Parathyroid Hormone Decreases HCO_3 Reabsorption in the Rat Proximal Tubule by Stimulating Phosphatidylinositol Metabolism and Inhibiting Base Exit

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Abstract

The mechanism of inhibition of HCO_3 transport by parathyroid hormone (PTH) in the proximal tubule is not clearly defined. Previous studies in vitro have suggested that this effect is mediated via cAMP generation, which acts to inhibit Na/H exchange, resulting in cell acidification. To examine this question in vivo, intracellular pH (pH_i) was measured in the superficial proximal tubule of the rat using the pH-sensitive fluoroprobes 4-methylumbelliferone (4MU) and 2',7'-bis(carboxyethyl)-(5, and 6)-carboxyfluorescein (BCECF). PTH was found to alkalinize the cell. This alkalinization suggested inhibition of basolateral base exit, which was confirmed by in situ microperfusion studies: lowering HCO_3 in peritubular capillaries acidified the cell, an effect blunted by PTH. Removal of luminal Na promoted basolateral base entry, alkalinizing the cell. This response was also blunted by PTH. Readdition of luminal Na stimulated the luminal Na/H exchanger, causing an alkalinization overshoot that was partially inhibited by PTH. cAMP inhibited luminal H secretion but did not alkalinize the cell. Stimulation of phosphatidylinositol-bis-phosphate turnover by PTH was suggested by the effect to the hormone to increase cell Ca. Blocking the PTH-induced rise in cell Ca blunted the effect of the hormone to alkalinize the cell, as did inhibition of phosphatidylinositol breakdown. Furthermore, stimulation of protein kinase C by a phorbol ester and a diacylglycerol applied basolaterally alkalinized the cell and inhibited luminal H secretion. The findings indicate that both arms of the phosphatidylinositol-bis-phosphate cascade play a role in mediating the effect of PTH on the cell pH. The results are consistent with the view that PTH inhibits base exit in the proximal tubule by activation of the phosphatidylinositol cascade. The resulting alkalinization may contribute, with cAMP, to inhibit apical Na/H exchange and the PTH-induced depression of proximal HCO_3 reabsorption. (*J. Clin. Invest.* 1992. 89: 1485–1495.)
Key words: acidification • cAMP • phosphatidylinositol metabolism • intracellular pH • intracellular calcium

Introduction

Previous studies (1) have shown that parathyroid hormone (PTH)[1] decreases sodium and HCO_3 reabsorption in isolated proximal segments of the rabbit. Little is known about the mechanism of this effect. Bicarbonate reabsorption in the proximal tubule occurs as a result of H secretion into the tubular lumen, predominantly via a luminal Na/H exchanger (2). Base generated in the cell exits the basolateral membrane largely via Na/HCO_3 cotransport (3). Studies by Kahn et al. (4) in brush border membrane vesicles (BBMV) harvested from rabbit renal tubules and by Pollock et al. (5) in cultured cells from the opossum kidney (OK) have shown that PTH inhibits Na/H exchange. Furthermore, PTH was reported to acidify the cell, an effect consistent with inhibition of the Na/H exchanger (5).

It has been proposed that PTH effects may be mediated by two separate cell signaling mechanisms. One pathway involves the cAMP cascade. The second mechanism depends on phosphatidylinositol-bis-phosphate (PIP_2) hydrolysis resulting in an increase in cell Ca and activation of protein kinase C (PKC). Concerning the inhibitory action by PTH on HCO_3 reabsorption, it has been accepted that this effect is mediated via cAMP insofar as this cyclic nucleotide has been shown to reproduce the effect of the hormone to decrease HCO_3 transport (2, 6) and mimic the ability of PTH to inhibit Na/H exchange in BBMV (4) and OK cells (5). The possibility that PTH could depress HCO_3 reabsorption by an increase in PIP_2 turnover has not been explored.

The present studies were undertaken to examine the effect of PTH to inhibit HCO_3 reabsorption in the proximal convoluted tubule of the rat. We further sought to study whether the decrease in proximal acidification by PTH is mediated primarily by a direct inhibition of the luminal Na/H exchanger or, alternatively, by a direct inhibitory effect on Na/HCO_3 cotransport, which, by increasing cell pH, would inhibit Na/H exchange. Lastly, studies were performed to determine the cellular mediators of the effect of PTH on HCO_3 transport. The results demonstrate that PTH inhibits base exit, an action that causes cell alkalinization and would inhibit the Na/H exchanger by alteration of driving forces and by allosteric inhibition of the exchanger. In addition, PTH has a direct action to inhibit the luminal Na/H exchanger, an effect reproduced by cAMP. However, the effect of PTH to alkalinize the cell is not reproduced by cAMP. Inhibition of base exit by PTH appears to be the result of stimulating PIP_2 hydrolysis. Both a rise in cell Ca and stimulation of basolateral PKC by PTH seem to play separate roles in inhibiting base exit. PTH may also activate a luminal pool of PKC. Activation of this pool stimulates luminal Na/H exchange, an action that would partially offset the inhibitory effect of cAMP on the exchanger.

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1. *Abbreviations used in this paper:* BAPTA, bis-(*o*-aminophenoxy)-*N,N,N',N'*-tetraacetic acid; BBMV, brush border membrane vesicles; BCECF, 2',7'-bis(carboxyethyl)-(5, and 6)-carboxyfluorescein; db-cAMP, dibutyryl cAMP; IP_3, inositol trisphosphate; 4MU, 4-methylumbelliferone; OAG, oleylacetyl-*sn*-glycerol; OK, opossum kidney; PI, phosphatidylinositol; PIP_2, phosphatidylinositol-bis-phosphate; PKC, protein kinase C; PTH, parathyroid hormone; TCO_2, total dissolved CO_2; TPTX, thyroparathyroidectomy.

Methods

Sprague-Dawley and Munich-Wistar rats maintained on a normal rat chow diet and weighing 180–200 g were anesthetized by an injection of sodium nembutal (50 mg/kg i.p.). A surgical thyroparathyroidectomy (TPTX) was performed as previously described (7). To maintain their body temperature, rats were placed on a feedback-regulated heating table. A tracheotomy was performed. Catheters were inserted into the external jugular vein for the administration of an isotonic solution containing NaCl and $NaHCO_3$ at a rate of 60 μl/min. The amount of $NaHCO_3$ was adjusted to maintain a HCO_3 concentration in blood of 25–28 mM. A catheter was inserted in the femoral artery to monitor blood pressure and arterial blood gases. Arterial P_{CO_2} was maintained at 40–45 mmHg using a rodent ventilator (Edco Scientific, Chapel Hill, NC). The left kidney was exposed as previously described (7) and both ureters catheterized for urine collection. pH and P_{CO_2} were measured on arterial blood and perfusion solutions using a blood gas analyzer (model 150, Corning Medical, Medfield, MA).

HCO_3 reabsorption studies

Studies were performed in Sprague-Dawley rats. On completion of surgery, appropriate amounts of [^{3}H]insulin were infused in 0.85% NaCl. After control whole kidney clearance and tubular fluid collections were obtained, 1–34 b-PTH was infused at a rate of 5×10^{-11} M/h after a priming dose of 5×10^{-11} M. After a 60-min equilibration, measurements were repeated.

Tubular fluid collections

Late proximal convolutions were identified by an intravenous injection of FD & C green dye. Timed tubular fluid collections were obtained after injecting a column of Sudan Black–stained mineral oil preequilibrated with a CO_2/air gas mixture to yield a P_{CO_2} of 60 mmHg. Collected tubular fluid was dispensed into mineral oil preequilibrated as above.

Analytical methods

Inulin concentration in tubular fluid, plasma, and urine was determined by liquid scintillation counting. Phosphorus concentration in plasma and urine was measured by the method of Chen et al. (8). Total dissolved CO_2 (TCO_2) in aliquots of plasma and tubular fluid was measured by microcalorimetry as described by Vurek et al. (9).

Fluoroprobe methods

4-Methylumbelliferone (4MU). Early proximal convolutions were identified by intravenous injection of FD & C green dye. Identification of more distal convolutions was determined by intratubular injection of isotonic saline stained with FD & C green dye. Proximal tubular cells in Sprague-Dawley and Munich-Wistar rats were loaded by superfusion with 4MU acetate as previously described (10). Briefly, the superfusate was allowed to flow continuously on the renal surface, forming an ultra-thin layer under a submicrometer-thick sheet of copolymer. The composition of the superfusate was (mM): 145 NaCl, 2.5 $CaCl_2$, 1.8 $MgSO_4$, 4.5 KCl, 4.5 Tris, and 0.2 4MU acetate (1% methanol), and pH adjusted to 6.5. Superfusates were bubbled with a 7% CO_2-93% O_2 gas mixture to obtain a P_{CO_2} of 60 mmHg, similar to that reported in the rat renal cortex (11). Appropriate amounts of $NaHCO_3$ were added to the perfusate to yield a pH of 6.5. For cell fluorescence measurement, 365 and 334 nm were chosen as the pH-discriminating excitation wavelengths. Fluorescence was measured at 460 nm.

Calibration of 4MU was performed as previously described (10). After the insertion of a wax block, lumen and peritubular capillaries were perfused with solution containing (mM): 25 Hepes, 60 K_2HPO_4 and adjusted to various pH (P_{CO_2} 60 mmHg and appropriate amounts of $NaHCO_3$ added for each pH value). 4MU (1 mM) was added to the luminal perfusate. K concentration was chosen to approximate the K activity reported in proximal tubular cells (12). Perfusates contained nigericin (10 μM), a K/H antiporter.

2',7'-bis(carboxyethyl)-(5, and 6)-carboxyfluorescein (BCECF). For cell pH (pH_i) measurement early proximal cells in Munich-Wistar rats were loaded with the fluoroprobe BCECF-AM by manual injection using an 8–10 μm o. d. pipette. BCECF-AM (10^{-5} M) prepared in DMSO (0.8%) was dissolved in a Tris buffer solution of composition identical to that used for superfusion in the 4MU studies, except pH was adjusted to 7.3. In these and all other subsequent studies, because of the need to perform micropuncture, the renal surface was bathed with Tris buffer, pH 7.4, and not covered by a sheet of copolymer. Cell loading was performed at a rate low enough to prevent overt dilatation of the lumen and lasted 10–15 min until the signal to background fluorescence at 460 nm excitation was > 20-fold. 460 and 490 nm were chosen as the pH-insensitive and -sensitive wavelengths. Changes in fluorescence measured at 535 nm as a function of pH were expressed as the ratio of 490/460 nm using an in vivo nigericin-K calibration as described for 4MU. Measurements were used only if fluorescence at 460 nm excitation was ≥ 15-fold greater than that of background.

Luminal pH measurements. Early proximal convolutions were perfused as described above with a solution containing 25 mM HCO_3 (Table I) and 2 mg/ml of BCECF-dextran (70,000 mol wt) at a rate of 20 nl/min using a Hampel pump. 490/460 fluorescence ratio was measured just distal to the perfusion pipette and in the late proximal convolution of the same nephron. Area of fluorescence measurement was limited to the center of the tubular lumen. Uptake of fluoroprobe into epithelial cells did not significantly contribute to the fluorescence derived from the lumen; in unblocked convolutions, fluorescence during perfusion with BCECF-dextran was ≥ 20-fold greater than that measured after the fluoroprobe cleared from the convolution. Measurements were obtained before and 2 min after either superfusion of Tris buffer containing dibutyryl-cAMP (db-cAMP, 7mM) or phorbol myristate acetate (PMA) (5×10^{-7}M) or luminal perfusion with PMA (5×10^{-7}M). In this latter case, after control measurements were obtained, the luminal perfusion pipette was removed and a pipette containing the same perfusate but with PMA added was reinserted at the same site. In situ pH calibration was obtained after each study with a perfusate identical to that used above for luminal perfusion, except that Na-containing salts were replaced with Na Hepes and pH adjusted to 6.8 or 7.4. Convolutions were perfused at a rapid rate achieved by connecting the perfusion pipette to a pressure source and fluorescence measured just distal to the pipette.

Fura 2. Early proximal cells in Munich-Wistar rats were loaded with the acetoxymethyl ester of Fura 2 (Fura 2 AM). Tris buffer containing 10^{-5} M Fura 2 AM and 0.8% DMSO was perfused luminally for 20 min. Fluorescence was highly homogeneous. Dye leak rate had a $t_{1/2}$ of ~ 30 min. 334 and 365 nm were chosen as the Ca-sensitive excitation wavelengths. Fluorescence measured at 495 nm, the wavelength of maximal fluorescence intensity in vivo, was expressed as a function of cell Ca using an in vitro calibration. Fluorescence intensity at 334 and 365 nm excitation exceeded background fluorescence by two- to three-,

Table I. Perfusion Solutions

	25 HCO_3	5 HCO_3	0 Na
	mM		
Na^+	148	148	0
K^+	5	5	5
$Choline^+$	0	0	148
Ca^{2+}	2.5	2.5	2.5
Mg^{2+}	1.8	1.8	1.8
Cl^-	130	130	130
HCO_3^-	25	5	25
PO_4^{-2}	3	3	3
SO_4^{-2}	1.8	1.8	1.8

Perfusates containing 25 or 5 mM HCO_3 were prepared by first titrating the solutions to pH 7.25 and 6.5, respectively, with 1 N Tris base. Subsequently, appropriate amounts of $NaHCO_3$ were added and the solutions were bubbled with 7% CO_2-93% O_2.

and four- to sixfold, respectively. Ratio measurements were not corrected for background because this did not change the ratio appreciably. After control measurements, readings were repeated at 2-min intervals after initiating superfusion or intravenous injection of test compounds. Measurements could not be obtained before 2 min because of a focus change due to doming of the superfusate under the renal capsule during superfusion, or motion of the kidney consequent to blood pressure changes during intravenous administration. Cell Ca concentrations were calculated according to the formula $Ca = R - R_{min}/R_{max} - R \times Sf_2/Sb_2 \times K_d$, as described by Grynkiewicz et al. (13), where R is the experimentally measured 334/365 nm fluorescence ratio, R_{max} is the maximal ratio obtained in the presence of a saturating Ca concentration (2 mM), R_{min} is the ratio obtained in the absence of Ca, Sf_2/Sb_2 is the ratio of fluorescence at 365 nm excitation in Ca-free and Ca-saturating solutions, and K_d is the dissociation constant of the Fura 2/Ca complex and was taken to be 224 nM (13).

An in vitro calibration was performed using the same optics used for the measurement of Fura 2 fluorescence in vivo. To avoid photobleaching, fluorescence in the Ca-free and Ca-saturating solutions was measured, as these were constantly perfused through quartz capillary tubing. R_{min} and Sf_2 were measured in a solution resembling the intracellular milieu and having the following composition (mM): 110 KCl, 17.5 NaCl, 1.0 NaH_2PO_4, 0.8 $MgSO_4$, 25 Hepes, 2 EGTA and adjusted to pH 7.2. For the measurement of R_{max} and Sb_2, 2 mM EGTA was replaced with 2 mM $CaCl_2$. The effect of varying Fura 2 concentration (10–500 μM) was studied. No effect on R_{min} was observed over this range of Fura 2 concentrations. R_{max} decreased by 8% over the range of concentrations studied.

Optical system

Fluorescence measurements were made using a modified microscope Labophot, (Nikon Inc., Garden City, NY) fitted with an epifluorescence attachment and a trinocular body tube. The microscope was attached to an X-Y-Z translational stage driven by remote control. A 100-W mercury lamp was used as the light source. Optics were modified by replacing the glass lenses with quartz elements. Focal distance was increased to permit micropuncture by insertion of a diverging quartz lens above the nosepiece. The epifluorescence attachment was modified to accommodate appropriate band pass excitation filters (Ditric Optics, Hudson, MA) and inconel-coated quartz windows (Corion Corp., Holliston, MA) to decrease the possibility of photodamage by attenuating the excitation light to the limits of detection.

In studies with BCECF, epillumination was achieved with a Nikon B filter cube cassette without the excitation filter and a ×20 CF objective. In studies with 4MU and Fura 2, light was filtered with a UG11 filter (Ealing Corp., South Natick, MA) situated in front of the 7-nm narrow-band excitation filters. For epillumination a Nikon UV cube cassette without the excitation filter and a ×20 (4MU studies) or ×10 (Fura 2 studies) Nikon Fluor objective were used.

Fluorescence was measured using a microscope spectrum analyzer (Farrand Optical Co., Inc., Valhalla, NY) with target spotting capability mounted on the phototube of the microscope. A 0.13-mm (4MU studies) or a 0.55-mm (BCECF and Fura 2 studies) pinhole and a 15-nm slit were placed on the apertures of the emission grating monochromator and photomultiplier tube, respectively. The area from which fluorescence was measured corresponded to 4 μm^2 (4MU studies) or 32 μm^2 (BCECF and Fura 2 studies). In the case of 4MU fluorescence, measurements were localized to the width of the epithelium, avoiding the lumen and peritubular capillaries. In BCECF and Fura 2 studies, measurements were localized to the width of the convolution. Precautions were taken to reduce the time of exposure and thus prevent cell damage.

Luminal and capillary perfusion (Table I)

For luminal perfusion an 8–10-μm o. d. pipette was inserted into the midportion of an early proximal convolution after insertion of a wax block in its proximal end. In those studies in which only one perfusate was to be tested, the pipette was attached to a modified Hampel pump and perfusion carried out at 40 nl/min. In studies in which rapid switching between two perfusates was required, the following setup was used. The perfusion pipette assembly consisted of a holder with two pieces of 31-gauge steel tubing inserted into the tip of the pipette. The tubing free ends were connected via PE20 and a switching valve to separate syringes containing the desired perfusates. The back ends of the syringes were connected via a three-way stopcock to a gas tank containing 7% CO_2-93% O_2. To change solutions the hydraulic pressure was switched from one tubing to the other while venting the pipette by transiently opening a relief outlet in the pipette holder. Switchover time was ~ 3 s.

For capillary perfusion studies requiring one perfusate only, a 10–12-μm o. d. pipette was inserted into a capillary adjacent to the convolution under study. Perfusion was achieved by connecting the pipette to a gas tank with the same composition as noted above for luminal perfusion. For sequential capillary perfusion with two different perfusates, the setup described for luminal perfusion was used. Hydraulic pressure was adjusted to prevent marked dilatation of the tubular lumen during luminal perfusion, and overt blanching of the capillaries over a large area, during capillary perfusion. Overperfusion resulted in rapid loss of BCECF from the cells and in progressive cell acidification, suggesting that increased pressure had an adverse effect on cell pH. Studies in which rapid leak rates of the fluoroprobe were noted were discarded.

Fluoroprobes and 5-5'dimethyl BAPTA-AM were obtained from Molecular Probes, Inc. (Eugene, OR). The protein kinase inhibitors 1-(5-isoquinoline-sulfonyl)-2-methylpiperazine dihydrochloride, (H_7) and N-[2-(methylamino)ethyl]-5-isoquinoline-sulfonamide dihydrochloride (H_8) were obtained from Seikagaku America Inc. (St. Petersburg, FL). All other biologicals and agents were obtained from Sigma Chemical Co. (St. Louis, MO). Statistical comparisons involving sequential measurements as a function of time were made by analysis of variance with repeated measures followed by Dunnett's a posteriori test. Otherwise, comparison of means was performed using t test for paired or unpaired data, as appropriate.

Results

PTH effect on HCO_3 reabsorption (Tables II and III)

PTH significantly increased the fractional delivery of TCO_2 to late proximal convolutions from 10 to 18.6%. The increased TCO_2 delivery by PTH signifies a decrease in tubular reabsorption of TCO_2. Both the plasma concentration of TCO_2 and single nephron glomerular filtration rate (SNGFR) were unaffected by the hormone. Calculated absolute reabsorption of TCO_2 declined from 869±40 to 798±50 pM/min after PTH, $P < 0.005$.

Table II. Results of Whole Kidney Function in Acutely TPTX Rats before and after PTH

	GFR	Plasma PO_4	FE PO_4	Arterial pH	Blood TCO_2
	μl/min per 100 g body wt	*mM/liter*	*%*		*mM/liter*
TPTX	374±33	2.8±0.1	2.2±1.0	7.35±0.01	28.6±0.8
PTH	419±19	2.3±0.1*	38.2±3.7‡	7.36±0.01	29.1±0.6

Values are means±SEM for 8 rats. Values are not significantly different from those in TPTX rats except where noted: * $P < 0.01$; ‡ $P < 0.001$.

Table III. Results of Proximal Tubular Fluid Collections before and after PTH in Acutely TPTX Rats

	$(TF/P)_{in}$	SNGFR	TCO_2	$FDTCO_2$
		nl/min	*mM/liter*	%
TPTX	2.63±0.09	33.8±1.9	7.2±0.6	10.0±0.8
PTH	1.85±0.05‡	33.4±1.5	9.8±0.6*	18.6±1.5‡

Values are means±SEM for 8 rats. * $P < 0.01$; ‡ $P < 0.001$.

Effect of PTH on pH_i

4MU studies. In a first series of studies, the effect of PTH on pH_i was studied during free-flow conditions in Sprague-Dawley and Munich-Wistar rats after the intravenous administration of 1-34 b-PTH (5×10^{-11} M as a bolus followed by 5×10^{-11} M/h). Results were not different in the two strains of rats and were pooled (Fig. 1 *A*). Before PTH administration, pH_i was higher in early (7.15±0.02) than in late proximal cells (7.11±0.01), a finding previously reported by us (10). At 20 min, PTH increased pH_i by a similar amount in early and late proximal cells (ΔpH_i = 0.06±0.01 and 0.05±0.01 in early and late proximal cells, respectively). The early and late proximal cells remained alkalinized at these levels at 50 min.

BCECF studies.[2] After superfusion of PTH (5×10^{-8} M) for 2 min, pH_i in early proximal cells increased significantly, as shown by the 490/460 ratio (Fig. 1 *B*). This increase was maximal at 2 min (7.27±0.08 in TPTX vs. 7.39±0.09 after PTH); and, although it fell slightly thereafter, it remained significantly higher than control up to 10 min (7.34±0.09). In a separate group of nephrons, early and late proximal convolutions perfused in situ with a luminal perfusate containing 25 mM HCO_3, PTH was found to increase cell pH in both early and late proximal cells by 0.08 and 0.07, respectively.

Mechanism of the cell alkalinization effect of PTH

The alkalinization produced by PTH suggests that a primary effect of the hormone is to inhibit base exit from the cell. Direct studies of base exit were therefore performed.

Effect of PTH on base exit (Fig. 2). In the absence of PTH, lowering HCO_3 concentration in the capillaries of early proximal cells from 25 (pH 7.25) to 5 mM (pH 6.5) resulted in a marked decrease in pH_i from 7.11 to 6.90. Administration of PTH given intravenous or by superfusion while perfusing the capillaries with 25 mM HCO_3 increased pH_i to 7.18, a value above that seen in TPTX. Lowering HCO_3 in the capillaries to 5 mM acidified the cell but to a lesser extent than that in the TPTX phase (6.96 vs 6.90). These results indicate that PTH inhibits base exit. In further support of this thesis, the initial rate of change of pH_i, $\Delta pH/min$, after changing from the 25 to 5 mM perfusate, was significantly slowed by PTH (2.60±0.24 in TPTX vs 1.87±0.18 after PTH, $P < 0.02$; Fig. 3). In time control studies in TPTX rats, after the first step decrease in HCO_3 concentration (25 to 5 mM), capillaries were perfused with 25 mM HCO_3 and measurements repeated after again lowering the HCO_3 concentration to 5 mM. Cell pH was not different during the second step lowering of peritubular HCO_3 concentration at both 25 mM (7.12±0.02 vs 7.10±0.02) and 5 mM HCO_3 (6.91±0.03 vs 6.87±0.04, $n = 9$). Furthermore, the initial rate of decline of pH_i after changing from 25 to 5 mM HCO_3 perfusates was not slowed during the second step change (2.43±0.29 vs 2.55±0.27 pH units/min).

Effect of PTH during inhibition of the Na/H exchanger. As an alternative approach to evaluating the effects of PTH on the luminal Na/H exchanger and basolateral $Na/(HCO_3)_{n>2}$ co-

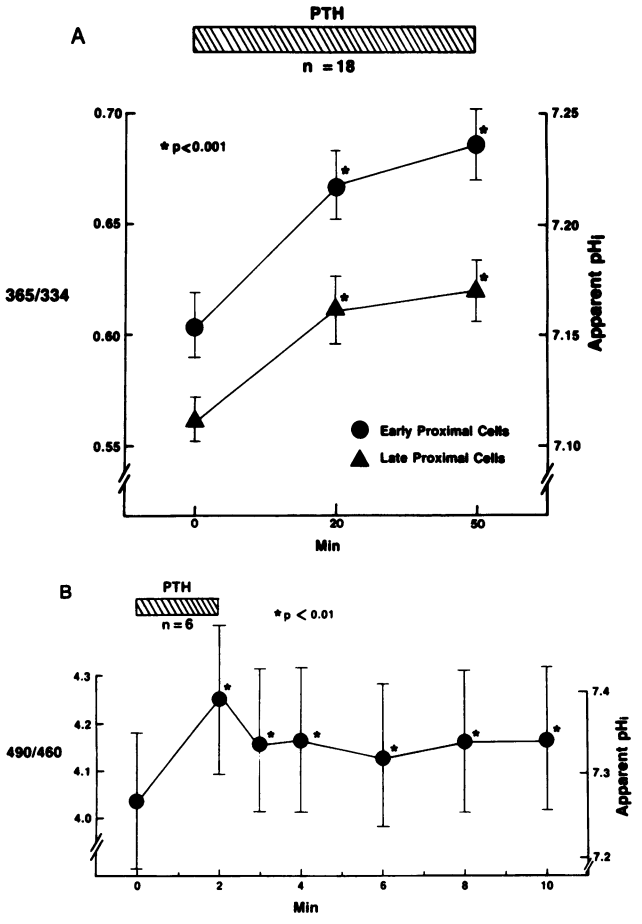


Figure 1. (*A*) Effect of intravenous administration of PTH on cell pH under free-flow conditions in early and late proximal cells loaded with 4MU. (*B*) Effect of PTH on cell pH in early proximal cells loaded with BCECF. The ordinate shows the fluorescence excitation ratio on the left and the calculated pH_i values on the right.

2. In this study pH_i was measured with two different fluoroprobes: 4MU and BCECF. Both fluoroprobes yielded identical results concerning the effect of PTH and db-cAMP on cell pH in early proximal convolutions. To be noted, however, is that pH_i values obtained with BCECF were significantly more alkaline by 0.1 to 0.15 pH units compared with those using 4MU. This discrepancy may be indicative of the different behavior of the two fluoroprobes in the cell milieu. 4MU fluorescence is highly homogeneous, suggesting that the fluoroprobe is largely localized in the cytosol and does not undergo intracellular compartmentalization and/or binding. By contrast, BCECF fluorescence, although highly homogeneous at low magnification, exhibits punctuate fluorescence at high magnification, which may be reflective of segregation of the fluoroprobe into intracellular organelles and/or binding. Indeed, the in vivo calibration of BCECF has been reported to be shifted to more alkaline values compared with the in vitro calibration (3). We believe, based on the above considerations, that although both fluoroprobes yield pH_i values that agree closely with those obtained with microelectrodes (14), pH_i values estimated by BCECF may be slightly biased in the alkaline direction.

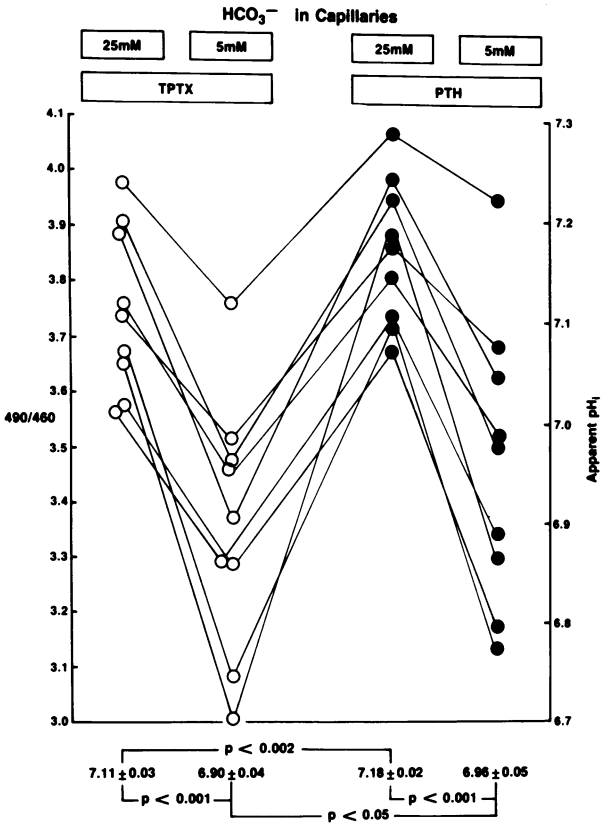


Figure 2. Effect of changing peritubular capillary HCO_3 on steady-state cell pH in the absence and presence of PTH. The lumen was perfused with a solution containing 25 mM HCO_3. The ordinate shows the fluorescence excitation ratio on the left and the calculated pH_i values on the right.

transporter, we examined the effects of removing and then restoring luminal Na using a protocol described by Alpern and Chambers (15). According to their description, removal of luminal Na first acidifies the cell by reversal of Na/H exchange. This lowers cell Na, causing a late alkalinization by base entry into the cell via reverse flux through the basolateral $Na/(HCO_3)_{n>2}$ cotransporter (15). In the present studies capillary flow was left uninterrupted throughout (See Fig. 4, *A* and *B*, for control series and Fig. 5, *A* and *B*, for PTH series). In the absence of PTH, two different responses were seen after removal of luminal Na. In 14 of the 23 convolutions there was rapid (~ 30 s) cell acidification ($\Delta pH_i = -0.07 \pm 0.01$) (Figs. 4 *A* and 5 *A*). In the remaining nine convolutions pH_i rose by 0.07±0.02 pH units (Figs. 4 *B* and 5 *B*). Convolutions showing no acidification corresponded to earlier sites than those convolutions in which acidification was observed (transit time by disappearance of FD & C dye was 2.6±0.1 vs 3.8±0.1 s, $P < 0.05$). It is also worth noting that the initial pH_i in the presence of Na was more acidic in the earlier sites (Figs. 4 and 5).[3]

3. We have previously found in the Sprague-Dawley rat that pH_i falls progressively along the accessible proximal nephron (10). In subsequent studies in the Munich-Wistar strain, similar measurements reveal that pH_i in the very earliest accessible proximal tubule was found to be significantly lower than in more distal convolutions (unpublished observations). This is also shown in these studies by comparing pH_i in the early vs. later proximal sites (7.35±0.03 vs. 7.42±0.03, $P < 0.05$, from baseline pH_i data of Fig. 4, *A* and *B*).

After this early phase, Na removal caused a sustained cell alkalinization by 2.5 min in all 23 convolutions. The change in pH_i from baseline in the presence of Na was not different in convolutions with early acidification ($\Delta pH_i = 0.08 \pm 0.02$, $n = 14$) compared with those in which alkalinization was seen ($\Delta pH_i = 0.13 \pm 0.02$, $n = 9$).

In the absence of luminal Na (Fig. 5, *A* and *B*) administration of PTH by superfusion for 2 min or intravenous infusion resulted in cell acidification ($\Delta pH_i = -0.05 \pm 0.01$, $n = 13$). This effect is consistent with direct inhibition of base entry into the cell. This thesis is supported by the findings with 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS) (Fig. 6). In a separate series, early proximal convolutions were luminally perfused with a Na-free solution while the capillaries were perfused with a solution containing 25 mM $NaHCO_3$. Addition of 0.5 mM SITS to the capillary perfusate lowered pH_i to a value not different from that in the presence of luminal Na (7.33 vs 7.32). It is of note that the decrease in pH_i after SITS was greater than that with PTH ($\Delta pH_i = -0.10 \pm 0.02$ vs -0.05 ± 0.01, $P < 0.02$), suggesting that PTH may only partially inhibit base entry.

Restoring luminal Na also allows an assessment of the apical and basolateral acid/base transport systems. In the absence of PTH, readdition of luminal sodium caused a rapid (within 30 s) increase in pH_i followed by a slower decline (~ 4 min) toward control values (Fig. 4, *A* and *B*). The transient pH_i overshoot is presumably caused by rapid H efflux via the Na/H exchanger. The return of pH_i toward baseline is due to the rise in cell Na leading to cessation of base entry as the Na/HCO_3

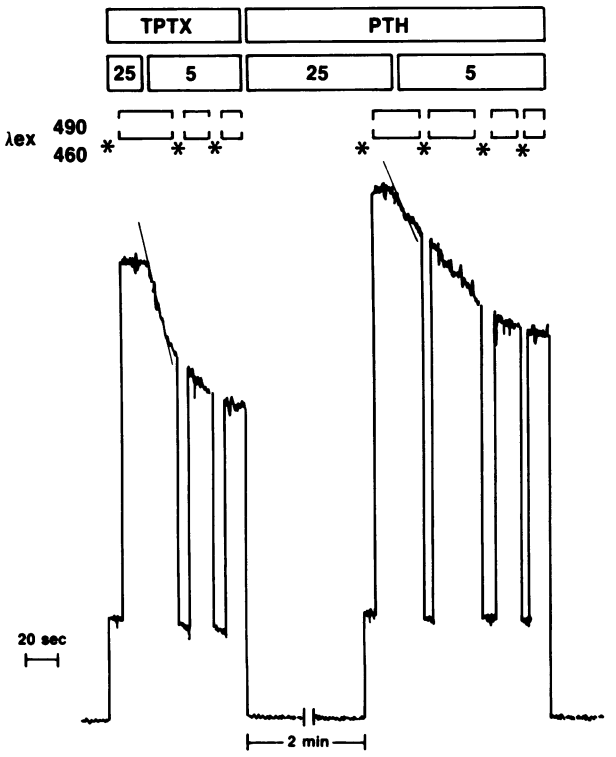
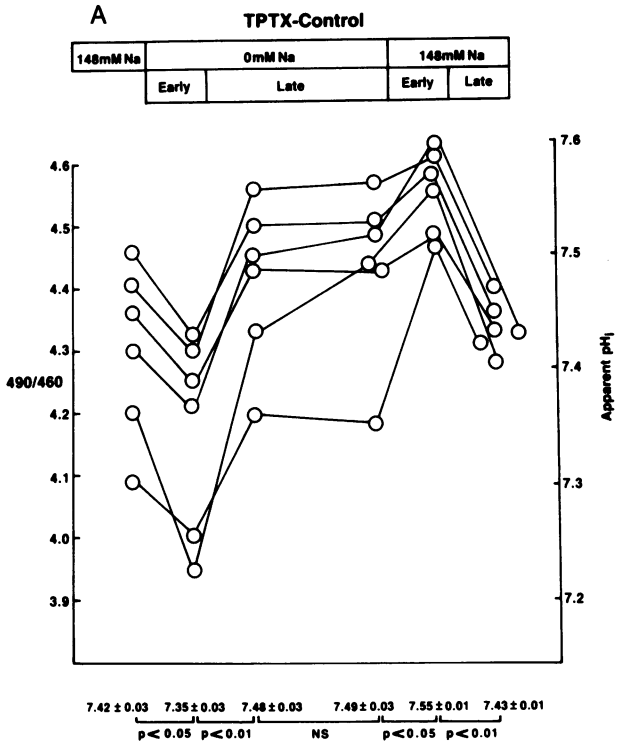


Figure 3. A representative study showing the effect of rapid changes of peritubular HCO_3 concentration on fluorescence in the absence and presence of PTH. Decreasing HCO_3 concentration in the peritubular capillaries from 25 to 5 mM caused a rapid decrease of fluorescence at 490 nm excitation. The initial rate of change before and after PTH is shown by a straight line.



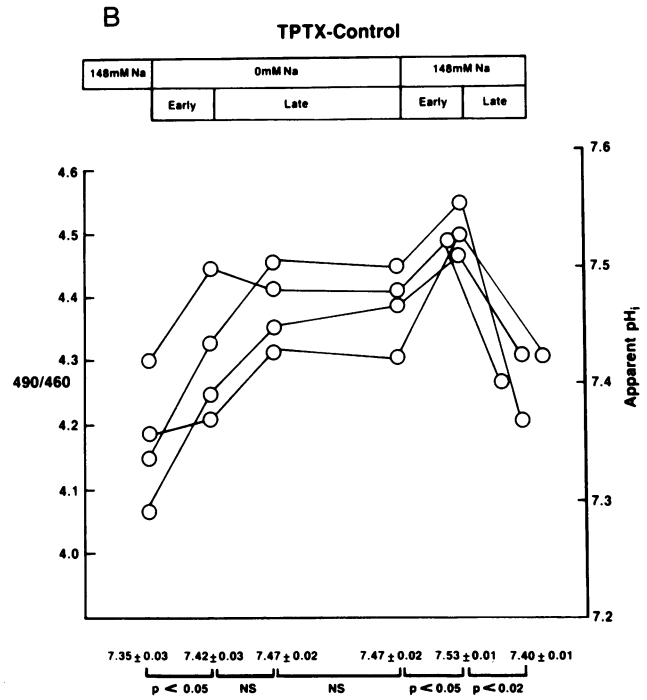
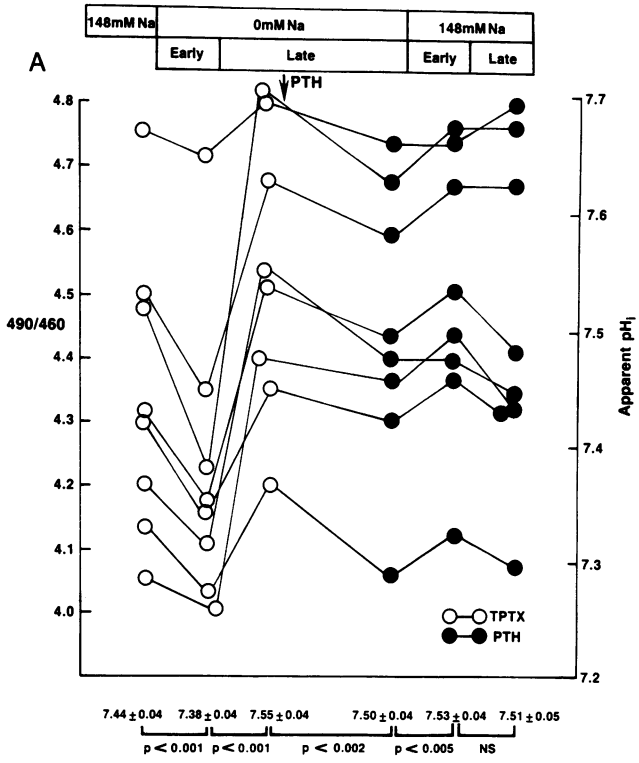


Figure 4. (*A*) Effect of luminal sodium removal and readdition on cell pH in TPTX control rats. After switching luminal perfusates, the trough or the peak of the transient change in cell pH (early phase) as well as the sustained effect (late phase) are shown. Represented here are cells in which an initial acidification after luminal sodium removal was seen. Capillary blood flow was left uninterrupted. The ordinate shows the fluorescence excitation ratio on the left and the calculated pH_i values on the right. (*B*) Effect of luminal sodium removal and readdition in TPTX control rats. Represented here are cells in which no acidification was observed after luminal sodium removal. For further details see legend of *A*.



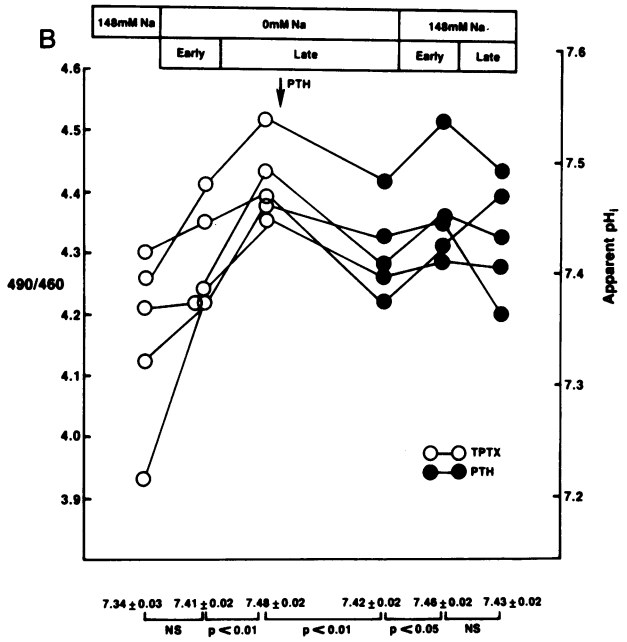


Figure 5. (*A*) Effect of PTH on cell pH after luminal sodium removal and readdition. Shown here are cells in which an initial acidification was seen after luminal sodium removal. (*B*) Effect of PTH on cell pH after luminal sodium removal and readdition. Represented here are cells in which no initial acidification after luminal sodium removal was observed. For further details see legend of Fig. 4 *A*.

cotransporter would start operating in a forward mode (HCO_3 efflux). This formulation is supported by the finding that SITS inhibits the late acidification phase (Fig. 6). In the presence of PTH, switching from a Na-free luminal perfusate to one containing 148 mM Na (Fig. 5, *A* and *B*) caused a signifi-

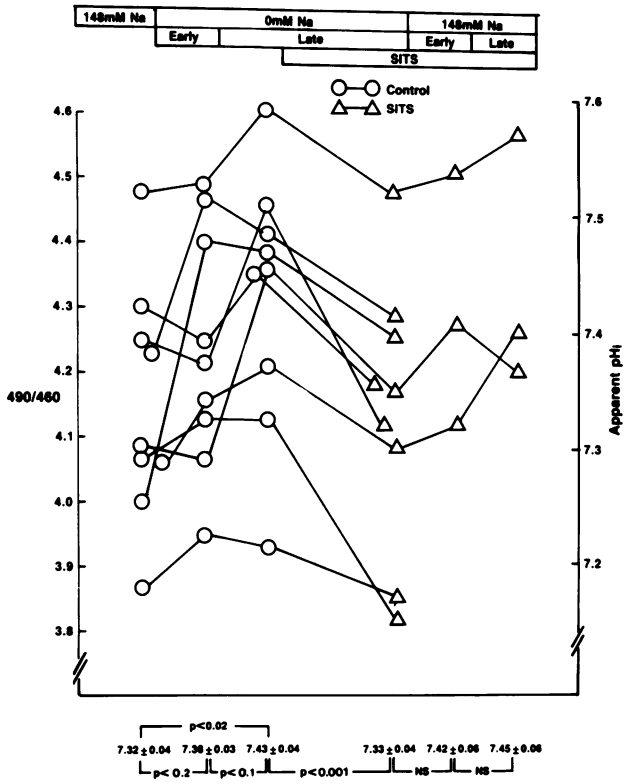


Figure 6. Effect of SITS on cell pH after luminal sodium removal and readdition. Shown here are cells in which an initial or no initial acidification was observed after luminal sodium removal. For further details see legend of Fig. 4 *A*.

cantly smaller pH_i overshoot compared with TPTX: ΔpH_i = 0.03±0.01 (n = 13) vs 0.07±0.01 (n = 10, $P < 0.05$). Furthermore, PTH essentially abolished the late acidification phase after the readdition of Na to the lumen: $\Delta pH_i = -0.12 \pm 0.01$ (n = 10) in TPTX compared with −0.02±0.01 (n = 13) after PTH ($P < 0.001$). Moreover, in cells loaded with the Na-sensitive fluoroprobe SBFI, PTH increased the 334/365 Na-sensitive excitation ratio from 0.70±0.02 to 0.78±0.02 (n = 5, $P < 0.01$). These results support the thesis that PTH has a separate action to directly inhibit the Na/H exchanger. In addition, the obliteration of the late acidification phase argues in favor of an inhibition of the base exit mechanism.

Cellular signal of the cell alkalinization by PTH

Role of cAMP. db-cAMP (7 mM) superfused for 2 min inhibited luminal acidification, as evidenced by a smaller decrease in luminal pH compared with control (ΔpH = 0.19±0.05 vs 0.32±0.08, Table IV). However, in cells loaded with 4MU, intravenous administration of db-cAMP (7×10^{-6} M as a bolus followed by 7×10^{-6} M/h) failed to increase pH_i in early and late proximal cells (Fig. 7 *A*). Similar results were also obtained in early proximal cells loaded with BCECF (Fig. 7 *B*). Superfusion of db-cAMP (7 mM) for 2 min also did not reproduce the effect of PTH to alkalinize early proximal cells. pH_i was unchanged at 2 min (7.35±0.04 vs 7.33±0.04 at baseline) and remained stable over 10 min, (7.32±0.02).

Role of phosphatidylinositol (PI) stimulation. 1) Inhibition of PI turnover. To evaluate whether the effect of PTH to alkalinize the cell might be due to stimulation of PI metabolism, the effect of PTH on pH_i was examined in rats given the aminoglycoside, gentamicin, at a dose of 100 mg/kg s.c. for 2 d. This protocol has been shown to prevent the effect of PTH to stimulate PIP_2 hydrolysis in the rat (16). Cell pH before PTH was 7.32±0.04 (n = 4), a value not different from that in rats not given gentamicin. In the presence of the aminoglycoside, PTH administered by superfusion failed to alkalinize the cell: cell pH values at 2 and 10 min were 7.33±0.04 and 7.31±0.04, respectively.

2) Role of cell Ca on the PTH action to alkalinize the cell. To evaluate the possibility that stimulation of PI turnover with a consequent increase in cell Ca might serve as the cellular mediator of the alkalinization effect of PTH, a first group of studies was performed to determine the effect of PTH on cell Ca. In the time control series, 334/365 nm fluorescence ratio was stable throughout the 10-min study period (Fig. 8). PTH administration caused a marked rise in cell Ca as demonstrated by the increase in the 334/365 fluorescence ratio (fluorescence at 334 nm excitation increased, whereas that at 365 nm decreased). The effect of PTH to increase cell Ca was detected immediately after the 2-min superfusion period with the hormone and reached a maximal response at 3 min: cell Ca rose from a baseline of 116±10 to 190±15 nM (n = 15). This effect persisted essentially unaltered for up to 10 min. Superfusion of 4×10^{-5} M ionomycin, a calcium ionophore, for 2 min had an effect on cell Ca similar to that seen with PTH (results not shown). To be noted is that superfusion of db-cAMP failed to alter cell Ca (n = 8).

To assess the role of the increase in cell Ca by PTH on the action of the hormone to alkalinize the cell, the effect of PTH on pH_i was examined when the effect of PTH to increase cell Ca was prevented by the calcium chelator 5-5′ dimethyl bis-(*o*-aminophenoxy)-*N,N,N′,N′*-tetraacetic acid (BAPTA). Loading of early proximal cells with Fura 2 AM and dimethyl BAPTA-AM (5×10^{-5} M) produced a slight but nonsignificant decrease in baseline cell Ca (96±1.8 nM), compared with cells not loaded with dimethyl BAPTA, and a complete obliteration of the effect of PTH to increase cell Ca (ΔCa at 2 min was 1.5±3 nM, n = 10, results not shown). The effect of PTH on pH_i while

Table IV. Effect of db-cAMP and PMA Administration, Basolateral and Luminal, on Luminal pH in Proximal Tubular Convolutions Perfused with BCECF-dextran

	Time control		db-cAMP		Basolateral PMA		Luminal PMA	
	Control	Control	Control	db-cAMP	Control	PMA	Control	PMA
ΔpH	0.31±0.09	0.30±0.10	0.32±0.08	0.19±0.05*	0.27±0.05	0.16±0.03‡	0.31±0.09	0.41±0.11*

ΔpH denotes the change in luminal pH from early proximal convolutions (7.25, at the perfusion site) to late proximal convolutions. Number of observations was 4 in all cases except luminal PMA, which was 5. * $P < 0.05$; ‡ $P < 0.02$.

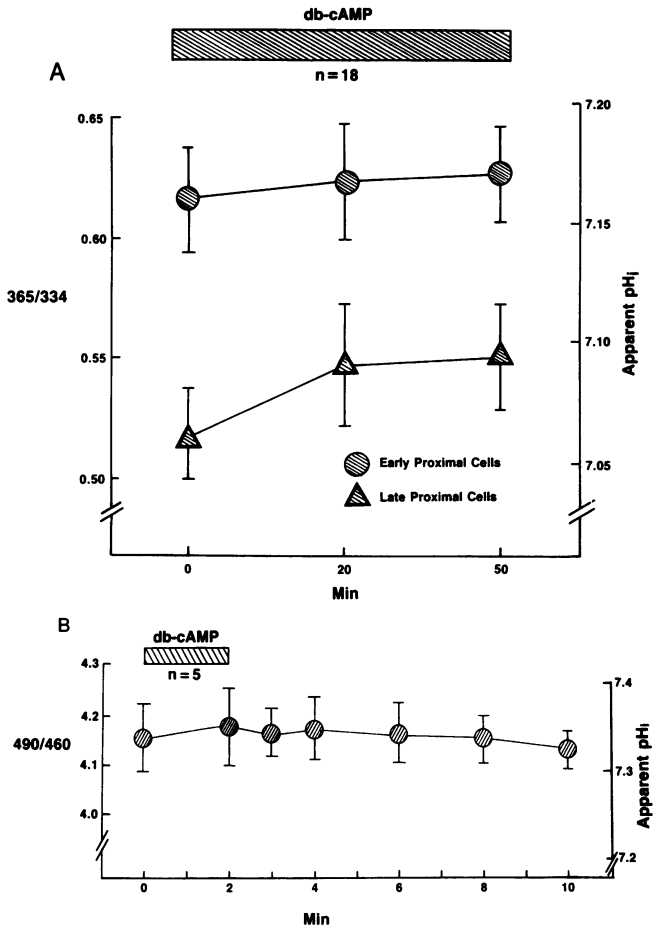


Figure 7. (*A*) Effect of intravenous administration of dibutyryl cAMP on cell pH under free flow conditions in early and late proximal tubular cells loaded with 4MU. (*B*) Effect of dibutyryl cAMP superfusion on cell pH in early proximal cells loaded with BCECF.

preventing the effect of the hormone to raise cell Ca was studied in early proximal cells loaded luminally with BCECF-AM (Fig. 9). In BAPTA-loaded cells the increase in pH_i after PTH was significantly blunted (ΔpH_i at 2 min was 0.06±0.01 [n = 14] vs 0.13±0.02 [n = 14] in the absence of BAPTA, $P < 0.001$).

3) Role of PKC on the effect of PTH to alkalinize the cell. To assess whether stimulation of PKC by PTH might be in part responsible for the effect of the hormone to increase cell pH, the effect on cell pH of direct stimulation of PKC by the phorbol ester, phorbol myristate acetate (PMA), and the diacylglycerol, oleylacetyl-*sn*-glycerol (OAG), was studied. In separate studies basolateral and apical cell surfaces were exposed to PMA (5×10^{-7} M) or OAG (2.5×10^{-4} M). In cells loaded with BCECF, PMA and OAG applied basolaterally caused a sustained increase in cell pH (ΔpH_i at 2 min was 0.10±0.02 [n = 12] with PMA [Fig. 10 *A*] and 0.15±0.03 [n = 10, $P < 0.01$] with OAG [results not shown]). Superfusion of DMSO, the vehicle used to prepare these two PKC activators, failed to alter pH_i (ΔpH_i at 2 min was 0.01±0.04 [n = 6]). Furthermore, superfusion of the inactive 4-alpha phorbol (5×10^{-7} M) did not alkalinize the cell (pH_i was 7.22±0.03 in control and 7.19±0.02 after phorbol [n = 4; results not shown]). In addition, the effect of basolateral application of PMA to alkalinize the cell was not mediated by increasing cell Ca (ΔCa at 2 min was 4.6±5.9 nM [n = 11, NS]).

To further evaluate the role of PKC on the effect of PTH to alkalinize the cell, the effect of the isoquinoline sulfonamide H_7, an inhibitor of PKC (K_i = 6 μM) was used (17). Because H_7 may also inhibit PKA, we also examined the effect of H_8, a relatively high specific inhibitor of PKA (K_i = 1.2 μM) (17). Superfusion of H_7 or H_8 failed to alter pH_i when given alone (results not shown). Administration of 15 μM H_7 by superfusion totally inhibited the effect of PTH to alkalinize the cell (pH_i 7.39±0.03 in control and 7.41±0.03 at 2 min in the presence of PTH and H_7 [n = 10, results not shown]). In contrast, H_8 at a concentration of 2.5 μM had no effect in preventing the alkalinization by PTH (ΔpH_i = 0.11±0.02 [n = 8] with PTH alone and ΔpH_i = 0.09±0.03 [n = 11] after PTH and H_8, $P < 0.01$). However, 25 μM H_8, a concentration that would be expected to inhibit PKC, blocked the effect of basolateral PMA (5×10^{-7} M) to alkalinize cell pH (n = 6; 7.38±0.07 in control and 7.38±0.06 at 2 min in the presence of PMA and H_8, results not shown). Superfusion with 25 μM H_8 also completely obliterated the cell alkalinization action by PTH (n = 8; 7.38±0.05 in control and 7.39±0.05 after PTH and H_8).

To elucidate whether cell alkalinization after PKC activation may inhibit the luminal Na/H exchanger, the effect of PMA applied basolaterally on luminal acidification was studied in convolutions perfused with BCECF-dextran. As shown in Table IV, the decrease in luminal pH after basolateral PMA was smaller than that in control (ΔpH = 0.16 vs 0.27). By contrast luminal administration of PMA (n = 15, Fig. 10 *B*)

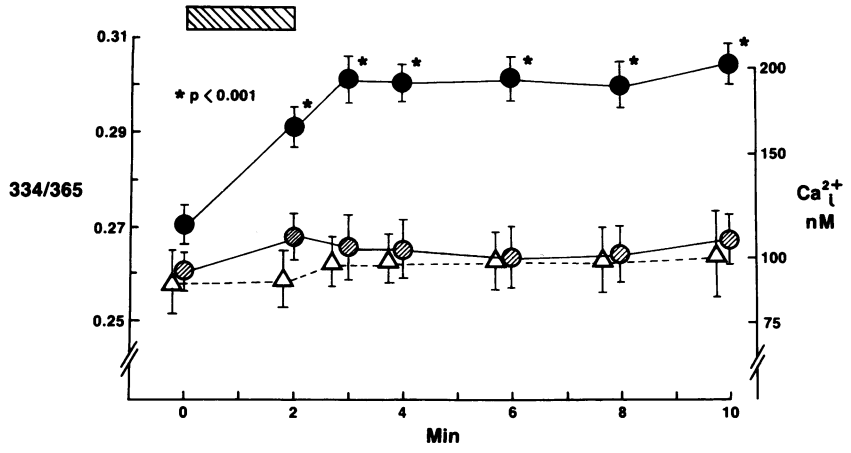


Figure 8. Effect of PTH and dibutyryl cAMP given by superfusion on cell calcium in early proximal cells loaded with Fura 2. The ordinate shows the fluorescence excitation ratio on the left and the calculated cell calcium values on the right. The horizontal bar denotes the duration of superfusion with any of the agents tested. ● — ●, PTH; ◍ — ◍, db-cAMP; △ — △, control.

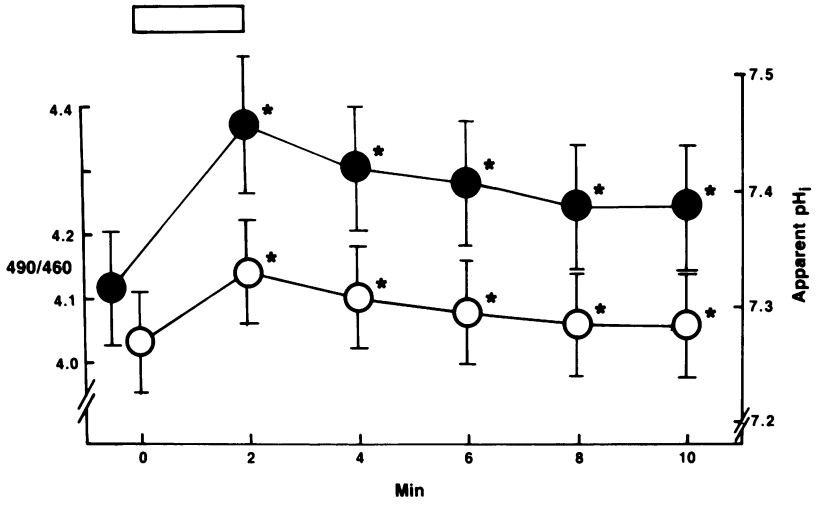


Figure 9. Effect of PTH on cell pH in early proximal cells loaded with dimethyl BAPTA and BCECF. The horizontal bar denotes the duration of superfusion with PTH in cells loaded or not loaded with BAPTA. ●, PTH; ○, PTH + BAPTA. *$P < 0.001$.

had no effect on cell pH. Luminal administration of OAG also failed to alter cell pH (pH$_i$ before and 2 min after OAG was 7.36±0.06 and 7.36±0.07, n = 12, results not shown). Vehicle had no effect on pH$_i$. Surprisingly, luminal PMA (Table IV) increased luminal H secretion, as evidenced by a larger decrease in luminal pH from early to late proximal convolutions compared with control (ΔpH = 0.41 vs 0.31, $P < 0.05$).

Discussion

Effect of PTH on HCO_3 reabsorption and pH$_i$

Under free flow conditions, PTH significantly decreased HCO_3 reabsorption along the proximal tubule, an effect that signifies an inhibition of the luminal Na/H exchanger. Studies in OK cells have shown that inhibition of the Na/H antiporter by PTH resulted in cell acidification, as measured by dimethyloxazolidinedione (5). In view of these findings, we were very much surprised to find that PTH caused cell alkalinization in early and late proximal cells. This finding clearly indicates that the effect of PTH on cell pH could not be mediated primarily by inhibition of the Na/H exchanger.

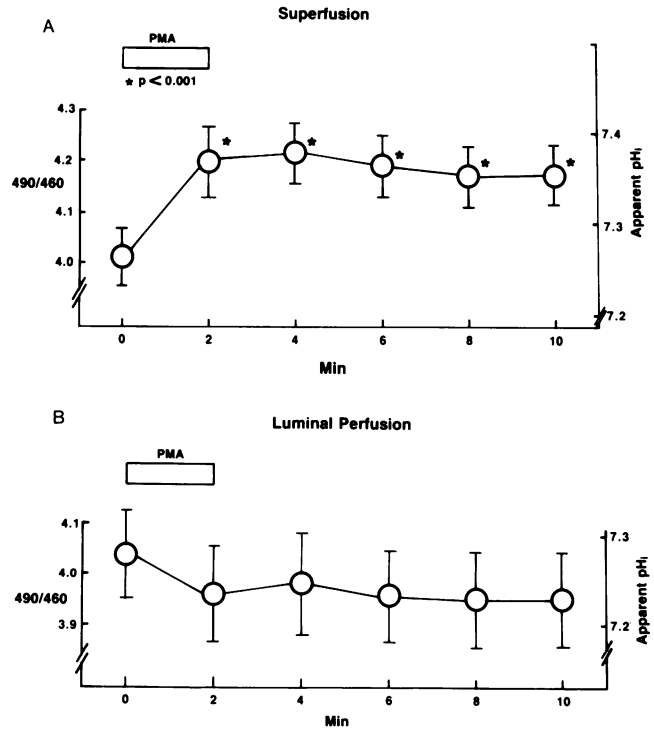


Figure 10. (*A*) Effect of PKC stimulation by PMA applied basolaterally on cell pH in early proximal cells. (*B*) Effect of luminal PMA on cell pH in early proximal cells.

Effect of PTH on basolateral base transport

The results clearly show that PTH alkalinizes the cell as a result of inhibiting base exit. This is supported by the findings that (*a*) PTH increases cell pH at both normal and reduced concentrations of peritubular HCO_3; (*b*) the initial rates of change of cell pH after step changes of capillary HCO_3 were decreased by PTH; (*c*) when luminal Na/H exchange was inhibited by luminal Na removal, the subsequent entry of base was inhibited by PTH; and (*d*) PTH increases cell Na.

The results after luminal Na removal are also of importance in another regard. An initial cell acidification phase is not seen in very early convolutions (presumed to correspond to the S_1 segment) but is observed in slightly more distal convolutions (S_2 segment). To explain this finding we propose that basolateral HCO_3 transport exhibits different sensitivity to changes in cell Na in the two segments. Cells belonging to the S_1 segment may have a greater number of $Na/(HCO_3)_{n>2}$ cotransporters or these cotransporters may have a greater affinity for external Na compared with S_2 cells, resulting in increased HCO_3 influx compared with cells in the S_2 segment.

Effect of PTH on the Na/H exchanger

The effect of PTH to inhibit the luminal Na/H exchanger is clearly demonstrated by the finding that the hormone significantly blunted the rapid overshoot in pH$_i$ after readdition of luminal Na. This overshoot is thought to represent sudden H removal from the cell by the Na/H exchanger (15). It is to be noted that the inhibitory effect of PTH was observed at a time when pH$_i$ (before Na addition) was significantly more acidic than in TPTX, a setting that would be expected, if anything, to enhance Na/H exchange and cause a higher degree of cell alkalinization. The blunting of the pH$_i$ overshoot by PTH would suggest that PTH may have a direct inhibitory effect on the exchanger. These findings suggest that under physiological

conditions the inhibitory effect of PTH on the Na/H exchanger may be mediated by a direct (cAMP-dependent) modification of the exchanger (see below), and as a consequence of inhibiting base exit by an increase of cell pH and an increase of cell Na. The alkaline shift in pH_i would be expected to inhibit the Na/H exchanger via an allosteric interaction (18).

Cellular signals

cAMP. Administration of cAMP mimicked the effect of PTH to inhibit luminal H secretion but did not reproduce the effect of the hormone to alkalinize the cell. These findings support the thesis that the direct inhibitory effect of PTH on the Na/H exchanger (see above) may be mediated by cAMP (4, 19). Furthermore, they imply that the inhibition of base exit by PTH is not mediated by cAMP. Failure of cAMP to acidify the cell suggests that its effect to inhibit the Na/H exchanger is accompanied by a simultaneous decrease in base exit. Such coupling of the two transport systems is well documented in the proximal tubule (3, 15, 20) and allows cell pH to remain constant by offsetting changes in the rate of Na/H exchange via compensatory changes in basolateral base exit. If cAMP has an effect to directly inhibit HCO_3 exit, it would have to be minor.

PI turnover. It is well established that PTH stimulates the PI cascade in proximal tubules. Hruska et al. (21) have shown that PTH induces a rapid increase in inositol trisphosphate (IP_3) and diacylglycerol in OK cells, dog proximal tubular cells in culture, and basolateral membranes from canine proximal tubular cells. Furthermore, the increase in IP_3 is associated with an increase in cell Ca (21) and activation of PKC (16). Our findings argue strongly in favor of a role of stimulation of PI metabolism in mediating the effect of PTH to alkalinize the cell, with both arms of the PIP_2 hydrolysis cascade (an increase in cell Ca and stimulation of PKC) playing a role. Moreover, the pool of PKC that is involved in this action appears to be segregated to the basolateral membrane, consistent with receptor-activated PKC stimulation.

Role of cell Ca

PTH was found to cause a sustained effect on cell Ca after exposing the cells to the hormone for 2 min. This action was not reproduced by cAMP, consistent with observations in vitro (22, 23). Although PTH has been reported previously to increase cell Ca in proximal tubular cells in vitro (21–25), this is to our knowledge the first in vivo demonstration of this effect. In vitro PTH causes a rapid (within seconds) but transient 1- (21) to 3-min (24) increase in cell Ca. In only one study was a sustained effect, lasting up to 15 min, reported (22). In our study, measurements of Fura 2 fluorescence could not be performed for technical reasons immediately after administration of PTH. Therefore, we were unable to determine the profile of cell Ca during this early phase. The mechanism responsible for the sustained increase in cell Ca by PTH must involve at least in part an increase in Ca entry into the cell. Studies in basolateral membrane vesicles suggest that PTH enhances Ca entry by a receptor-operated Ca channel or a second-mesenger–operated Ca channel stimulated by IP_3 or its metabolites (26, 27).

The effect of PTH to increase cell Ca was found to play an essential role in the alkalinization action of the hormone. The blunted rise in pH_i after PTH in BAPTA-loaded cells would not be expected to be due to a significant H-buffering effect by BAPTA. First, on the basis of the reported cell accumulation of Fura 2 (28), a close analogue of BAPTA, of $\sim$ 100-fold over that of the external medium, BAPTA concentration in cells would not be expected to exceed 2–3 mM. Since the cell buffering capacity is 20–30-fold greater (29) than this concentration of BAPTA, the added effect of this buffer on the cell buffering capacity would be minimal. Furthermore, in the absence of PTH, pH_i was not lowered in BAPTA-loaded cells compared with cells not loaded with BAPTA.

Role of PKC

The results are consistent with the thesis that a basolateral pool of PKC is involved in mediating the alkalinization effect of PTH. This is supported by the findings that (*a*) basolateral administration of PMA and OAG alkalinizes the cell and (*b*) administration of the PKC inhibitor H_7 prevents the cell alkalinization action of PTH. The thesis that a pool of PKC localized to the basolateral membrane regulates basolateral HCO_3 is also supported by the observation by Hammerman et al. (30) that phorbol esters specifically phosphorylate a basolateral membrane protein. The finding that activation of basolateral PKC and the increase in cell Ca by PTH act in concert but separately to inhibit base exit suggests that PKC and Ca-calmodulin–dependent protein kinases act by phosphorylation of different membrane proteins, which may represent the HCO_3 transporter or transporter-associated proteins. Alternatively, PKC activation may inhibit HCO_3 exit by decreasing the driving force for electrogenic HCO_3 exit, i.e., cell potential. Miyauchi et al. (31) have reported that PTH causes cell depolarization in OK cells, an effect that may be mediated by inhibition of basolateral K conductance.

The action of basolateral PMA to inhibit luminal acidification clearly indicates that the stimulation of the basolateral pool of PKC by virtue of its effect to alkalinize the cell plays a crucial role in inhibiting luminal Na/H exchange. This finding is in agreement with the observation by Baum and Hays (32) that in the in vitro perfused rabbit proximal tubule basolateral exposure to a phorbol ester decreases HCO_3 reabsorption.

The observation that luminal PMA increases H secretion is consistent with the presence of a luminal pool of PKC that when activated serves to stimulate Na/H exchange, an effect opposite to that mediated by activation of the basolateral pool of the kinase. A similar observation has been made by Liu and Cogan (33) in the rat proximal tubule. Stimulation of Na/H exchange by a phorbol ester was also observed by Mellas and Hammerman (34) in suspensions of canine proximal tubular cells and by Weinman and Shenolikar (35) in BBMV. The stability of cell pH after luminal PMA is not necessarily surprising (see above for cAMP) and indicates that the stimulation of Na/H exchange was balanced by a compensatory increase in basolateral base exit. It is important to acknowledge that PTH is likely to stimulate the luminal pool of PKC, as supported by the finding that this hormone increases PI turnover in BBMV (36), an effect that might be mediated via an increase in cell calcium. Therefore, the resulting stimulation of Na/H exchange would mitigate the more dominant effect of PTH to inhibit the exchanger consequent to cell alkalinization and increased cAMP.

Cell model

To explain the mechanism of inhibition of HCO_3 reabsorption by PTH, we propose a model in which both the cAMP and PI turnover cascades play separate roles (Fig. 11). Stimulation of PI metabolism through an increase in cell Ca and activation of

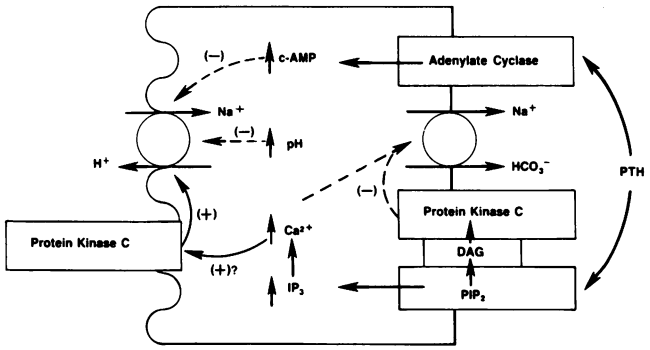


Figure 11. Cell model depicting the signal pathways activated by PTH and their effect on H and HCO_3 transport systems.

basolateral PKC act in concert to inhibit base exit. Inhibition of base exit serves to alkalinize the cell and inhibit the Na/H exchanger by alteration of driving forces and allosteric inhibition. In addition, PTH via cAMP may further inhibit the luminal Na/H exchanger. Activation of an apical pool of PKC by PTH (36) stimulates the Na/H exchanger, but this stimulatory effect is not the dominant one because the final expression of PTH effect is inhibition of the exchanger.

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