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J Clin Invest. 1993;92(4):1632-1638. https://doi.org/10.1172/JCI116748.

Research Article

Parathyroid hormone, dopamine, alpha-adrenergic catecholamines, and angiotensin II regulate renal Na excretion, at least in part through modulation of acute cyclic (c)AMP-induced proximal tubule Na/H antiporter inhibition. The present studies examined the effect of chronic increases in cell cAMP on Na/H antiporter activity in OKP cells. Whereas 8-bromo cAMP acutely inhibited Na/H antiporter activity, chronic application for 6 h led to a 24% increase in Na/H antiporter activity measured 16-20 h after cAMP removal. This chronic persistent activation of the Na/H antiporter required > 2 h exposure. This effect was not a nonspecific effect of 8-bromo cAMP, in that addition of forskolin or forskolin + 3-isobutyl-1-methylxanthine for 6 h also led to a chronic persistent increase in Na/H antiporter activity. Inhibition of protein synthesis with cycloheximide prevented 8-bromo cAMP-induced Na/H antiporter stimulation. Although 8-bromo cAMP addition decreased cell pH by 0.15-0.20 pH U, Na/H antiporter stimulation could be dissociated from cell acidification. In summary, while cAMP acutely inhibits Na/H antiporter activity, it chronically increases antiporter activity. This chronic activation occurs with exogenous addition or endogenous generation of cAMP. These results imply that for hormones that modulate renal Na excretion and proximal tubule Na/H antiporter activity via cAMP and protein kinase A, acute effects may not predict chronic effects.

Cyclic Adenosine Monophosphate Acutely Inhibits and Chronically Stimulates Na/H Antiporter in OKP Cells

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Abstract

Parathyroid hormone, dopamine, α-adrenergic catecholamines, and angiotensin II regulate renal Na excretion, at least in part through modulation of acute cyclic (c)AMP-induced proximal tubule Na/H antiporter inhibition. The present studies examined the effect of chronic increases in cell cAMP on Na/H antiporter activity in OKP cells. Whereas 8-bromo cAMP acutely inhibited Na/H antiporter activity, chronic application for 6 h led to a 24% increase in Na/H antiporter activity measured 16–20 h after cAMP removal. This chronic persistent activation of the Na/H antiporter required > 2 h exposure. This effect was not a nonspecific effect of 8-bromo cAMP, in that addition of forskolin or forskolin + 3-isobutyl-1-methylxanthine for 6 h also led to a chronic persistent increase in Na/H antiporter activity. Inhibition of protein synthesis with cycloheximide prevented 8-bromo cAMP–induced Na/H antiporter stimulation. Although 8-bromo cAMP addition decreased cell pH by 0.15–0.20 pH U, Na/H antiporter stimulation could be dissociated from cell acidification. In summary, while cAMP acutely inhibits Na/H antiporter activity, it chronically increases antiporter activity. This chronic activation occurs with exogenous addition or endogenous generation of cAMP. These results imply that for hormones that modulate renal Na excretion and proximal tubule Na/H antiporter activity via cAMP and protein kinase A, acute effects may not predict chronic effects. (*J. Clin. Invest.* 1993. 92:1632–1638.) Key words: cyclic AMP • Na/H antiporter • protein kinase A • protein synthesis • OKP cells

Introduction

The apical membrane Na/H antiporter of the proximal tubule mediates a significant component of renal Na absorption. Many hormones and neurotransmitters that regulate renal Na excretion do so by regulating this Na/H antiporter. In many cases, this regulation is believed to occur through modulation of adenylyl cyclase and secondary inhibition of the Na/H antiporter by cAMP-dependent protein kinase (1, 2). Parathyroid hormone (PTH)[1] and dopamine inhibit the Na/H antiporter at least in part by activating adenylyl cyclase (3–8), and α-adrenergic catecholamines and angiotensin II stimulate the antiporter in part by inhibiting adenylyl cyclase (9–11). Studies demonstrating this regulation have of necessity been short term, examining the acute effects of agonist addition. However, chronic changes in hormone levels are more physiologically relevant. Tissue culture offers a setting in which chronic regulation can be studied in the absence of complicating changes in hemodynamics, nerve activity, and hormone levels.

The purpose of the present studies was to compare the acute and chronic effects of cAMP on the Na/H antiporter. Studies were performed in OKP cells, a clone of an opossum kidney cell line with many characteristics of the proximal tubule (12). These cells have been used extensively to study regulation of the Na/H antiporter by cAMP (7). The present results demonstrate that while acute application of cAMP inhibits the Na/H antiporter, chronic increases in cAMP stimulate the Na/H antiporter. This latter effect is inhibited by cycloheximide, and is not secondary to changes in cell pH. These results imply that acute effects of PTH, dopamine, angiotensin II, and α-adrenergic catecholamines on renal Na excretion and proximal tubule Na/H antiporter activity may not predict chronic effects.

Methods

Cell culture. OKP cells (gift from Dr. K. Hruska) are a clonal subline of the opossum kidney (OK) cell line, originally described by Cole et al. (12). OKP cells were passaged in DME (4.5 mg/ml glucose) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS. For experimentation, OKP cells between passages 45 and 55 were grown to confluence on glass coverslips, rendered quiescent by serum deprivation for 48 h, and then studied.

Measurement of intracellular pH and Na/H antiporter activity. Continuous measurement of cytoplasmic pH (pH_i) was accomplished using the intracellularly trapped pH-sensitive dye 2′,7′-bis(2-carboxyethyl)-5-(and -6)carboxyfluorescein (BCECF). Cells were loaded with the acetoxymethyl ester of BCECF (10 μM) for 35 min at 37°C. After washing, the coverslip was placed in a plastic cuvette in a computer-controlled spectrofluorometer (8000C; SLM Instruments Inc., Urbana, IL) at a 30° angle to the excitation beam. pH_i was estimated from the ratio of fluorescence with excitation at wavelengths of 500 and 450 nm with emission at 530 nm (13, 14). Slit widths were 4 nm. Background fluorescence was measured before dye loading and subtracted from fluorescence intensity at each excitation wavelength. Calibration of the BCECF excitation ratio was accomplished using the nigericin technique as described (13).

Na/H antiporter activity was assayed as the initial rate of Na-dependent pH_i increase after an acid load in the absence of CO_2/HCO_3. For this assay, Hepes-buffered solution contained (mM): 130 Na, 5.0 K, 1.1 Ca, 1.5 Mg, 140.2 Cl, and 30 Hepes. In Na-free solutions, Na was replaced with choline. All solutions were adjusted to pH 7.4 with *N*-methyl-D-glucammonium hydroxide at 37°C. Cells were first bathed in the Na-containing solution, and baseline pH_i was measured. The bath was then changed to a Na-free solution containing 13 μM nigericin for 4 min, which caused pH_i to decrease to ~ 6.4. Nigericin was then removed and the cells washed with 1% (wt/vol) dialyzed human albu-

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Received for publication 5 November 1992 and in revised form 24 March 1993.

1. *Abbreviations used in this paper:* BCECF, 2′,7′-bis(2-carboxyethyl)-5-(and -6)carboxyfluorescein; IBMX, 3-isobutyl-1-methylxanthine; pH_i, cytoplasmic pH; PTH, parathyroid hormone.

J. Clin. Invest.

0021-9738/93/10/1632/07 $2.00
Volume 92, October 1993, 1632–1638

min for 2 min. Cells were then bathed in the Na-free perfusate for 30 s. Subsequent addition of Na caused a rapid pH_i recovery that is due to the Na/H antiporter (15). The initial rate of this Na-dependent pH_i change (dpH_i/dt) was calculated by drawing a tangent to the initial deflection (over 20 s). In all studies, control and experimental cells were from the same passage and were assayed on the same day.

To calculate buffer capacity, cells were pulsed with 10 mM NH_4Cl in the Na-free perfusate (replacing choline chloride) at the trough pH_i (after washing out albumin). The pH_i decrease caused by the removal of NH_4 (ΔpH_i) was used to calculate the cell buffer capacity (β), using the formula:

$$\beta = \frac{[NH_4]_i}{\Delta pH_i},$$

where $[NH_4]_i$ was calculated from the pH_i just before NH_3/NH_4 removal, the extracellular $[NH_4]$, and the extracellular pH, as described (16). Buffer capacity was not affected by any of the experimental maneuvers (data not shown). Therefore, Na/H antiporter activity is expressed as dpHi/dt.

For the measurement of pH_i during control and cAMP incubation, cells were bathed in CO_2/HCO_3-containing solutions similar to the incubation culture media but without phenol red and vitamins.

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted as follows: penicillin and streptomycin from Whittaker M.A. Bioproducts (Walkersville, MD); culture media from GIBCO BRL (Gaithersburg, MD); and BCECF-AM from Molecular Probes, Inc. (Eugene, OR). 8-Bromo cAMP and forskolin were dissolved in ethanol and 3-isobutyl-1-methylxanthine (IBMX) was dissolved in DMSO. In all studies, vehicle was diluted 1:1,000 and was also added to control cells.

Statistics. Data are reported as mean±SEM. Statistical significance was assessed using the unpaired Student's *t* test or analysis of variance (ANOVA), where appropriate.

Results

cAMP acutely inhibits and chronically stimulates Na/H antiporter activity. In the first series of experiments, we examined the acute effect of cAMP on the Na/H antiporter. 10^{-4} M 8-bromo cAMP or vehicle was applied to OKP cells for 45 min, and Na/H antiporter activity assayed as the rate of Na-dependent pH_i recovery from an acid load. In agreement with previous studies (7), 8-bromo cAMP acutely inhibited Na/H antiporter activity by 31% (Fig. 1).

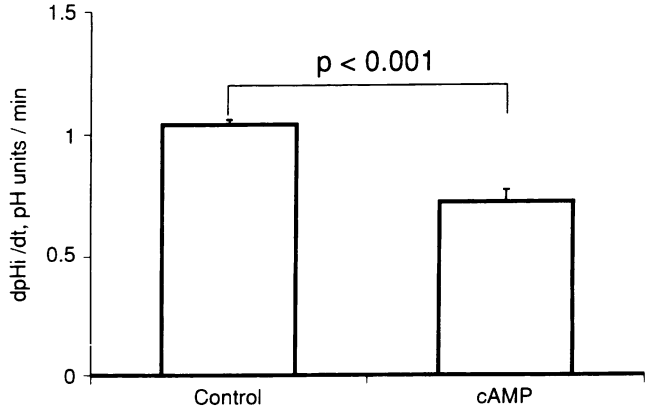

Figure 1. 8-Bromo cAMP acutely inhibits Na/H antiporter activity. 8-Bromo cAMP or vehicle was applied to OKP cells for 45 min and Na/H antiporter activity examined. The *y*-axis shows Na/H antiporter activity assayed as the initial rate of Na-dependent pH_i recovery from an acid load. Control, $n = 4$; cAMP, $n = 5$.

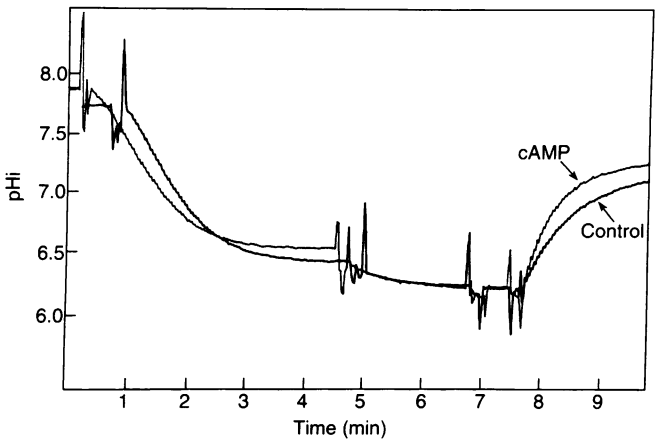

Figure 2. 8-Bromo cAMP chronically stimulates Na/H antiporter activity: typical tracing. OKP cells were treated with either 10^{-4} M 8-bromo cAMP or vehicle for 6 h, and then studied 16–20 h after removal of the agent. Cells were acidified by addition of nigericin at ~ 1 min. Nigericin was removed 4 min later and albumin added for 2 min. Sodium was then added ~ 30 s after albumin removal, and Na/H antiporter activity was assessed as the initial rate of increase in cell pH.

To examine the effect of chronic increases in cAMP, 10^{-4} M 8-bromo cAMP or vehicle was applied to OKP cells for 24 h. Somewhat unexpectedly, chronic exposure to 8-bromo cAMP caused Na/H antiporter activity to increase by 23% (dpH_i/dt = 1.35±0.08, control [$n = 7$] vs. 1.66±0.05, cAMP [$n = 8$]; $P < 0.005$). These results suggested that the acute and chronic effects of cAMP are opposite in direction.

To study the chronic effect of cAMP in the absence of any acute effects, 10^{-4} M 8-bromo cAMP or vehicle was applied to OKP cells for 6 h and then removed. On the next day, 16–20 h after removal of 8-bromo cAMP, Na/H antiporter activity was measured. Fig. 2 shows a typical tracing, and Fig. 3 shows mean results. cAMP-treated cells demonstrated a 24% increase in Na/H antiporter activity. Once again, this increase is opposite in direction to that observed with acute application of cAMP. Fig. 4 shows results with 1, 2, and 6 h of 8-bromo cAMP appli-

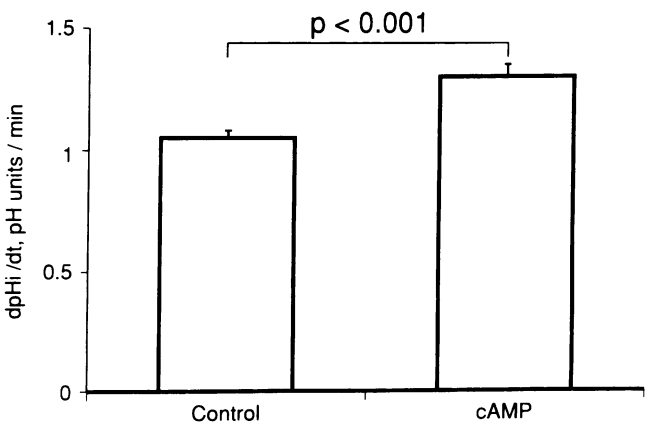

Figure 3. 8-Bromo cAMP chronically stimulates Na/H antiporter activity. OKP cells were treated with either 10^{-4} M 8-bromo cAMP or vehicle for 6 h, and then studied 16–20 h after removal of the agent. *y*-axis is as described in Fig. 1. Control, $n = 17$; cAMP. $n = 17$.

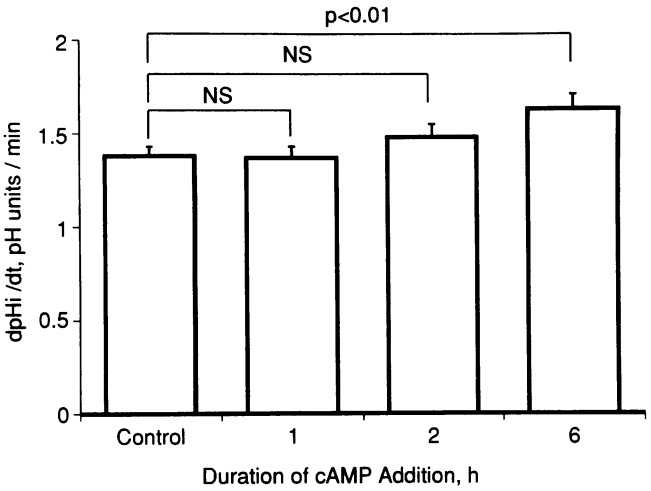

Figure 4. 8-Bromo cAMP chronically stimulates Na/H antiporter activity: time course. OKP cells were treated with 8-bromo cAMP for 1, 2, or 6 h, after which Na/H antiporter activity was assayed 16–20 h later. *y*-axis is as described in Fig. 1. Control, $n = 11$; 1 h, $n = 11$; 2 h, $n = 9$; 6 h, $n = 11$.

cation. Once again, Na/H antiporter activity was measured 16–20 h after removal of 8-bromo cAMP. As can be seen, only the 6-h timepoint achieved statistical significance.

To examine the possibility that this chronic effect may represent a nonspecific effect of 8-bromo cAMP, we examined other methods of raising cell cAMP concentration. Fig. 5 shows results with 10^{-4} M 8-bromo cAMP, 10^{-5} M forskolin, and 10^{-5} M forskolin plus 2 mM IBMX. In all cases, agents were applied for 6 h, and Na/H antiporter activity measured 16–20 h after removal. 8-Bromo cAMP caused a 52% increase in Na/H antiporter activity, forskolin caused a 32% increase, and forskolin plus IBMX caused a 51% increase in Na/H antiporter activity. Thus, either exogenous administration or endogenous generation of cAMP causes a chronic persistent increase in Na/H antiporter activity.

Chronic cAMP activation of the Na/H antiporter is inhibited by cycloheximide. To inhibit protein synthesis, 100 μM cycloheximide was added to the cell cultures 1 h before and during cAMP or vehicle addition, and during the following 16–20 h before antiporter assay. This dose of cycloheximide inhibited [^{3}H]leucine incorporation by 94% in OKP cells; lower concentrations of cycloheximide were not as effective in inhibiting protein synthesis in these cells. As can be seen in Fig. 6, cycloheximide inhibited basal Na/H antiporter activity and inhibited the cAMP-induced increase in antiporter activity. The effect on basal rates is most likely due to protein turnover, but nonspecific effects cannot be ruled out. These results suggest that protein synthesis is required for chronic activation, but do not determine whether the protein synthesized is the Na/H antiporter.

cAMP-induced Na/H antiporter activation is not secondary to cell acidification. We and others have previously shown that chronic decreases in extracellular and intracellular pH cause a protein synthesis–dependent increase in Na/H antiporter activity in a number of renal cell lines (14, 15, 17). We reasoned that acute inhibition of the Na/H antiporter by cAMP could lead to cell acidification, which could secondarily cause the chronic increase in Na/H antiporter activity. To examine this possibility, the effect of 10^{-4} M 8-bromo cAMP on pH_i was examined in the presence of CO_2/HCO_3-containing media. Fig. 7 shows that cAMP acidified the cells by ~ 0.15–0.20 pH U, and that this effect persisted throughout the 6-h incubation. This cell acidification is likely due to Na/H antiporter inhibition, and suggests that inhibition persists for 6 h.

We next examined the effect of decreasing extracellular fluid pH by 0.3 pH U. When culture media was acidified for 24 h and then antiporter activity measured, Na/H antiporter activity was increased 66% in acid incubated cells (Fig. 8). These

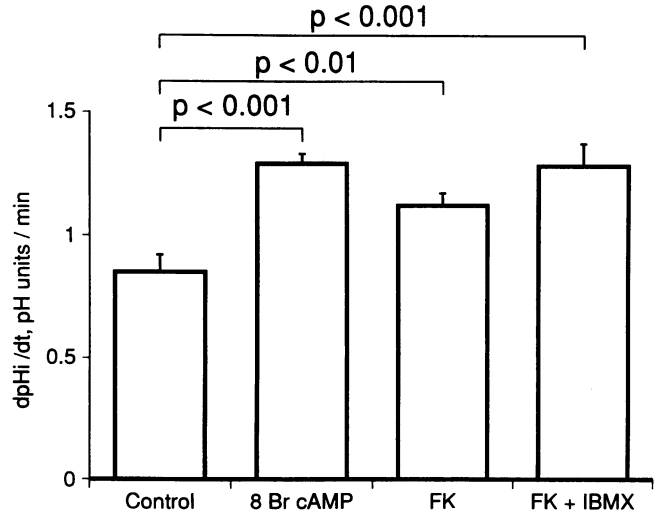

Figure 5. Exogenous and endogenous cAMP chronically stimulate Na/H antiporter activity. OKP cells were incubated with either 10^{-4} M 8-bromo cAMP, 10^{-5} M forskolin, or 10^{-5} M forskolin + 2 mM IBMX for 6 h. Na/H antiporter activity, assayed 16–20 h after removal of the agents, is plotted on the *y*-axis as described in Fig. 1. Control, $n = 7$; 8 Br cAMP, $n = 6$; FK + IBMX, $n = 5$.

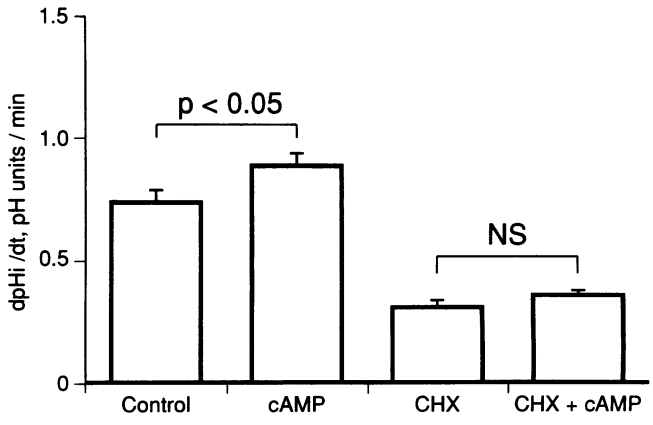

Figure 6. Cycloheximide blocks 8-bromo cAMP–induced chronic Na/H antiporter stimulation. Cells were treated with 8-bromo cAMP or vehicle for 6 h, and Na/H antiporter activity was assayed 16–20 h later. Cycloheximide-treated cells were incubated with 100 μM cycloheximide starting 1 h before 8-bromo cAMP addition, and remaining until Na/H antiporter activity was assayed. *y*-axis is as described in Fig. 1. Control, $n = 6$; cAMP, $n = 6$; CHX, $n = 8$; CHX + cAMP, $n = 8$.

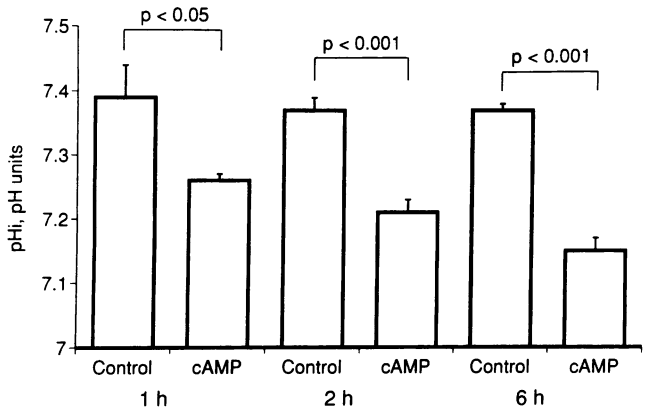

Figure 7. 8-Bromo cAMP acidifies OKP cells. OKP cells were incubated with 8-bromo cAMP or vehicle for 1, 2, or 6 h. Baseline pH_i, plotted on the *y*-axis, was measured under the same conditions as the incubation, in the presence of CO_2/HCO_3-containing media. 1 h: control, $n = 4$; cAMP, $n = 5$. 2 h: control, $n = 5$; cAMP, $n = 5$. 6 h: control, $n = 5$; cAMP, $n = 5$.

results confirm our previous observations with a similar protocol in OKP cells (15). We next examined whether incubation of cells in acid media for 6 h, followed by 16–20 h of incubation in control media (similar to the 8-bromo cAMP protocol) was sufficient to activate the antiporter. With this protocol, Na/H antiporter activity was increased by 13% (Fig. 8). This latter result suggests that cell acidification due to only 6 h of cAMP exposure may be sufficient to chronically increase Na/H antiporter activity.

We next examined the effect of inhibiting cAMP-induced cell acidification on cAMP-induced antiporter activation. Cells

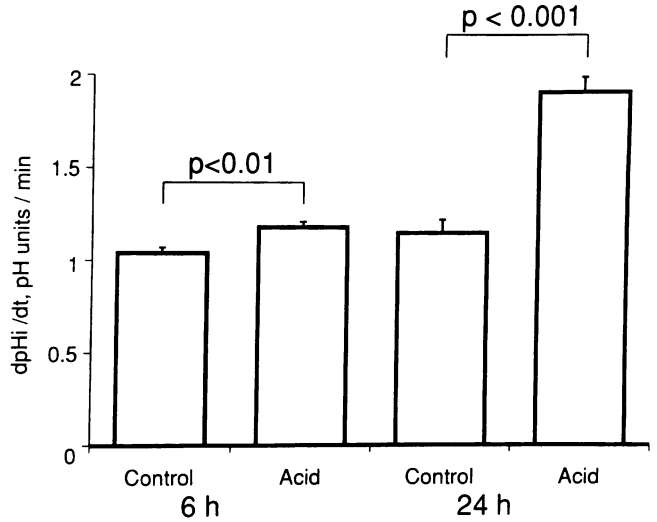

Figure 8. Acid incubation chronically stimulates Na/H antiporter activity in OKP cells. OKP cells were incubated in control or acid media ($\Delta pH = 0.3$ pH U) (14, 15). (*Left two bars*) Cells were incubated for 6 h and then were placed in control media for 16–20 h before assay of Na/H antiporter activity. (*Right two bars*) Cells were incubated either in control or acid media for 24 h, and then placed in control media for 1 h before assay of Na/H antiporter activity. *y*-axis is as in Fig. 1. 6 h: control, $n = 15$; acid, $n = 14$. 24 h: control, $n = 7$; acid, $n = 7$.

were incubated either in control (pH 7.3) or alkaline media (pH 7.6) for 6 h, either in the presence or absence of 8-bromo cAMP. This was then followed by 16–20 h of incubation in control media. Fig. 9 *A* shows the effect of these maneuvers on pH_i at 6 h. As can be seen, incubation in alkaline media increased pH_i, and incubation in cAMP decreased pH_i. The effects of alkaline media and cAMP were additive. It should be noted that the cells incubated in alkaline media with cAMP (*fourth bar*) had a higher pH_i than control cells (*first bar*).

Fig. 9 *B* shows results of Na/H antiporter activity assayed 16–20 h after the 6-h incubations described above. Compared with controls, incubation in alkaline media had no effect on Na/H antiporter activity. In addition, cAMP stimulated Na/H antiporter activity in cells incubated in control or in alkaline media. If one compares cells incubated in alkaline media and treated with 8-bromo cAMP (*fourth bar*) with those incubated in control media (*first bar*), it can be seen that in the former group Na/H antiporter activity was increased while pH_i during incubation was increased. Thus, this increase in Na/H antiporter activity cannot be attributed to a decreased pH_i. The antiporter stimulation is also not attributable to the alkaline media, in that this might be expected to inhibit the Na/H antiporter, and in fact had no measurable effect on the antiporter (Fig. 9 *B, first* vs. *third bars*). Thus, in this setting where cAMP incubation was not associated with a lower pH_i, cAMP was still able to stimulate the Na/H antiporter. Therefore, we conclude that increases in cell cAMP for 6 h lead to an increase in Na/H antiporter activity that is present 16–20 h after removal of cAMP, and is independent of cell acidification. There is likely an additional component of the cAMP-induced chronic Na/H antiporter stimulation that is attributable to cell acidification.

Discussion

We and others have shown that acute activation of adenylyl cyclase or short-term exogenous administration of cAMP inhibits Na/H antiporter activity in proximal tubule cells, brush border membrane vesicles, or OKP cells (1–7). In the present study, we demonstrate that chronic increases in cell cAMP levels for 6 h lead to a stimulation of the Na/H antiporter, when measured 16–20 h later. This effect was not evident after 1 or 2 h of 8-bromo cAMP exposure.

Many examples exist in biology whereby chronic activation of a signaling pathway leads to chronic biological effects that are qualitatively similar to acute effects, but occur by distinct mechanisms. We previously found in primary rabbit proximal tubule cell cultures that activation of protein kinase C with a 5-min application of PMA acutely increased Na/H antiporter activity, but did not affect Na/H antiporter activity measured 24 h later, whereas a 2-h application of PMA led to a persistent increase in antiporter activity that was dependent on protein synthesis (18). Similar results have been found in studies examining the cellular basis of memory in *Aplysia* sensory neurons. Application of cAMP to these cells for 5 min led to a short-lived inhibition of K channel activity, whereas a 2-h application of cAMP caused a persistent protein synthesis–dependent inhibition of K channel activity measured 24 h later (19, 20). The present results are somewhat unique in that acute and chronic increases in cAMP have opposite effects on the Na/H antiporter. The chronic stimulation does not represent an artifact

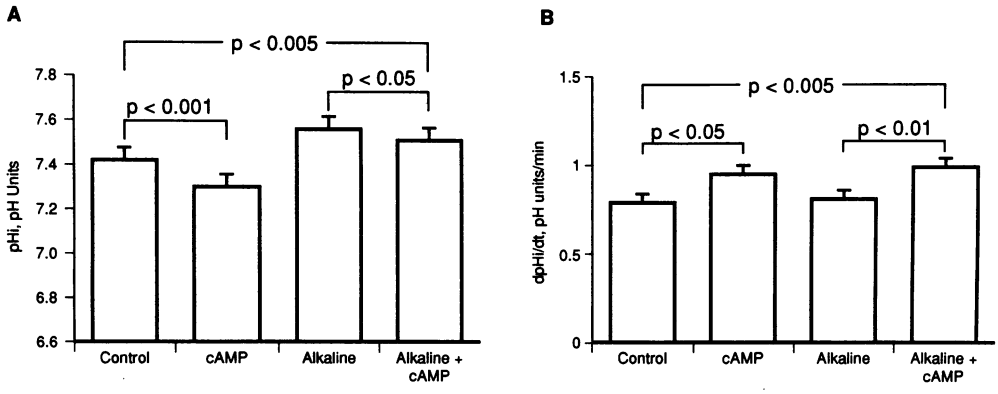

Figure 9. 8-Bromo cAMP–induced chronic stimulation of Na/H antiporter activity is not mediated by cell acidification. OKP cells were incubated either in vehicle, 10^{-4} M 8-bromo cAMP, alkaline media, or alkaline media + 10^{-4} M 8-bromo cAMP for 6 h. A. pH_i (*y*-axis) was measured at the end of the 6-h incubation. (*B*) Na/H antiporter activity measured 16–20 h after incubation. *y*-axis is as in Fig. 1. (*A*) Control, $n = 7$; cAMP, $n = 5$; alkaline, $n = 8$; alkaline + cAMP, $n = 5$. (*B*) Control, $n = 7$; cAMP, $n = 7$; alkaline, $n = 8$; alkaline + cAMP, $n = 8$.

of 8-bromo cAMP, in that similar results were obtained with a 6-h application of forskolin or of forskolin with IBMX.

The chronic effect of cAMP on the Na/H antiporter was inhibited by cycloheximide, suggesting a dependence on protein synthesis. These studies do not address whether the protein synthesized is the Na/H antiporter, or a regulatory protein. Indeed, in *Aplysia* sensory neurons chronic regulation of K channel activity is mediated by protein synthesis–dependent changes in kinase activity (21). Chronic protein synthesis–dependent stimulation of the Na/H antiporter by protein kinase C in rabbit proximal tubule cells was associated with an increase in NHE-1 mRNA, which likely encodes the amiloride-sensitive basolateral membrane Na/H antiporter in the proximal tubule (18).

In previous studies we were unable to detect NHE-1 mRNA expression under basal conditions in OKP cells (15). This is in contrast to most other renal tubuloepithelial cell lines in which NHE-1 expression is easily detected (15, 17, 18). To examine whether chronic exposure to cAMP induced expression of NHE-1 mRNA, we performed Northern blotting at high stringency with the human NHE-1 cDNA probe (22). NHE-1 mRNA was undetectable in poly(A)$^+$ RNA from control OKP cells or OKP cells treated with 10^{-4} M 8-bromo cAMP for 6 h and then harvested 16–20 h later (data not shown).

The Na/H antiporter in OKP cells is amiloride resistant, and in this respect is similar to the proximal tubule apical membrane Na/H antiporter (15). An Na/H antiporter isoform, NHE-3, whose mRNA is abundant in renal cortex, has recently been cloned in rat and rabbit (23, 24). This may correspond to the isoform present in OKP cells and allow molecular studies of its regulation. Unfortunately, we have been unable to detect NHE-3 mRNA in poly(A)$^+$ RNA from any renal cell line (OKP, LLC-PK$_1$, MDCK, MCT, and a series of cell lines generated in our laboratory from mice transgenic for SV-40 Large T antigen) using the rat cDNA probe, although we have detected NHE-3 mRNA in rat and rabbit renal cortical poly(A)$^+$ RNA. NHE-3 mRNA also was not detected in poly(A)$^+$ RNA from OKP cells treated with 8-bromo cAMP for 6 h and harvested 16–20 h later (data not shown). It is presently not clear whether the amiloride-resistant Na/H antiporter of OKP cells is encoded by a distinct isoform or is encoded by an NHE-3 isoform that is undetectable by Northern blotting because of interspecies divergence. While NHE-1 has been markedly conserved in evolution, this may not be the case with NHE-3. Last, it is possible that levels of NHE-3 mRNA expression in cultured cells are too low to detect by Northern blot.

We and others have previously shown that chronic incubation of primary rabbit proximal tubule cell cultures, MCT cells, LLC-PK1, and OKP cells in acid media for 24–48 h leads to an increase in Na/H antiporter activity that persists after the cells are removed from acid media and is dependent on protein synthesis (14, 15, 17). Because the response to chronic increases in cAMP was similar to the response to chronic acid incubation, we asked whether they may occur through a common mechanism. Indeed, addition of 8-bromo cAMP for 6 h caused a decrease in pH_i, likely secondary to phosphorylation-induced inhibition of the Na/H antiporter. While this cell acidification likely contributes to the chronic stimulation of the Na/H antiporter, it does not provide the entire explanation. When cells were exposed for 6 h to cAMP while in alkaline media, cell acidification was prevented and, in fact, pH_i increased (compared with controls). Nevertheless, Na/H antiporter activity was still increased 16–20 h later. Thus, cAMP is able to chronically increase Na/H antiporter activity independent of cell acidification. We have also found that acid-induced Na/H antiporter activation cannot be attributed to chronic increases in cAMP, as acid incubation decreases cAMP production in OKP cells (unpublished observation).

Increases in cAMP can regulate protein synthesis by a number of general mechanisms. Chronic regulation by cAMP could be transcriptional. Frequently, this occurs through protein kinase A–induced phosphorylation of the cAMP response element binding protein (CREB), modulating its ability to activate a cAMP response element (CRE) in the promoter/enhancer region of cAMP-responsive genes (25, 26). Indeed, chronic regulation of K channels by cAMP in *Aplysia* sensory neurons has been demonstrated to be mediated through CREB (27). In addition to CREB, a number of other proteins have been identified that can bind to the CRE and potentially regulate transcription (28–30). Last, cAMP regulation of transcrip-

tion can be mediated by activation of transcription factors binding to AP-1 (31, 32) or AP-2 (26, 33) binding sites. We have recently found in MCT cells, a mouse proximal tubule cell line, that cAMP increases the abundance of mRNAs encoding c-*fos*, c-*jun*, and junB, components of AP-1 (34). cAMP regulation of protein synthesis may also occur through a posttranscriptional mechanism, but we are not aware of instances where this has been found. Last, increases in cAMP may modulate other signaling pathways that may regulate protein synthesis transcriptionally or posttranscriptionally.

The opposing acute and chronic effects of cAMP may be of significant physiologic importance. PTH, dopamine, α-adrenergic catecholamines, and angiotensin II are believed to regulate proximal tubule Na/H antiporter activity at least in part through regulation of adenylyl cyclase, cAMP, and protein kinase A. However, this is based on studies that have examined only the acute effects of these hormones. The present studies raise the possibility that chronic effects may be very different from acute effects.

Evidence in favor of the importance of these findings is derived from comparing the acute and chronic effects of PTH. Whereas acute hyperparathyroidism inhibits proximal tubule acidification and apical membrane Na/H antiporter activity (3–6, 35, 36), chronic hyperparathyroidism causes metabolic alkalosis and increases in renal acidification (37–39). In fact, in human subjects acute increases in PTH cause metabolic acidosis while chronic increases cause metabolic alkalosis (39). The acute Na/H antiporter inhibition is believed to be mediated by cAMP (3–7). Chronic increases in PTH may increase renal acidification by stimulating H secretion in segments other than the proximal tubule, or by increasing proximal tubule H secretion through mechanisms other than cAMP-induced regulation of the Na/H antiporter. The present studies raise the intriguing possibility that the converse effects of acute and chronic hyperparathyroidism may be due to time-dependent changes in the effect of cAMP on Na/H antiporter activity.

Acknowledgments

We appreciate the technical assistance of Martha Ferguson. We are grateful to Michel Baum, R. Tyler Miller, and Orson Moe for suggestions and discussions during the performance of these studies, and for their reading of the manuscript.

This work was supported by National Institutes of Health grant RO1 DK-39298, a Grant-in-Aid from the American Heart Association, Texas Affiliate (90R-121), and grants from the National Kidney Foundation of Texas to R. J. Alpern and P. Preisig. A. Cano is the recipient of a Clinician Scientist Award from the American Heart Association.

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