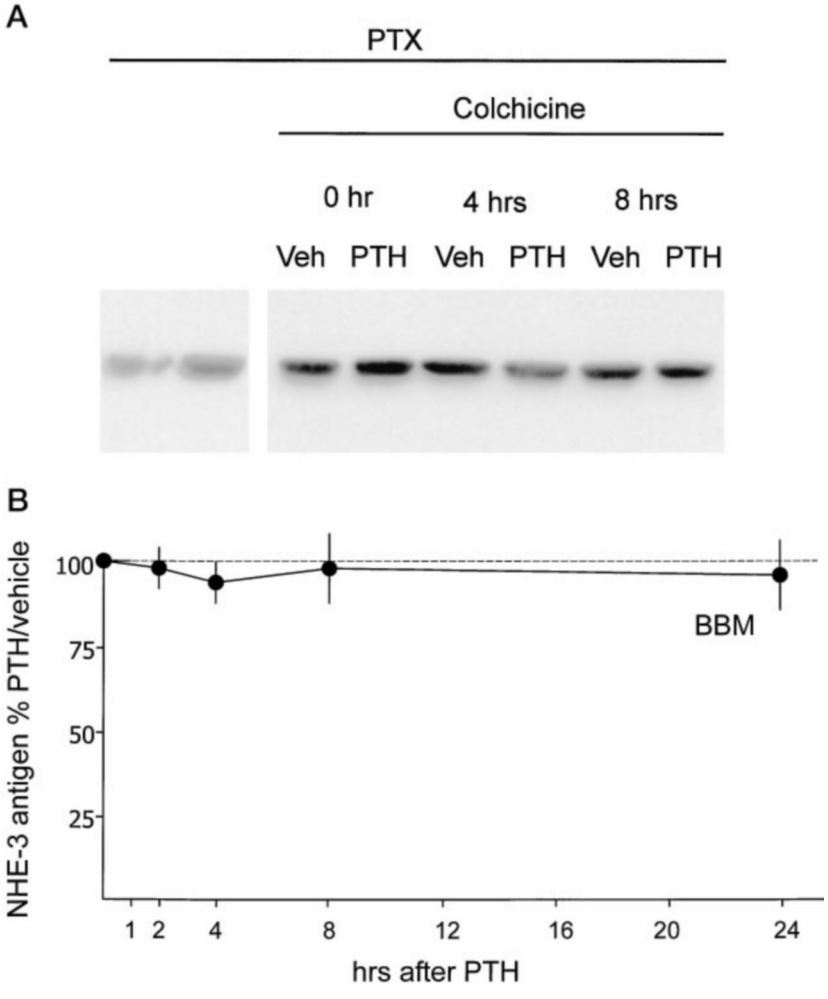


FIG. 7. **Effect of PTH on NHE-3 antigen: pretreatment with colchicine.** Animals were given colchicine prior to PTH infusion. *A*, immunoblot from a typical experiment. Experimental groups are as described: *PTX*, parathyroidectomy; *Colchicine*, animals pretreated with colchicine prior to PTH bolus; *PTH* or *Veh*, either PTH or vehicle was given for the stated time points. *B*, summary of all experiments. NHE-3 antigen in renal cortical apical brsuh border membranes (*BBM*) in PTH-treated animals was expressed as a percentage of NHE-3 antigen in vehicle-treated animals for each time point. *Bars* and *error bars* depict the means ± S.E. Number of animals (vehicle/PTH): 2 h, 4/4; 4 h, 6/6; 8 h, 8/8; 24 h, 6/6.

kidney after 30 min of PTH further substantiate the importance of NHE-3 phosphorylation in its functional regulation.

The time profile of inhibition of NHE-3 activity did not parallel that of NHE-3 antigen in the apical membrane. The decrease in NHE-3 activity within 2 h of PTH administration was not associated with decreased apical membrane NHE-3 antigen. There are other examples where NHE-3 activity is regulated likely without changes in surface NHE-3 antigen. Na/H exchange activity is inhibited by direct addition of cAMP analogues or hormones coupled to adenylyl cyclase to apical membrane vesicles, which are unlikely to retain competence for protein trafficking (54–56). Weinman and co-workers found decrease in Na/H exchange activity in proteoliposomes reconstituted from solubilized apical membrane proteins phosphorylated in solution by PKA *in vitro* (57–59). In OK cells, 15 min of PTH application altered the $pH_i$ sensitivity ($K_H$) of native NHE-3 in addition to a $V_{max}$ effect (31), and a recent study showed similar findings with cAMP addition to fibroblasts expressing NHE-3 (53). A change in the $K_H$ of NHE-3 is compatible with the hypothesis that mechanisms other than changes in plasma membrane NHE-3 protein may be operational in modifying NHE-3 activity. Our data from intact animals support the notion that changes in the intrinsic activity of NHE-3 may be one mechanism responsible for physiologic regulation of

NHE-3 function. Substrate kinetics were not examined in this study, but with the imposed gradients on the vesicles (internal pH, 5.5; external, pH 7.5; external [sodium], 0.1 mM), one is unlikely to be observing a pure $K_H$ effect. In fibroblasts transfected with NHE-3 and in OK cells, activation of PKA can acutely decrease NHE-3 activity in the intact cell without changing NHE-3 antigen abundance on the plasma membrane.[3,4] One preliminary report indicated early inhibition of native NHE-3 by PTH in OK cells can also occur without changes in plasma membrane NHE-3 antigen (60).

After 4 h of PTH administration, we clearly observed a decrease in apical membrane NHE-3 without changes in total cortical NHE-3 compatible with redistribution of NHE-3 protein from the apical membrane to another pool. Protein trafficking has been implied to explain regulation of apical membrane Na/H exchange (41, 61). Hensley and co-workers (41) fractionated homogenates from renal tubules treated with PTH and showed that PTH decreased Na/H exchange activity in a fraction enriched with alkaline phosphatase and increased Na/H exchange activity in a fraction enriched with acid phosphatase and galactosyltransferase. Although no antigenic data was available, this finding is compatible with trafficking of NHE-3 from an apical to an intracellular compartment. There are two major discrepancies between this study and that of Hensley and co-workers. First, the time course of redistribution NHE-3 antigen is much slower in this study than that inferred by activity measurements in Hensley's study. This may be due to systemic administration of PTH in this study *versus* direct incubation of tubules with PTH in Hensley's paper. Second, the Hensley experiment suggests that the redistributed Na/H exchanger is rapidly destroyed due to loss of Na/H exchange activity in the acid phosphatase fraction, whereas the present study shows minimal decrease in whole cortex NHE-3 after PTH infusion. It is difficult to correlate Na/H exchange activity in various membrane fractions to NHE-3 antigen. The measured Na/H exchange activities can be due to NHE-3, other NHE isoforms, or parallel Na and H conductances. Biemesderfer and co-workers (21) have shown NHE-3 residing in subapical vesicles in native rat kidneys. Zhang and co-workers (42, 43) showed that the acute decrease in proximal tubule apical membrane NHE-3 activity in a rat model of pressure natriuresis is associated with redistribution of NHE-3 antigen from the apical membrane to a subapical region. Preliminary reports have shown that the acute activation of NHE-3 activity by acidosis extracellular pH or endothelin are both associated with increase in plasma membrane NHE-3 antigen (46, 62). PTH induces dramatic change in the distribution of the type II sodium phosphate cotransporter NaPi-2 (44, 45).[2] There are marked differences between the responses of NHE-3 and NaPi-2 to PTH. Within 30 min of PTH infusion when no change in NHE-3 antigen can be detected, apical membrane NaPi-2 antigen is dramatically reduced. In addition to the difference in kinetics, the internalized NaPi-2 is rapidly targeted for lysosomal degradation, whereas no detectable decrease in total cortical NHE-3 antigen occurred in the study period. These findings suggest that PTH modulates distinct trafficking pathways for NaPi-2 and NHE-3 involving different vesicle populations destined for different fates.

The present study shows that immediate inhibition of renal cortical apical membrane NHE-3 activity is associated with phosphorylation of NHE-3 without detectable changes in apical membrane NHE-3 antigen. With sustained PTH, NHE-3 is distributed away from the apical membrane with no change in total cortical NHE-3 antigen. At present, we cannot establish the role of NHE-3 phosphorylation in mediating the change in apical membrane NHE-3 antigen, nor do we know whether the decrease in apical membrane NHE-3 antigen is mediated by decreased insertion or increased removal. With chronic changes in PTH levels such as parathyroidectomy, total renal NHE-3 antigen is altered.

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[3] O. W. Moe, Unpublished observation.
[4] S. Grinstein, personal communication.

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