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Regulation of Renal Na-HCO_3 Cotransporter: VIII. Mechanism of Stimulatory Effect of Respiratory Acidosis

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Abstract. We examined the effect of respiratory acidosis on the Na-HCO_3 cotransporter activity in primary cultures of the proximal tubule of the rabbit exposed to 10% CO_2 for 5 min, 2, 4, 24 and 48 hr. Cells exposed to 10% CO_2 showed a significant increase in Na-HCO_3 cotransporter activity (expressed as % of control levels, 5 min: 142 ± 6, 2 hr: 144 ± 13, 4 hr: 145 ± 11, 24 hr: 150 ± 15, 48 hr: 162 ± 24). The increase in activity was reversible after 48 hr. The role of protein kinase C (PKC) on the stimulatory effect of respiratory acidosis on the cotransporter was examined in presence of PKC inhibitor calphostin C or in presence of PKC depletion. Both calphostin C and PKC depletion prevented the effect of 10% CO_2 for 5 min or 4 hr to increase the activity of the cotransporter. 10% CO_2 for 5 min or 4 hr increased total and particulate fraction PKC activity. To examine the role of phosphotyrosine kinase (PTK) on the increase in cotransporter activity we studied the effect of two different inhibitors, 2-hydroxy-5-(2,5-dihydroxylbenzyl) aminobenzoic acid (HAC) and methyl 2,5-dihydroxycinnamate (DHC) which inhibit phosphotyrosine kinase in basolateral membranes. Cells were pretreated either with vehicle or HAC or DHC and then exposed to 10% CO_2 for 5 min or 4 hr. In cells treated with vehicle, 10% CO_2 significantly increased cotransporter activity as compared to control cells exposed to 5% CO_2. This stimulation by 10% CO_2 was completely prevented by HAC or DHC at 5 min (5% CO_2: 1.8 ± 0.2, 10% CO_2: 2.6 ± 0.2, 10% CO_2 + HAC: 1.6 ± 0.2, 10% CO_2: +DHC: 2.0 ± 0.3 pH unit/min) and also at 4 hr. The protein synthesis inhibitors actinomycin D and cycloheximide appear to prevent the effect of 10% CO_2 for 4 hr on the cotransporter. Our results show that early respiratory acidosis stimulates the Na-HCO_3 cotransporter through PKC and PTK-dependent mechanisms and the late effect appears to be mediated through protein synthesis.

Key words: Na-HCO_3 cotransporter — Respiratory acidosis — Protein kinase C — Phosphotyrosine kinase

Introduction

Respiratory acidosis is associated with increased HCO_3 reabsorption in the proximal tubule and enhanced activity of the brush border Na-H antiporter [6, 23]. We have previously shown that respiratory acidosis is also associated with stimulation of the basolateral Na-HCO_3 cotransporter [15]. The mechanism responsible for the enhanced activity of the cotransporter was not clarified. However, in the last few years we have characterized in detail the basolateral Na-HCO_3 cotransporter and have shown that in primary cultures of the proximal tubule, the cotransporter is regulated by protein kinase C, protein kinase A, calcium-calmodulin kinase II [16], angiotensin II [19], parathyroid [20], and glucocorticoid hormones [18]. We have previously shown that protein kinase C plays an important role in the adaptive increase in Na-H antiporter in respiratory acidosis [11]. Other investigators have recently shown that the adaptation of the Na-H antiporter in metabolic acidosis is mediated, at least in part, through phosphotyrosine kinase activation [28]. Because the activity of the Na-H antiporter and Na-HCO_3 cotransporter are regulated in a parallel and symmetric fashion [1, 4, 5, 9, 12, 15, 17, 18, 20, 21, 26, 27], we investigated the role of protein kinase C, phosphotyrosine kinase, and protein synthesis, in the adaptive increase in the activity of the Na-HCO_3 cotransporter in respiratory acidosis. In the present study, we used primary cultures of the proximal tubule to study the effect of respiratory acidosis on the activity of the cotransporter and the mechanism by which this effect is mediated.

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Materials and Methods

Isolation and Culture of Rabbit Proximal Tubule Cells

The separation and culture of proximal tubules from New Zealand white rabbits was done as previously described [4, 10]. The cultures were maintained at 37°C in a 5% CO_2 incubator and pH was maintained at 7.40. Media were changed regularly every 3–4 days. Confluency was reached after 8–10 days and then cells were trypsinized and replated on clear plastic coverslips. Confluency was reached 2–3 days after and the cells which were rendered quiescent for 48 hr were used to measure the Na-HCO_3 cotransporter activity fluorometrically. We have previously shown that these cultures have morphologic, enzymatic, and transport functions very similar to the original epithelium [4, 18].

Respiratory acidosis was induced by incubating the cells in 10% CO_2, and 90% compressed air for various periods of time (5 min, 2, 4, 24, 48 hr). Control cells were incubated in 95% compressed air and 5% CO_2 for comparable periods of time. The cells were removed from the incubator and measurement of the Na-HCO_3 cotransporter activity was performed with the cells perfused with a physiologic solution (*see below*), pH 7.40, and bubbled with compressed air and 5% CO_2.

Measurement of Intracellular pH (pH_i) in Cultured Proximal Tubule Cells and Fluorometric Assay of Na-HCO_3 Cotransporter Activity

Intracellular pH was continuously measured using the pH fluorescent probe, BCECF [1,2,7 biscarboxyethyl-5 (6)-carboxyfluorescein], as previously described [18]. For assay of the Na-HCO_3 cotransporter, the cells were perfused first with Cl-free physiologic solution containing (in mM): 25 $NaHCO_3$, 110 Na gluconate, 5 K gluconate, 9 HEPES, 2 $CaSO_4$, 1 KH_2PO_4, 0.5 $MgSO_4$, 10 glucose, and 1 amiloride at a rate of 20 ml/min at 37°C. The extracellular pH was maintained constantly at pH 7.40. Once a stable fluorescence signal was reached, Na was removed by replacing Na gluconate and $NaHCO_3$ by equimolar amounts of choline gluconate and choline HCO_3, respectively, at pH 7.40. With removal of Na, the intracellular pH decreases and when Na was re-added, there was rapid recovery of intracellular pH to baseline levels. Na-HCO_3 cotransporter activity was assayed as the initial rate of pH recovery following the addition of Na-HCO_3 in cells perfused in the absence of Na and was calculated from the slope of the line drawn tangent to the initial deflection over a period of 1 min (the time it took the solution to reach the cell was approximately 40 sec). This recovery of intracellular pH observed with addition of Na was inhibited by DIDS. In the absence of chloride and in the presence of DIDS, this pH recovery was attributed to the Na-HCO_3 cotransporter [2], Fig. 1*A*. Buffer capacity of the cells was determined from the intracellular pH change as described [14].

Preparation of Plasma Membranes from Cultured Proximal Tubule Cells

Plasma membranes from cultured proximal tubule cells were prepared as described [22] with some modifications. Cells were exposed either to 5% CO_2 or to 10% CO_2 for 5 min or pretreated with 50 μM calphostin C and then exposed to 10% CO_2 for 5 min were washed twice with phosphate buffered saline containing 1.6 mM Ca gluconate. Cells were then scraped, transferred to a pre-chilled 50 ml tube and pelleted at 4°C at 5000 rpm for 3 min. The cell pellets were resuspended in an ice-cold buffer containing (in mM): 60 Tris, pH 7.00, 250 sucrose, 10 EGTA, 2 EDTA, 0.1 Na_2VO_4, 10 β-mercaptoethanol, 5 μg/ml phenylmethylsulfonyl fluoride, and 5 μg/ml aprotinin and sonicated for 45 sec on ice. The homogenates were centrifuged at 100,000 × *g* for 1 hr at 4°C. The resulting pellets were resuspended in the above buffer containing 1% Triton X-100 and centrifuged at 100,000 × *g* for 1 hr at 4°C. The supernatant was the crude membrane fraction consisting of nuclear, plasma, and mitochondrial membranes.

PKC Assay

PKC activity was assayed based on the phosphorylation of acetylated myelin basic protein [8, 30]. In brief, PKC in the cytosol and particulate fractions prepared from rabbit proximal tubule cell previously exposed to 5% or 10% CO_2 for 5 min or pretreated with calphostin C and then exposed to 10% CO_2 is partially purified by ion exchange chromatography. The reaction is started by addition of 10 μl, 20 μCi/ml [γ^{32}P]ATP to a reaction mixture consisting of 20 mM Tris, pH 7.5, 20 mM $MgCl_2$, 1 mM $CaCl_2$, 20 μM ATP, 50 μM Ac-MBP and lipid mixture containing 0.28 mg/ml phosphatidylserine and Triton X-100 mixed micelles or an inhibitor solution containing 20 μM PKC peptide (19–36) and incubated at 30°C for 5 min. The reaction is stopped by spotting 25 μl of the mixture to a phosphocellulose paper. After washing the paper with 1% phosphoric acid and water, radioactivity is measured with a scintillation counter.

Statistical Analysis

Results are expressed as mean ± SEM. The results were analyzed by paired *t* test or analysis of variance as appropriate.

Results

Effect of Respiratory Acidosis on the Na-HCO_3 Cotransporter Activity

We examined the effect of 10% CO_2 at various times of incubation on the activity of the Na-HCO_3 cotransporter. The cells were exposed to 90% compressed air and 10% CO_2 for 5 min or 2, 4, 24 or 48 hr. Control cells were exposed to 95% air and 5% CO_2. The cells were removed from the incubator and loaded in vitro with intracellular pH probe and perfused with physiologic solution of pH 7.40, bubbled with compressed air and 5% CO_2. During the exposure to 10% CO_2, the extracellular pH decreased to 7.10–7.20. Figures 1*B* and 2 show that 10% CO_2 significantly increased the activity of the Na-HCO_3 cotransporter (expressed as % of control levels in Fig. 2. After 5 min of respiratory acidosis, the activity of Na-HCO_3 cotransporter was 142 ± 6% above control levels normalized at 100%. The activity remained increased at this level after 2 and 4 hr. After 24 and 48 hr, the activity increased further to 157 ± 15 and 162 ± 26% of control levels. The values at 48 hr were significantly higher than those at 5 min or 4 hr indicating an additional late increase in the activity of the cotransporter. The

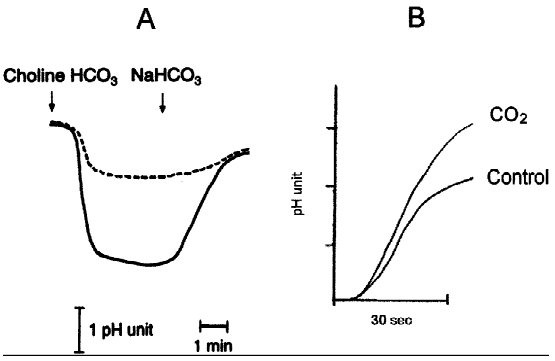

Fig. 1. Intracellular pH measurement in proximal tubule cells and fluorometric assay of Na-HCO_3 cotransporter. (*A*) Cells were continuously perfused with physiologic solution, pH 7.40 containing 25 mM $NaHCO_3$ and 1 mM amiloride. $NaHCO_3$ was removed and replaced with choline HCO_3 (first arrow) and when a decrease in pH_i was observed, choline HCO_3 was replaced with $NaHCO_3$ (second arrow), (control, solid line and pretreatment with DIDS, dashed line) as previously described (reference 18). (*B*) Representative experiment showing the effect of 10% CO_2 on the recovery of intracellular pH.

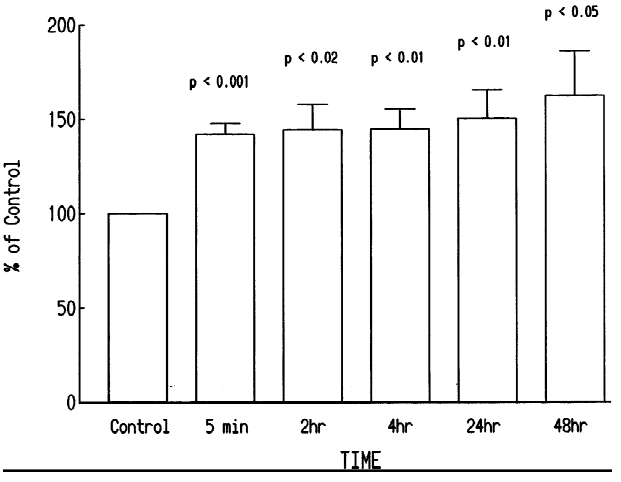

Fig. 2. Effect of varying incubation times with 10% CO_2 on the activity of the Na-HCO_3 cotransporter. Primary cultures of rabbit proximal tubule were exposed either to 5% CO_2 (control) or to 10% CO_2 for 5 min, 2, 4, 24 or 48 hr and then Na-HCO_3 cotransporter activity assayed fluorometrically. Each bar represents mean ±SEM expressed as per cent of control of 5–9 experiments.

intracellular pH and the buffer capacity were not different between control cells and cells exposed to 10% CO_2 for 5 min and 4 hr and then withdrawn from the 10% CO_2 (Table 1).

We also examined the reversibility of the effect of respiratory acidosis by exposing cells to 10% CO_2 for 5 min or 24 hr and then cells were exposed to 5% CO_2 for 48 hr. Na-HCO_3 cotransporter activity was measured after 48 hr in 5% CO_2. Figure 3 shows that the activity of Na-HCO_3 cotransporter decreased significantly and returned to near normal after 48 hr (5 min: 141.9 ± 6.1,

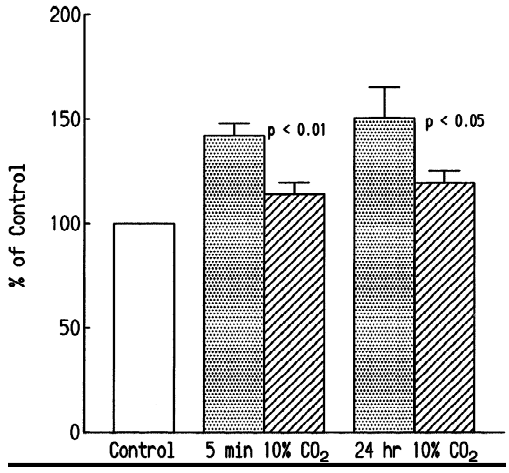

Fig. 3. Effect of exposure to 10% CO_2 for 5 min and 24 hr (speckled bars) and then to 5% CO_2 for 48 hr (diagonal bars) on the Na-HCO_3 cotransporter activity. Each bar represents mean ± SEM of 5–6 different experiments.

after 48 hr in 5% CO_2, 114 ± 5.4; 24 hr; 150.3 ± 15.0, after 48 hr in 5% CO_2, 119.3 ± 5.9, expressed as percent of control). This same effect of 10% CO_2 after 48 hr has been observed with Na-H antiporter [29].

Role of Protein Kinase C on the Adaptive Increase in Na-HCO_3 Cotransporter Activity

We have previously shown that protein kinase C stimulation by phorbol ester has two distinct effects on the Na-HCO_3 cotransporter activity: an early effect (5 min) and a late effect (2 hr) which appear to be mediated by phosphorylation of the cotransporter and increased immunoreactive protein content respectively [21]. To examine the role of PKC in respiratory acidosis, we utilized the inhibitor, calphostin C, which does not alter the baseline activity of the cotransporter [21]. Control cells were incubated with either calphostin C overnight or with the vehicle and then exposed to respiratory acidosis for 5 min or 4 hr. Figure 4*A*, shows that calphostin C, 50 μM, prevented the effect of 5-min respiratory acidosis to increase the activity of the cotransporter (control: 1.76 ± 0.18, 10% CO_2: 2.77 ± 0.39, 10% CO_2 + calphostin C: 1.82 ± 0.19). Calphostin C also blocked the effect of 4-hr respiratory acidosis (control: 1.79 ± 0.2, 10% CO_2: 2.67 ± 0.23, 10% CO_2 + calphostin C: 1.90 ± 0.14 pH unit/min), Fig. 4*B*.

The role of PKC was also examined in PKC-depleted cells. PKC depletion was accomplished by incubating the cells with 10^{-6} M PMA for 72 hr. We have previously shown that this protocol resulted in approximately 80% depletion of PKC activity in cytosol and particulate fraction without affecting cell viability [11]. Control cells were treated with the vehicle, DMSO, for

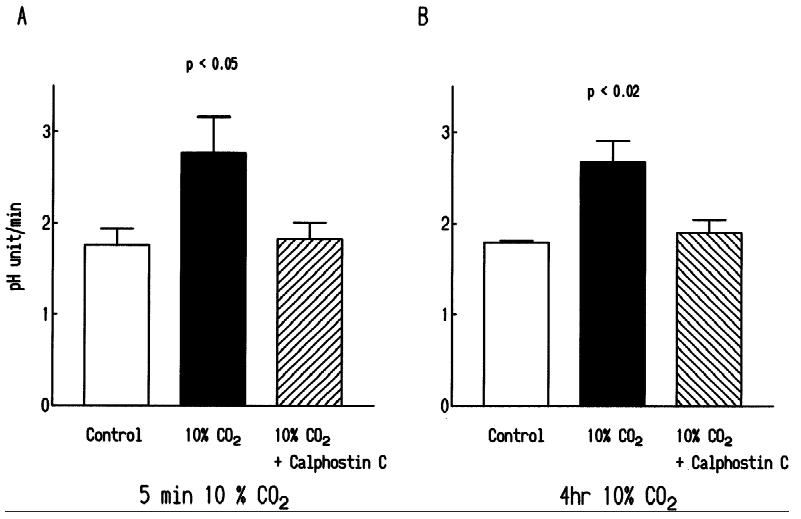

Fig. 4. Effect of calphostin C on the stimulation of Na-HCO_3 cotransporter by respiratory acidosis. Proximal tubule cells were pretreated with either vehicle (open bar) or with calphostin C overnight (diagonal bar) and then exposed to 10% CO_2 (solid and diagonal bar) for 5 min (*Panel A*) or for 4 hr (*Panel B*) and then Na-HCO_3 cotransporter activity measured fluorometrically. Each bar represents mean ± SEM of 5–7 different experiments.

the same period of time. After 72 hr, cells were exposed to respiratory acidosis for 5 min or 4 hr. Figure 5 shows that in cells treated with DMSO the activity of the Na-HCO_3 cotransporter increased significantly when exposed to respiratory acidosis for 5 min (*A*) or 4 hr (*B*). In the presence of PKC depletion respiratory acidosis of either 5 min or 4 hr duration failed to increase the activity of the cotransporter.

The effect of respiratory acidosis on PKC activity in cytosolic and particulate fractions is shown in Table 2. Respiratory acidosis tended to cause an increase in the total PKC activity but the difference did not achieve statistical significance. There was a significant increase in the particulate fraction with a decrease in the cytosolic fraction.

Effect of Respiratory Acidosis on the Cotransporter in the Presence of PTK Inhibitors

To examine the role of PTK on the increase in Na-HCO_3 cotransporter activity in respiratory acidosis, we utilized two dissimilar inhibitors of PTK, 2-hydroxy-5(2,5-dihydroxybenzyl) aminobenzoic acid (HAC) and methy 2,5-dihydroxycinnamate (DHC) which we have previously shown to prevent phosphorylation of synthetic substrate for PTK, poly-GT [7, 12, 24]. Genistein was not used because we have been unable to show inhibition of poly GT by this compound. In the presence of 5% CO_2, HAC (1 μM) and DHC (5 μM) did not affect baseline activity of the Na-HCO_3 cotransporter (in pmol/mg protein/5 min; control: 1.54 ± 0.18, HAC: 1.85 ± 0.18, DHC: 1.56 ± 0.21). Figure 6 shows the effect of respiratory acidosis in the presence of HAC or DHC. Respiratory acidosis was associated with a significant increase in the activity of the cotransporter, both at 5 min (*A*) and 4 hr (*B*). HAC and DHC significantly inhibited the adaptive increase in cotransporter activity at 5 min (5% CO_2: 1.55 ± 0.18, 10% CO_2: 2.55 ± 0.15, 10% CO_2 + HAC: 1.64 ± 0.24, 10% CO_2 + DHC: 2.01 ± 0.25 pH unit/min) and at 4 hr (5% CO_2: 1.54 ± 0.18, 10% CO_2: 2.64 ± 0.31, 10% CO_2 + HAC: 1.90 ± 0.10, 10% CO_2 + DHC: 1.79 ± 0.06 pH unit/min).

Effect of Protein Synthesis Inhibitors on the Stimulatory Effect of Respiratory Acidosis on the Na-HCO_3 Cotransporter

We have previously shown that the late effect (4 hr) of PKC activation on the Na-HCO_3 cotransporter was prevented by protein synthesis inhibitors [21]. Figures 7 and 8 show the effect of actinomycin D, 10 μM, or cycloheximide, 7 μM, respectively, on the effect of 10% CO_2 for 5 min or 4 hr on the activity of the Na-HCO_3 cotransporter. Actinomycin D or cycloheximide alone had no effect on the activity of the cotransporter (Actinomycin D: control, 1.36 ± 0.07; actinomycin D, 1.58 ± 0.06 pH unit/min. Cycloheximide: control, 1.36 ± 0.08; cycloheximide, 1.40 ± 0.08 pH unit/min). Actinomycin D or cycloheximide did not block the early effect of respiratory acidosis (5 min) on the cotransporter but completely prevented the late effect. Not shown is the fact that actinomycin D or cycloheximide did not affect buffer capacity.

Discussion

The present studies were aimed at investigating the cellular mechanisms responsible for the adaptive increase in HCO_3 absorption in respiratory acidosis. We have previously shown that the effect of CO_2 is specific for acid-base transport, i.e., Na-H antiporter and Na-HCO_3 cotransporter, and does not affect other transport systems

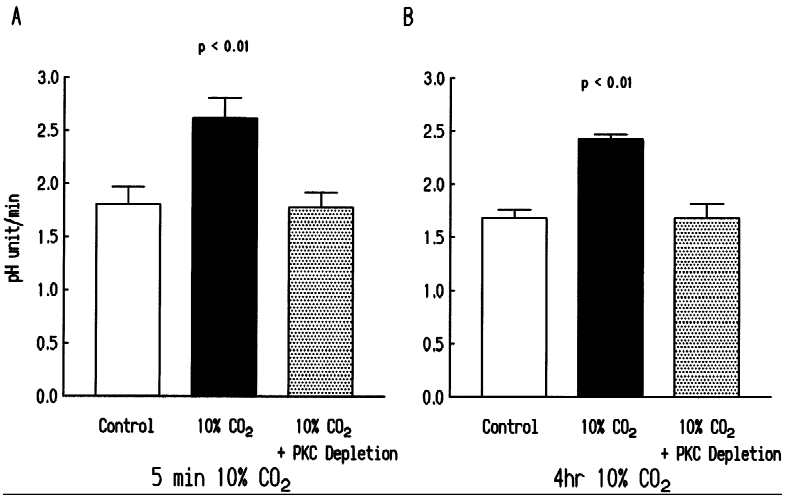

Fig. 5. Effect of PKC depletion on the stimulatory effect of respiratory acidosis on the Na-HCO_3 cotransporter. Proximal tubule cells were pretreated with DMSO (open bar) or with 1 μM PMA for 72 hr (speckled bar) and then exposed to 10% CO_2 (solid and speckled bar) for 5 min (*Panel A*) or 4 hr (*Panel B*). Na-HCO_3 cotransporter activity was subsequently measured. Each bar represents mean ± SEM, $n = 6$.

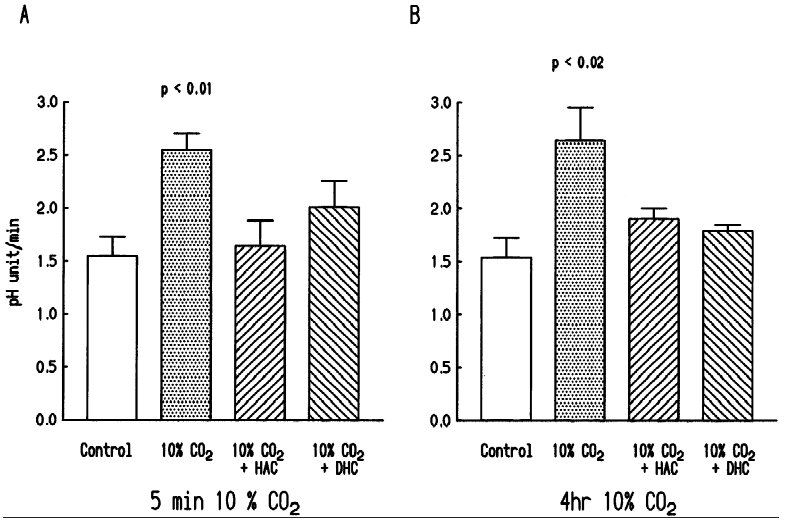

Fig. 6. Effect of PTK inhibitors on the stimulatory effect of respiratory acidosis on the Na-HCO_3 cotransporter activity. Proximal tubule cells were pretreated with either vehicle (open bar) or with HAC or DHC (diagonal bars) and then exposed to 10% CO_2 (speckled and diagonal bars) for 5 min (*Panel A*) or 4 hr (*Panel B*) and Na-HCO_3 cotransporter activity measured subsequently. Each bar represents mean ± SEM, $n = 5$–8.

such as Na-glucose transport [3, 29]. Respiratory acidosis is associated with increased activity of the Na-H antiporter in brush border membranes and in primary cultures of the proximal tubule [6, 23]. The increase in the activity of the brush border Na-H antiporter in respiratory acidosis appears to be associated with enhanced PKC activity in this membrane which seems to play a role in the adaptation [11]. We have also previously shown that respiratory acidosis is associated with a parallel rise in the basolateral Na-HCO_3 cotransporter [15] but the mechanism responsible for this increase was not clarified. In the present study, we utilized primary cultures of the proximal tubule of the rabbit to examine the mechanism responsible for this adaptation. These cultures have Na-HCO_3 cotransporter activity which is regulated by cAMP [16], protein kinase C [21], G protein [17], angiotensin II [19], glucocorticoid [18], and parathyroid hormones [20]. We have recently demonstrated that PKC activates the Na-HCO_3 cotransporter in these cells by two distinct mechanisms: an early effect (5 min) which seems to be related to phosphorylation of the cotransporter, and a late effect (4 hr) which appears to be mediated by enhanced synthesis of the cotransporters [21].

The results of these present studies show that cells exposed to 10% CO_2 for various periods of time and then removed from the hypercapnic environment had an increase in Na-HCO_3 cotransporter activity which was measured in the presence of 5% CO_2 and normal extracellular pH. The increase in Na-HCO_3 cotransporter was not related to a decrease intracellular pH because when the measurement of the Na-HCO_3 cotransporter activity was done, intracellular pH had returned to normal (Table 1). This finding does not exclude, however, that the initial decrease in intracellular pH played a role in the adaptive increase in Na-HCO_3 cotransporter activity.

The increase in Na-HCO_3 cotransporter activity was time-dependent with an initial increase in the first few

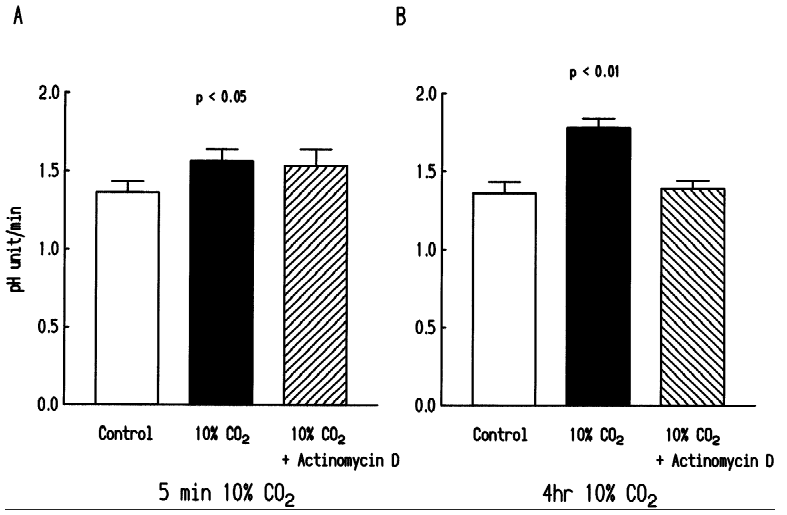

Fig. 7. Effect of actinomycin D on the stimulatory effect of respiratory acidosis on the Na-HCO_3 cotransporter. Cells were pretreated with either vehicle (open bar) or with 10 μM actinomycin D for 1 hr and then exposed to 10% CO_2 (solid and diagonal bars) for 5 min (*Panel A*) or 4 hr (*Panel B*) and then Na-HCO_3 cotransporter activity measured fluorometrically. Each bar represents mean ± SEM, $n = 6–12$.

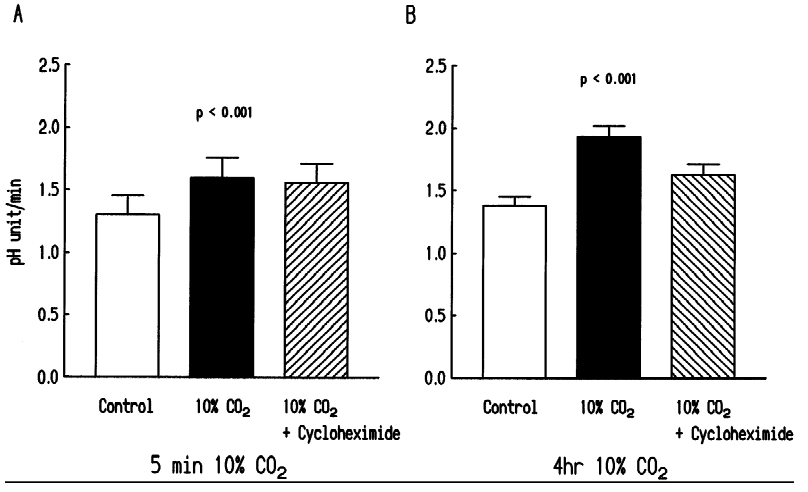

Fig. 8. Effect of cycloheximide on the stimulatory effect of respiratory acidosis on the Na-HCO_3 cotransporter. Cells were pretreated with either vehicle (open bar) or 7 μM cycloheximide (diagonal bar) for 1 hr and then exposed to 10% CO_2 (solid and diagonal bars) for 5 min (*Panel A*) or 4 hr (*Panel B*) and then activity of the Na-HCO_3 cotransporter measured. Each bar represents mean ± SEM, $n = 6–10$.

Table 1. Effect of respiratory acidosis on pH_i and buffer capacity

	pH_i	Buffer capacity mmol/pH unit
Control	7.33 ± 0.07	26.7 ± 1.9
10% CO_2, 5 min	7.35 ± 0.17	26.7 ± 1.2
10% CO_2, 4 hr	7.30 ± 0.07	27.0 ± 3.5

Each data point represents mean ± SEM of 4–8 experiments.

hours and additional increase at 48 hr. Based on the studies mentioned above of the effect of PKC on the cotransporter, we examined the role of PKC on the effect of respiratory acidosis on the cotransporter at 5 min and 4 hr. Respiratory acidosis was associated with an increase in total and particulate fraction PKC activity at both 5 min and 4 hr. In addition, the PKC inhibitor, calphostin C, or PKC depletion prevented the increase in Na-HCO_3 cotransporter activity supporting an important role of PKC in the adaptation to respiratory acidosis. Both calphostin C and PKC depletion did not affect baseline activity of the cotransporter. The concentration of calphostin C used does not inhibit other kinases [24] and the protocol used to induce depletion caused 80% reduction in PKC activity without affecting cell viability [11].

We have recently shown the presence of receptor and nonreceptor activated PTK in basolateral membranes of the rabbit kidney [13]. PTK seems to play a role in the adaptation of the Na-H antiporter in metabolic acidosis and also seems to modulate, at least in part, the effect of the cholinergic agent carbachol, insulin, and epidermal growth factor on the cotransporter. The two PTK inhibitors alone blocked the increase in phosphorylation of the synthetic substrate for PTK poly-GT [13]. These inhibitors also did not affect the baseline activity of the cotransporter but completely prevented the increase in cotransporter activity by respiratory acidosis at both 5 min and 4 hr. These results are compatible with the notion that PTK plays a role in the adaptive increase of the cotransporter in respiratory acidosis. The mechanism

Table 2. PKC activity

	Total activity (pmol/mg prot/min)	% of total	
		Cytosol	Particulate
Control	1244 ± 218	80.9 ± 5.7	19.1 ± 5.7
10% CO_2, 5 min	1758 ± 231	14.2 ± 6.5*	85.8 ± 6.5*
10% CO_2, 5 min +Calphostin C	980 ± 211	55.8 ± 10.0	44.2 ± 10.0

Each data point represents mean ± SEM of 4–5 experiments.
* $P < 0.001$, control cytosol *vs.* 10% CO_2 cytosol, control particulate *vs.* 10% CO_2 particulate.

whereby CO_2 affects PTK is not clarified by the present study. Additional experiments will be needed to determine whether CO_2 stimulation activates PTK.

The late effect of PKC activation to enhance Na-HCO_3 cotransporter appears to be mediated by enhanced synthesis of the cotransporter as suggested by the fact that protein synthesis inhibitors prevented the late but not the early effect of PMA [21]. We therefore studied the role of protein synthesis inhibitors on the stimulatory effect of respiratory acidosis at 5 min or 4 hr duration on the cotransporter. The cells were incubated with the inhibitors for 1 hr prior to exposure to respiratory acidosis. The inhibitors did not alter the baseline activity of the cotransporter at the concentration used. Both actinomycin D and cycloheximide prevented the effect of 4-hr respiratory acidosis. The effect of 5-min respiratory acidosis was not, as expected, blocked by actinomycin D or cycloheximide.

In conclusion, the results of the present studies demonstrate that respiratory acidosis is associated with increased activity of the Na-HCO_3 cotransporter. The increase in activity appears to be mediated by PKC and PTK dependent mechanism but the mechanism of interaction between the two systems was not clarified by the present studies. Future studies will be needed to define the interaction between PKC and PTK. The late effect of respiratory acidosis appears to be dependent on protein synthesis.

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References

1. Akiba, T., Rocco, V.K., Warnock, D.G. 1987. Parallel adaptation of the rabbit renal cortical sodium/proton antiporter and sodium/bicarbonate cotransporter in metabolic acidosis and alkalosis. *J. Clin. Invest.* **80:**308–315
2. Alpern, R.J. 1985. Mechanism of basolateral membrane $H^+/OH^-/HCO_3^-$ transport in the rat proximal convoluted tubule. *J. Gen. Physiol.* **86:**613–636
3. Arruda, J.A.L., Ruiz, O.S. 1991. Adaptation to acid-base disorders: Insight into the mechanisms as derived from vesicle studies. *Seminars Nephrol.* **11:**65–72
4. Arruda, J.A.L., Wang, L.J., Pahlavan, P., Ruiz, O.S. 1993. Glucocorticoid and the renal Na-H antiporter. Role in respiratory acidosis. *Regulatory Peptides* **48:**329–336
5. Baum, M., Moe, O.W., Gentry, D.L., Alpern, R.J. 1994. Effect of glucocorticoids on renal Na/H exchangers. *Am. J. Physiol.* **267:**F437–F442
6. Cogan, M.G. 1984. Chronic hypercapnia stimulates proximal bicarbonate reabsorption in the rat. *J. Clin. Invest.* **74:**1942–1947
7. Gazit, A., Yaish, P., Gilon, C., Levitzki, A. 1989. Tyrophostins I: Synthesis and biological activity of protein tyrosine kinase inhibitors. *J. Med. Chem.* **32:**2344–2352
8. House, C., Kemp, B.E. 1987. Protein kinase C contains a pseudosubstrate prototype in its regulatory domain. *Science* **238:**1726–1728
9. Khan, A., Dolson, G.M., Hise, M.K., Bennett, S.C., Weinman, E.J. 1985. Parathyroid hormone and dibutyrl cAMP inhibits Na/H exchange in renal brush border vesicles. *Am. J. Physiol.* **248:**F212–F218
10. Kniaz, D., Pahlavan, P., Valaitis, D., Arruda, J.A.L. 1991. High affinity binding sites for VIP in renal cortical membranes: Possible role of VIP in renal transport. *Kidney Int.* **39:**266–272
11. Pahlavan, P., Wang, L.J., Sack, E., Arruda, J.A.L. 1993. Role of protein kinase C in the adaptive increase in Na-H antiporter in respiratory acidosis. *J. Am. Soc. Nephrol.* **4:**1079–1086
12. Preisig, P.A., Alpern, R.J. 1991. Increased Na/H antiporter and Na/3HCO_3 symporter activities in chronic hyperfiltration. *J. Gen. Physiol.* **97:**195–217
13. Qiu, Y.Y., Cardoso, L.R., Wang, L.J., Ruiz, O.S., Arruda, J.A.L. 1995. Tyrosine kinase (TK) modulation of the renal Na-HCO_3 cotransporter. *J. Am. Soc. Nephrol.* **6:**313 (*Abstr.*)
14. Roos, A., Boron, W.F. 1991. Intracellular pH. *Physiol. Rev.* **61:**296–434
15. Ruiz, O.S., Arruda, J.A.L., Talor, Z. 1989. Na-HCO_3 cotransport and Na-H antiporter in chronic respiratory acidosis and alkalosis. *Am. J. Physiol.* **256:**F414–F420
16. Ruiz, O.S., Arruda, J.A.L. 1992. Regulation of the renal Na-HCO_3 cotransporter by cyclic AMP on Ca-dependent protein kinases. *Am. J. Physiol.* **262:**F560–F565
17. Ruiz, O.S., Qiu, Y.Y., Wang, L.J., Arruda, J.A.L. 1995. Regulation of renal cortical Na-HCO_3 cotransporter. II. Role of G protein. *Am. J. Physiol.* **268:**F461–F467
18. Ruiz, O.S., Wang, L.J., Pahlavan, P., Arruda, J.A.L. 1995. Regulation of the renal Na-HCO_3 cotransporter: III. Presence and modulation by glucocorticoid in primary cultures of the proximal tubule. *Kidney Int.* **47:**1669–1676
19. Ruiz, O.S., Qiu, Y.Y., Wang, L.J., Arruda, J.A.L. 1995. Regulation of the renal Na-HCO_3 cotransporter: IV. Mechanism of the stimulatory effect of angiotensin II. *J. Am. Soc. Nephrol.* **6:**1202–1208
20. Ruiz, O.S., Qiu, Y.Y., Wang, L.J., Arruda, J.A.L. 1996. Regulation of the renal Na-HCO_3 cotransporter: V. Mechanism of the inhibitory effect of parathyroid hormone. *Kidney Int.* **49:**396–402
21. Ruiz, O.S., Wang, L.J., Bernardo, A., Arruda, J.A.L. 1996. Regulation of the renal Na-HCO_3 cotransporter: VI. Mechanism of the stimulatory effect of protein kinase C. *Kidney Int.* **49:**696–704
22. Saxena, R., Saksa, B.A., Fields, A.P., Ganz, M.B. 1993. Activation of Na/H exchanger in mesangial cells is associated with translocation of PKC isoforms. *Am. J. Physiol.* **265:**F53–F60
23. Talor, Z., Yang, W.C., Shuffield, J., Sack, E., Arruda, J.A.L. 1987.

Chronic hypercapnia enhances Vmax of Na-H antiporter of renal brush border membranes. *Am. J. Physiol.* **253:**F394–F400

24. Tamaoki, T., Nakano, H. 1990. Potent and specific inhibitors of protein kinase C of microbial origin. *Biotechnology* **8:**732–735
25. Tremblay, L., Beliveau, R. 1994. Tyrosine phosphorylation in plasma membranes of rat kidney cortex. *Am. J. Physiol.* **267:**F415–F422
26. Weinman, E.J., Shenolikar, S., Kahn, M. 1987. cAMP-associated inhibition of Na^+-H^+ exchanger in rabbit kidney brush border membranes. *Am. J. Physiol.* **252:**F19–F25
27. Weinman, E.J., Dubinsky, W.P., Fisher, K., Steplock, D., Dinh, W., Chang, L., Shenolikar, S. 1988. Regulation of reconstituted Na^+/H^+ exchanger by calcium-dependent protein kinase. *J. Membrane Biol.* **103:**237–244
28. Yamaji, Y., Amemiya, M., Cano, A., Preisig, P.A., Tyler, M.R., Moe, O.W., Alpern, R.J. 1995. Overexpression of csk inhibits acid-induced activation of NHE-3. *Proc. Natl. Acad. Sci. USA* **92:**6274–6278
29. Yang, W.C., Arruda, J.A.L., Talor, Z. 1987. Na-H antiporter in post-hypercapneic state. *Am. J. Physiol.* **253:**F833–F840
30. Yasuda, I., Kishimato, A., Tanaka, S., Tominaga, M., Sakurai, A., Nishizuka, Y. 1990. A synthetic peptide substrate for selective assay of protein kinase C. *Biochem. Biophys. Res. Comm.* **166:**1220–1227