Acute hypotension induced by aortic clamp vs. PTH provokes distinct proximal tubule Na⁺ transporter redistribution patterns


Am J Physiol Regul Integr Comp Physiol 287: R878–R885, 2004. First published June 17, 2004; 10.1152/ajpregu.00180.2004.—Renal parathyroid hormone (PTH) action is often studied at high doses (100 μg PTH/kg) that lower mean arterial pressure significantly, albeit transiently, complicating interpretation of studies. Little is known about the effect of acute hypotension on proximal tubule Na⁺ transporters. This study aimed to determine the effects of acute hypotension, induced by aortic clamp or by high-dose PTH (100 μg PTH/kg), on renal hemodynamics and proximal tubule Na/H exchanger isoform 3 (NHE3) and type IIa Na-Pi cotransporter protein (NaPi2) distribution. Subcellular distribution was analyzed in renal cortical membranes fractionated on sorbitol density gradients. Aortic clamp-induced acute hypotension (from 100 ± 3 to 78 ± 2 mmHg) provoked a 62% decrease in urine output and a significant decrease in volume flow from the proximal tubule detected as a 66% decrease in endogenous lithium clearance. There was, however, no significant change in glomerular filtration rate (GFR) or subcellular distribution of NHE3 and NaPi2. In contrast, high-dose PTH rapidly (<2 min) decreased arterial blood pressure to 51 ± 3 mmHg, decreased urine output, and shifted NHE3 and NaPi2 out of the low-density membranes enriched in apical markers. PTH at much lower doses (<1.4 μg·kg⁻¹·h⁻¹) did not change blood pressure and was diuretic. In conclusion, acute hypotension per se increases proximal tubule Na⁺ reabsorption without changing NHE3 or NaPi2 subcellular distribution, indicating that trafficking of transporters to the surface is not the likely mechanism; in comparison, hypotension secondary to high-dose PTH blocks the primary diuretic effect of PTH but does not inhibit the PTH-stimulated redistribution of NHE3 and NaPi2 to the base of the microvilli.

kidney; lithium clearance; glomerular filtration rate; blood pressure; rats; parathyroid hormone

PARATHYROID HORMONE (PTH) is a potent inhibitor of renal proximal tubule sodium reabsorption that has a marked hypertensive effect when administered at high doses (32, 33). PTH mediates rapid retraction of type Ila Na-Pi cotransporter protein (NaPi2) and Na/H exchanger isoform 3 (NHE3) from the plasma membrane (5, 9, 18, 19, 48) not unlike that observed during acute hypertension (44, 46, 47), and both acute hypertension and PTH treatment provoke natriuresis and diuresis (46, 48). Although we have previously demonstrated that most, if not all, renal effects of PTH (including diuresis, natriuresis, increase in cAMP excretion, and redistribution of proximal tubule NaPi2 and NHE3) can be induced at doses with no depressor effect (48), PTH at doses that lower blood pressure has been routinely used to address the primary action of PTH on renal NHE3 and NaPi2 (9, 12, 19, 23, 41, 42). Little is known about the effect of acute hypotension on the subcellular distribution of proximal tubule NaPi2 and NHE3 and whether this effect will antagonize or blunt the primary effect of PTH on renal function.

The effects of reductions in arterial pressure on renal function are studied conventionally by aortic clamp (7, 39, 43). It has been demonstrated that acute changes in renal arterial pressure can alter inversely proximal tubule reabsorption (4, 27) without altering glomerular filtration rate (GFR) or renal blood flow (RBF). At the cellular and molecular level, we have shown that when blood pressure increases there is redistribution of NHE3 and NaPi2 out of the apical microvilli to the base and intermicrovillar cleft regions that accompanies the rapid decrease in proximal tubule sodium reabsorption (44, 45, 47, 49). The cellular and molecular mechanisms responsible for the antidiuretic response to acute hypotension have not been investigated. We hypothesized that the response to hypotension may be the reciprocal of the response to hypertension, namely, recruitment of proximal tubule transporters to the apical microvilli where they would increase proximal tubule reabsorption.

The aims of the present study were to determine the effects of acute hypotension, induced by either aortic clamp or by high-dose PTH (100 μg PTH/kg), on renal hemodynamics and on proximal tubule transporter density distribution patterns. We found that clamp-mediated hypotension increases proximal tubule reabsorption and decreases urine output without a change in the subcellular distribution of NHE3 and NaPi2. This suggests that other molecular mechanisms besides transporter recruitment to the apical membrane are responsible for the increase in proximal tubule reabsorption. On the other hand, acute arterial pressure drop induced by high-dose PTH provoked a significant antidiuretic effect and a removal of proximal tubule NHE3 and NaPi2 from the apical microvilli. This demonstrates that acute hypotension associated with high-dose PTH blocks the primary diuretic effect of PTH that is observed at nondenpressor doses but does not block the redistribution of NHE3 and NaPi2 to the base of the microvilli.

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HYPOTENSION AND PTH EFFECTS ON PROXIMAL TUBULE Na$^+$ TRANSPORTERS

EXPERIMENTAL PROCEDURES

Animal preparation and surgical protocols. Experiments were performed on male Sprague-Dawley rats (315 ± 5 g body wt) that were kept under diurnal light conditions and with free access to food and water. Rats were anesthetized intramuscularly with ketamine (Fort Dodge Laboratories) and xylazine (Miles) (1:1, vol/vol) and placed on a thermostatically controlled operating table to maintain the body temperature at 37°C throughout experimentation. Polyethylene catheters (PE-50) were placed into the carotid artery and/or femoral artery for blood pressure monitoring and into the jugular vein for infusion of drugs and 4.0% BSA in 0.9% NaCl at 50 μl/min to maintain euvoelma. The left ureter was cannulated with a Surflo IV Catheter (Terumo) for urine collection. All animal experiments were approved by the University of Southern California Keck School of Medicine and conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Series I study: Effects of acute hypotension. Rats were anesthetized, and a clamp was placed on the aorta immediately proximal to the junction of the right renal artery to regulate renal perfusion pressure to both kidneys. Acute hypotension was induced for 30 min with the clamp, and decreases in renal perfusion pressure were monitored by a catheter inserted into the left femoral artery. Urine samples were collected at 10-min intervals to assess urine output, GFR, and endogenous lithium clearance (ClLi). Kidneys were removed at 30 min after induction of acute hypotension for density gradient fractionation.

Series II study: Effects of graded doses of PTH. The effects of PTH were investigated using the synthetic bovine PTH fragment bPTH-(1–34) (peptide content 71%; Peninsula Lab, Belmont, CA) dissolved in 0.14% saline (pH 7.4), and fluorescence was measured with a GENios microplate reader (TECAN) against known FITC-inulin standards. GFR was calculated as [inulin]u × V/[inulin]p, where V is the urine volume, [inulin]u is the urine inulin concentration, and [inulin]p is the plasma inulin concentration.

Density gradient fractionation. The procedure for collection, homogenization, and subcellular fractionation of total cortical membranes has been described in detail previously (46, 47). In brief, cortical tissue homogenate was subjected to centrifugation at 100,000 g for 5 h in a hypotonic sorbitol gradient. 12 fractions were collected, and then membranes were pelleted and resuspended. To simplify analysis for some samples, gradient fractions with similar membrane characteristics were pooled into three “windows” (Win I–Win III). Win I, fractions 3–5, is enriched in apical membrane markers alkaline phosphatase and dipeptidyl peptidase IV, Win II, fractions 6–8, is enriched in the apical membrane marker alkaline phosphatase and the intermicrovillar cleft marker megalin; and Win III, fractions 9–11, is enriched in the endosomal marker rab5a and the lysosomal membrane marker β-hexosaminidase (44). Under the current membrane window assignment, redistribution of apical transporters from Win I to Win II provides evidence of retraction of transporters from the apical microvilli to the base of the microvilli and/or intermicrovillar clefts. Further redistribution to Win III provides evidence for further retraction of apical transporters to the intermicrovillar clefts, coated pits, and endosomal and lysosomal membrane compartments.

Immunoblot analysis and antibodies. To determine the density distribution of NHE3 and NaPi2, a constant volume of sample from each membrane fraction or each pooled membrane “window” was resolved by SDS-PAGE on 7.5% gels and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore) according to standard methods. NHE3 was detected with the polyclonal NHE3-C00 (44). NaPi2 was detected with the polyclonal anti-NaPi2 antibody generated by J. Biber and H. Murer (Univ. of Zurich, Zurich, Switzerland) against synthetic 12-mer NH2-terminal NaPi2 peptide. NaPi2 is the Na-Pi cotransporter type IIa (NaPi-IIa) protein found in the rat kidney (20). We focused on NaPi2 because NaPi-IIa is the major transporter in renal P, reabsorption (25), and it is expressed exclusively in the apical membrane of the proximal tubule (6). Another Na-Pi cotransporter, NaPi type IIC, which is also localized in the apical membrane of the proximal tubule in weaning animals but has reduced role in adult rats (36), was not addressed in the present study. Both antibodies (anti-NHE3 and anti-NaPi2) were used at 1:2,000 dilution and detected with Alexa 680-labeled goat anti-rabbit secondary antibody (Molecular Probes, Eugene, OR) using the Odyssey Infra-red Imaging System and quantitation software (LI-COR, Lincoln, NE).

Statistical analysis. Data are expressed as means ± SE. ANOVA was used for multiple-group comparison. If a significant difference among groups was concluded by ANOVA, further pairwise comparisons were assessed by two-tailed Student’s t-test. Comparisons between two data groups were assessed directly by pairwise two-tailed Student’s t-test. P value < 0.05 was considered significant.

RESULTS

Effect of acute hypotension induced by aortic clamp on renal function. Aortic clamp exerted a significant decrease in renal perfusion pressure measured at femoral artery from 100 ± 3 to 78 ± 2 mmHg (Fig. 1A) that sustained for 30 min. GFR showed a transient drop from 0.49 ± 0.09 ml/min (baseline) to 0.14 ± 0.05 ml/min at 10 min but quickly returned to baseline level (0.40 ± 0.13 ml/min) by 20 min of hypotension (Fig. 1B),
indicative of effective autoregulation of GFR during clamp-
mediated acute hypotension. Endogenous C\(_{Li}\), a measure of
volume flow from the proximal tubule, also decreased from a
baseline level of 62 ± 11 to 21 ± 6 \(\mu\)l/min by 30 min (Fig.
1C). Since there was no sustained decrease in GFR, the
decrease in C\(_{Li}\) indicates an increase in proximal tubule Na\(^+\)
reabsorption. Finally, during hypotension, urine output de-
creased from 6.9 ± 1.1 \(\mu\)g/min (baseline) to 2.6 ± 0.9 \(\mu\)g/min
(at 30 min) (Fig. 1D).

**Effect of acute hypotension on the density distribution of
NHE3 and NaPi2.** Because we previously demonstrated that
acute hypertension provokes redistribution of NHE3 and NaPi2
out of the top of the microvilli to the base of the microvilli and
intermicrovillar cleft (NHE3) and endosomal membrane do-

ments (NaPi2) (15, 44, 47), we tested the hypothesis that the
increase in proximal tubule Na\(^+\) reabsorption (decreased C\(_{Li}\))
and decrease in urine output (Fig. 1) were associated with a
redistribution of NHE3 and NaPi2 into the apical membranes
of the microvilli that are enriched in low-density membranes
after fractionation on sorbitol density gradients. Contrary to
this hypothesis, 30-min acute hypotension induced by aortic
clamp did not increase the abundance of NHE3 (Fig. 2A) or
NaPi2 (Fig. 2B) in the enriched microvillar membranes (frac-
tions 3–5), nor did it decrease abundance of the transporters in
the enriched intermicrovillar membranes (fractions 6–8) or in
the endosomal enriched membranes (fractions 9–12). These
findings suggest that the increase in proximal tubule sodium
reabsorption is not the simple inverse of the response to
hypertension and may involve transporter activation.

**Effects of graded doses of PTH on renal function.** bPTH-
(1–34) at 0.7 and 1.4 \(\mu\)g PTH·kg\(^{-1}\)·h\(^{-1}\) has no effect on
arterial blood pressure (Fig. 3A) as previously established (48)
but caused dose-dependent increases in C\(_{Li}\) (Fig. 3B), urine
output (Fig. 3C), and urinary cAMP excretion (Fig. 3D). C\(_{Li}\)
increased from 47 ± 4 \(\mu\)l/min (baseline) to 92 ± 12 \(\mu\)l/min (at 1.4
\(\mu\)g PTH·kg\(^{-1}\)·h\(^{-1}\)) (Fig. 3B), and urine output doubled
from 4.9 ± 1.0 \(\mu\)g/min (baseline) to 11.4 ± 2.3 \(\mu\)g/min (at 1.4
\(\mu\)g PTH·kg\(^{-1}\)·h\(^{-1}\)) (Fig. 3C). Urinary cAMP excretion in-
creased with PTH infusion from 40 ± 3 pmol/min (baseline)
to 93 ± 8 pmol/min (at 1.4 \(\mu\)g PTH·kg\(^{-1}\)·h\(^{-1}\)) (Fig. 3D). These
results establish that at these low doses, PTH causes increases
in both C\(_{Li}\) and urine output in the absence of a change in
arterial pressure. As established previously (48), these in-
creases are associated with a retraction of NHE3 and NaPi2
from the low density apical microvilli to the intermediate
density membranes enriched in intermicrovillar clefts (NHE3)
and to dense membranes enriched in endosomes (NaPi2).

**Effect of high-level PTH on renal function and cAMP ex-
cration.** A PTH bolus of 100 \(\mu\)g PTH/kg induced a rapid acute
transient decrease in mean arterial pressure (Fig. 4A): arterial
pressure decreased to 51 ± 3 mmHg in <2 min and then
returned to baseline by 10 min and remained stable for up to 30
min. In contrast to the diuretic effect of low-dose PTH infu-
sion (Fig. 3C), 100 \(\mu\)g PTH/kg induced a transient but significant
decline in urine output from 5.0 ± 1.7 \(\mu\)l/min (baseline) to
2.7 ± 1.6 \(\mu\)l/min (11–20 min after bolus) followed by a return
to 5.6 ± 2.7 \(\mu\)l/min (21–30 min after bolus) (Fig. 4B). In this
experiment, the marked antidiuretic effect precluded reliable

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Fig. 1. Effects of acute hypotension induced by aortic clamp on renal function. After a 30-min baseline period, acute hypotension
was induced by aortic clamp for 30 min. Each bar represents mean value over a 10-min interval. *\(P < 0.05\) compared with
responding basal values at 20- to 30-min time interval. A: renal perfusion pressure recorded from femoral artery. Values are
means ± SE (n = 6). B: glomerular filtration rate (GFR) was measured as clearance of infused FITC-labeled inulin. Values are
means ± SE (n = 4). C: endogenous lithium clearance (C\(_{Li}\)) was calculated as \([\text{Li}^+]/[\text{Li}^-]_n \times V/[\text{Li}^-]_p\), where V is the urine
volume, [\text{Li}^+]_n is the urine \text{Li}^+ concentration, and [\text{Li}^-]_p is the plasma \text{Li}^- concentration. Values are means ± SE (n = 4). C\(_{Li}\), at 0- to
10-min time interval was not measured. D: urine output was measured gravimetrically. Values are means ± SE (n = 6).
GFR determination because during zero urine flow, the accumulation of FITC-inulin within the kidney may cause subsequent urine samples to have erroneously high FITC-inulin concentrations. However, we predict there is a significant decrease in GFR that parallels the very marked drop in arterial pressure to values (51 ± 3 mmHg) out of the autoregulatory range for GFR (37, 38). In fact, our C\textsubscript{Lr} data indicate that the volume flow from the proximal tubule did not increase significantly from baseline (75 ± 16 µl/min) even by 21–30 min after PTH bolus (51 ± 15 µl/min) despite significant redistribution of NHE3 (Fig. 5) and NaPi2 (Fig. 6) from the apical membrane. This finding supports the notion of a persistent decrease in GFR during high-level PTH treatment.

High-level PTH increased urinary cAMP excretion from 33 ± 4 pmol/min (baseline) to 278 ± 39 pmol/min after 20–30 min (Fig. 4C). Despite the 70-fold higher PTH dose, there was only a 3-fold increase in cAMP compared with the low-dose PTH set (93 ± 8 vs. 278 ± 39 pmol/min), suggesting saturation of urinary cAMP stimulation by PTH at 100 µg PTH·kg\textsuperscript{-1}·h\textsuperscript{-1}.

Effect of high-level PTH on the density distribution of NHE3 and NaPi2. Because the acute hypotension associated with high-level PTH blunted the PTH-associated diuresis, we tested the hypothesis that it would also blunt the redistribution of NHE3 from the low-density apical microvilli membranes to higher density intermicrovillar clefts and blunt the redistribution of NaPi2 to subapical and endosomal membrane pools (48), especially at the point where blood pressure was very low. In contrast to our prediction, the high dose of PTH (100 µg/kg), which transiently lowers blood pressure, did not blunt the redistribution of NHE3 and NaPi2 out of the apical microvilli, even at the point where blood pressure was at the lowest (51 ± 3 mmHg at 2 min). This indicates that the redistribution of NHE3 and NaPi2 in response to PTH is pressure independent. To simplify analysis and quantitation, the fractionated membranes were pooled into three windows corresponding to apical microvilli enriched (Win I), intermicrovillar clefts (Win II), and subapical and endosomal (Win III) membrane pools.

Fig. 2. Effect of acute hypotension on density distribution of Na/H exchanger isoform 3 (NHE3) and type IIa Na-P\textsubscript{i} cotransporter protein (NaPi2) in renal cortex. Comparison of NHE3 distribution (A) and NaPi2 distribution (B) between control (○, n = 4) and 30 min of induced hypotension by aortic clamp (●, n = 5). Immunoreactivity in each fraction is expressed as the percentage of total signal in all 12 fractions. Values are means ± SE. Shown below the graphs are representative immunoblots from typical experiments with NHE3 detected at 80 kDa and NaPi2 detected between 80 and 90 kDa.

Fig. 3. Effect of graded doses of parathyroid hormone (PTH) on mean arterial pressure and renal function. PTH-(1–34) was infused progressively from low to high infusion rates (0, 0.7, 1.4 µg PTH·kg\textsuperscript{-1}·h\textsuperscript{-1}) at 20-min intervals. Data for 0.7 and 1.4 µg PTH·kg\textsuperscript{-1}·h\textsuperscript{-1} were collected during the last 10 min of the 20-min interval to allow for equilibration to the new PTH dose. Baseline (0 µg PTH·kg\textsuperscript{-1}·h\textsuperscript{-1}) was calculated as the mean of the two 10-min intervals before PTH infusion. All values are means ± SE (n = 4). A: mean arterial pressure recorded from carotid artery. B: endogenous C\textsubscript{Lr} was calculated as [Li\textsuperscript{+}]\textsubscript{p} × V/[Li\textsuperscript{+}]\textsubscript{p}. C: urine output was measured gravimetrically. D: urinary cAMP excretion was measured by radioimmunoassay. *P < 0.05 compared with values at baseline.
PTH bolus infusion (100 µg PTH/kg) also induced redistribution of NaPi2 out of apical membrane-enriched Win I from 15 ± 2% of total (controls) to 9 ± 1% and 8 ± 1% at 2 and 30 min, respectively (Fig. 6). However, the NaPi2 redistribution pattern was distinct from that of NHE3 (Fig. 5); specifically, PTH induced a time-dependent internalization of NaPi2 to the endosomal pools in Win III: by 30 min after PTH infusion NaPi2 immunoreactivity in Win II decreased from 65 ± 3% of total (controls) to 45 ± 3% accompanied by a significant increase in Win III from 20 ± 2% (controls) to 48 ± 3% of total NaPi2 (Fig. 6). Two-way ANOVA of the NaPi2 distribution pattern revealed three significantly distinct patterns between control, 2-min, and 30-min groups. In addition, the total NaPi2 pool size in cortical homogenate decreased by 32 ± 10% by 30 min after PTH infusion as previously reported (19). In summary, in response to high-level PTH there was a dramatic decrease in blood pressure along with the predicted decrease in urine output, but even at the lowest blood pressure point, the retraction of NHE3 and NaPi2 from the microvilli was the same as that seen previously at doses of PTH that cause diuresis and no change in blood pressure.

Fig. 4. Effects of high-level PTH on mean arterial pressure and renal function. PTH-(1–34) was infused at 100 µg PTH/kg as a single bolus (<2 min) through the jugular vein. Arterial pressure was traced continuously, and urine was collected over 10-min intervals. A: mean arterial pressure was recorded from a chart recorder but are presented at 10-min intervals except between 0 and 10 min after PTH bolus infusion when data are presented at 1-min intervals. Values are means ± SE (n = 5). B: urine output was measured gravimetrically. Values are means ± SE (n = 5). C: urinary cAMP excretion was measured by radioimmunoassay. Values are means ± SE (n = 4–5). *P < 0.05 relative to baseline values during the 10 min before PTH treatment.

Fig. 5. Effects of high-level PTH on NHE3 density distribution. PTH-(1–34) was infused as a single bolus of 100 µg PTH/kg through the jugular vein, and kidneys were removed at 2 or 30 min after the bolus. Kidneys from paired saline-infused controls were collected in tandem. Renal cortex was fractionated on sorbitol density gradients, collected as 12 fractions and pooled into 3 windows (Win I–III). NHE3 abundance in each window is expressed as the percentage of the total signal in all 3 windows. A: NHE3 distribution in each window in control (open bars), 2 min after PTH bolus (shaded bars), and 30 min after PTH bolus (solid bars). Values are means ± SE (n = 4). *P < 0.05 compared with corresponding controls in the same window. B: representative immunoblots of membranes in Win I–Win III in the 3 treatment groups. Volumes assayed were adjusted to ensure that signals were within the linear range of detection, and 2 to 3 different volumes were loaded to validate quantitation in the linear range.
Fig. 6. Effects of high-level PTH on NaPi2 density distribution. PTH-(1–34) was infused as a single bolus at 100 μg PTH/kg through the jugular vein and kidneys taken at 2 min or 30 min after the bolus. Kidneys from paired saline-infused controls were collected in tandem. Renal cortex was fractionated on sorbitol density gradients, collected as 12 fractions, and pooled into 3 windows (Win I = fractions 3–5, Win II = fractions 6–8, Win III = fractions 9–11). NaPi2 abundance in each window is expressed as the percentage of the total signal in all 3 windows. A: NaPi2 distribution in each window in control (open bars), 2 min after PTH bolus (shaded bars), and 30 min after PTH bolus (solid bars). Values are means ± SE (n = 4). *P < 0.05 compared with corresponding control values in the same window; #P < 0.05 compared with corresponding PTH 2-min values in the same window. B: representative immunoblots of membranes in Win I–Win III in the 3 treatment groups. Volumes assayed were adjusted to ensure that signals were within the linear range of detection, and 2 to 3 different volumes were loaded to validate quantitation in the linear range.

DISCUSSION

Many studies have investigated the effect of arterial pressure on proximal sodium and volume reabsorption in rats (4, 8, 13, 28) and dogs (8, 16, 26, 37). One complication in dissecting the effect of pressure on tubule sodium transport is that changes in pressure may cause parallel changes in GFR. If beyond the autoregulatory range, a change in GFR per se may change proximal tubule sodium reabsorption. Navar and coworkers (26) studied reductions in arterial pressure within the autoregulatory range in dogs and observed increases in fractional water and sodium reabsorption. These results indicate an effect of hypotension independent of GFR on proximal tubule reabsorption. In a reciprocal study, Chou and Marsh (4) reported in rats that an acute increase in arterial pressure significantly reduces proximal tubular fluid reabsorption. Together, these studies establish that, within the autoregulatory range, there is a strong inverse relationship between renal arterial pressure and proximal tubular reabsorption. We have shown that during acute hypertension apical NHE3 and NaPi2 redistribute out of the tops of the microvilli to the intermicrovillar cleft membranes (NHE3) and endosomes/lysosomes (NaPi2) (45, 49).

Because the responses of proximal tubular sodium transporters to an acute drop in arterial pressure have never been previously studied, we tested the hypothesis that it would be the inverse of the response to acute hypertension, namely, a redistribution of proximal tubular transporters from the base to the top of the microvilli.

This study demonstrates that acute hypotension induced by aortic clamp significantly increases proximal tubule sodium and fluid reabsorption (measured by a decrease in C Li ) but did not provoke any redistribution of NHE3 and NaPi2 into the apical microvilli membranes (Fig. 2). This suggests that other molecular mechanisms besides recruitment of Na+ transporters to the apical membrane, such as change in transporter activity by phosphorylation (22) and/or change in associated proteins such as Na/H exchanger regulatory factor and cytoskeleton (10), are responsible for the increased proximal sodium reabsorption that provides the tubuloglomerular feedback error signal to autoregulate GFR.

This study focused on understanding the interactions between the primary effects of PTH and the secondary hypotensive effect of commonly used high doses of PTH on proximal tubule transporters. PTH at 100 μg bolus/kg causes a rapid drop in arterial pressure that poses an interesting physiological dilemma for the proximal tubule. It is well documented that PTH inhibits proximal tubule reabsorption (1, 35), whereas acute hypotension stimulates proximal tubule reabsorption (26, 27). How these antagonistic influences interact in the proximal tubule during high-level PTH treatment has not been previously addressed. In the present study, we compared 1) the renal effects of acute hypotension alone (by aortic clamp) to 2) the renal effects of a high dose of PTH that induces acute hypotension to 3) the renal effects of nondepressor doses of PTH. From a comparison of these three series, we conclude the following. Acute hypotension induced by aortic clamp provokes an increase in proximal tubule reabsorption and antidiuresis without a redistribution of NHE3 and NaPi2. At low doses (0.7 and 1.4 μg PTH·kg⁻¹·h⁻¹), PTH has no depressor effect and is diuretic. On the other hand, high-dose (100 μg bolus/kg) PTH that induces acute drop in arterial pressure also provokes antidiuresis not unlike that observed during clamp-induced acute hypotension. This suggests that the antidiuretic effect of hypotension (and the likely fall in GFR) predominates over the primary diuretic effect of PTH. In contrast, the rapid retraction of NHE3 and NaPi2 from the apical microvilli during high-dose PTH mimics the previously established effect of low-dose PTH to retract these transporters (48). This indicates that the PTH influence on transporter redistribution predominates over any influence of hypotension per se induced by high-dose PTH. Finally, the primary effects of PTH on both renal function and transporter distribution can be investigated independent of blood pressure changes at “low levels” of PTH. In the present study, a direct comparison between low-dose and high-dose PTH on transporter redistribution was not carried out because the detailed effect of low-dose PTH on retraction of NHE3 and NaPi2 from the microvilli has been established and reported elsewhere (48). Our comparison reveals that low-dose and high-dose PTH provokes indistinguishable redistribution patterns for NHE3 and NaPi2.

At these low doses, PTH increases C Li, urine output, and cAMP excretion without any depressor effect (Fig. 3). A change in GFR is not likely to mediate the diuresis and the
decrease in proximal tubule reabsorption (i.e., an increase in GFR) since it has been shown that PTH (1–34) infused into rats at 4.1 μg·kg⁻¹·h⁻¹ did not have a significant sustained effect on GFR (50). In contrast, a PTH bolus of 100 μg/kg induced a rapid drop in mean arterial pressure to 51 ± 3 mmHg along with a decrease in urine output (Fig. 4). As explained in RESULTS, the marked antidiuretic effect induced by high-level PTH precluded accurate determination of GFR with FITC-inulin as a tracer. However, the antidiuretic effect can likely be attributed to a fall in GFR secondary to the acute hypotension because renal blood flow and GFR are known to fall to near zero when arterial pressure is decreased to <60 mmHg (37, 38) (i.e., beyond the autoregulatory range), and decreased filtration is associated with an increase in fractional sodium reabsorption (2, 3, 14, 26, 27), culminating in decreased urine flow rate and sodium excretion (26, 34). In addition, there was not a significant increase in volume flow from the proximal tubule, measured by Cl₂, even by 30 min after high-dose PTH treatment despite significant redistribution of NHE3 (Fig. 5) and NaPi2 (Fig. 6) out of the top of the microvilli that is usually associated with a decrease in Na⁺ reabsorption. This further supports the notion of a persistent decrease in GFR during high-level PTH treatment.

NHE3 is the primary Na/H exchanger isoform responsible for proximal tubular apical NaCl and NaHCO₃ reabsorption (22, 30, 31). Fan and coworkers (9) reported that bolus injection of 100 μg PTH/kg decreased apical NHE3 activity in brush-border membranes (BBMs) within 30 min. Our studies demonstrate that PTH induces a far more rapid redistribution of NHE3 protein out of the apical membranes (Win I), as soon as 2 min after injection (Fig. 5). Hensley and coworkers (11) also reported in isolated rat proximal tubules a rapid redistribution of Na/H exchanger activity from apical to intracellular membranes by 2 min after PTH treatment. The delayed inhibition of activity seen by Fan et al. (9) may be that the acute transient hypotension temporarily masks and delays the decrease in NHE3 activity. In contrast with PTH, acute hypotension induced by aortic clamp does not provoke a redistribution of NHE3 in the proximal tubule and so, naturally, does not mask the effects of PTH on NHE3 distribution.

In the current study, we studied the effect of the same PTH dose on the subcellular distribution of renal cortical NaPi2 and observed a significant disappearance of NaPi2 from the apical membranes (Win I) as early as 2 min after PTH injection (Fig. 6) when the hypotensive effect was the most prominent (Fig. 4). This again provides evidence that the primary action of PTH on NaPi2 retrieval from the apical membranes is not masked by accompanying hypotension (Fig. 2). The rapid (<2 min) PTH-induced retrieval of NaPi2 from Win I also strongly suggests that most, if not all, PTH regulatory effects on proximal tubular Na-Pi cotransport are exerted by directly altering Na-Pi transporter protein abundance in the plasma membrane (24). Using a BBM isolation protocol, Lhotscher and coworkers (19) demonstrated that 100 μg PTH/kg induced a significant decrease in the renal cortical BBM NaPi2 abundance after 15 min of PTH injection although a drop in BBM NaPi2 was already “faintly detectable” after 5 min. The more rapid (<2 min) redistribution of NaPi2 observed in this study compared with Lhotscher et al. (19) could be explained by the fact that our subcellular fractionation scheme separates source and target membranes (e.g., intermicrovillar cleft) that were both present in the isolated total BBM used by Lhotscher et al. (19). Here we also reported that there was no significant increase in NaPi2 abundance in the intracellular membrane pools (Win III) until after 30 min of PTH injection (Fig. 6). At this point there was also a significant fall in total cortical NaPi2 protein abundance. This is consistent with the findings of the immunohistochemical study by Traebert and coworkers (41) that 15 min after bolus injection of 100 μg PTH, NaPi2 protein abundance decreased in the BBM and increased in the endocytic vacuoles and lysosomes where NaPi2 is destined to lysosomal degradation without recycling (19, 21, 29).

In summary, this study demonstrates that the proximal tubule response to acute hypotension is physiologically the opposite of the response to acute hypertension, namely, an increase in proximal tubule Na⁺ and volume reabsorption. However, at the molecular level the response to hypotension does not involve the reversal of the response to acute hypertension, namely, enrichment of NHE3 and NaPi2 in the microvilli. Because there is no redistribution of NHE3 or NaPi2 during acute hypotension, the transient hypotension that accompanies high-level PTH treatment does not alter the PTH-driven retraction of NHE3 and NaPi2 from the apical microvilli, but it does antagonize the PTH-associated diuresis. The molecular basis for the increase in proximal tubule sodium transport during hypotension remains to be determined. It also remains to be determined whether the transient hypotension accompanying high-dose PTH antagonizes the PTH-induced decrease in transporter activity.

GRANTS
This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-54316. P. K. K. Leong and L. E. Yang were supported by postdoctoral and predoctoral support, respectively, from the American Heart Association, Western States Affiliate.

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