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Overexpression of csk inhibits acid-induced activation of NHE-3

(Na^+/H^+ antiporter/src/acidosis/proximal tubule)

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ABSTRACT **Opossum kidney OKP cells express an apical membrane Na^+/H^+ antiporter that is encoded by *NHE-3* (for Na^+/H^+ exchanger 3) and is similar in many respects to the renal proximal tubule apical membrane Na^+/H^+ antiporter. Chronic incubation of OKP cells in acid medium for 24 hr increases Na^+/H^+-antiporter activity and NHE-3 mRNA abundance. The increase in Na^+/H^+-antiporter activity was not prevented by H7, a protein kinase C/protein kinase A inhibitor, but was prevented by herbimycin A, a tyrosine kinase inhibitor. Incubation of cells in acid medium increased c-src activity, and this was inhibited by herbimycin A. To determine the role of the src family of nonreceptor protein-tyrosine kinases, Csk (for *c*arboxyl-terminal *src* *k*inase), a physiologic inhibitor of these kinases, was overexpressed in OKP cells. In three clones overexpressing csk, acid-induced increases in Na^+/H^+-antiporter activity and NHE-3 mRNA abundance were inhibited. In these clones, inhibition of acid activation of Na^+/H^+-antiporter activity paralleled inhibition of acid activation of c-src. Neither herbimycin A nor overexpression of csk inhibited dexamethasone-induced increases in Na^+/H^+-antiporter activity. These studies show that decreases in pH activate c-src and that the src family nonreceptor protein-tyrosine kinases play a key role in acid activation of NHE-3.**

Chronic metabolic acidosis causes an increase in the activity of the renal proximal tubule apical membrane Na^+/H^+ antiporter that persists after the transporter is removed from the acidotic environment (1–3). This effect represents a direct effect of pH, in that chronic incubation of cultured proximal tubule cells in acid medium leads to increases in the activities and mRNA abundance of two Na^+/H^+-antiporter isoforms, NHE-1 and NHE-3 (Na^+/H^+ exchangers 1 and 3) (4–7). While NHE-1 mRNA encodes the basolateral membrane antiporter that likely effects housekeeping functions in the proximal tubule, NHE-3 mRNA encodes the apical membrane Na^+/H^+ antiporter that mediates transepithelial bicarbonate absorption (see *Discussion*). Thus, activation of NHE-3 represents a key step in increased transepithelial bicarbonate absorption in acidosis.

Incubation of MCT cells, a simian virus 40-transformed mouse proximal tubule cell line, in acid medium leads to transcriptional activation of immediate early genes including c-*fos*, c-*jun*, *junB*, and *egr-1* (8). This effect is not blocked by inhibiting protein kinase C or preventing increases in cell Ca^{2+}, but is blocked by tyrosine kinase inhibitors. Incubation of these cells in acid medium leads to increased phosphotyrosine content of 60- to 70- and 120-kDa proteins and activation of c-src (9). The purpose of the present studies was to determine whether tyrosine kinase pathways, and c-src in particular, contribute to acid activation of NHE-3.

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MATERIALS AND METHODS

Materials. All chemicals were purchased from Sigma, unless otherwise noted as follows: penicillin and streptomycin, from BioWhittaker; culture media, herbimycin A, lipofectin, and G-418, from GIBCO/BRL; monoclonal anti-src antibody 327, from Oncogene Science; protein G-agarose, from Calbiochem; polyclonal anti-c-src antibody (SRC 2), from Santa Cruz Biotechnology (Santa Cruz, CA); enhanced chemiluminescence (ECL) kit, from Amersham; BCECF-AM [2′,7′-bis(2-carboxyethyl)-5(and 6)-carboxylfluorescein, acetoxymethyl ester], from Molecular Probes; phorbol 12-myristate 13-acetate (PMA) and H7, from LC Services (Woburn, MA); and [γ-^{32}P]ATP, from New England Nuclear.

Cell Culture. Opossum kidney OKP cells were passaged in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units of penicillin and 100 μg of streptomycin per ml (Pen/Strep) and 10% (vol/vol) fetal bovine serum. For experimentation, OKP cells were grown to confluence and serum-deprived in DMEM/Ham's F-12 medium, 1:1 (vol/vol) containing Pen/Strep for 2 days prior to study. To study the effect of chronic acid incubation, cells were incubated in control (pH 7.4) or acid (pH 7.0; titrated with HCl) medium for 24 hr. To study the effects of dexamethasone, cells were incubated in 0.1 μM dexamethasone or vehicle (ethanol) for 24 hr. Herbimycin A (1 μM) was added to cells 18 hr prior to study and control cells received vehicle (dimethyl sulfoxide). H7 was added to cells 1 hr prior to study, and control cells received vehicle (water).

Chicken csk cDNA in a pcDNAI expression vector (pcDNAI-csk) (10) and plasmid pSV2-neo were cotransfected at a ratio of 20:1 into OKP cells by using Lipofectin according to the manufacturer's instructions. Neomycin-resistant cells were selected by growth in medium containing G-418 at 400 μg/ml and were maintained in medium containing G-418 at 200 μg/ml. Clonal cell lines were isolated by limiting dilution. For experimentation, G-418 was removed at the time of serum deprivation, 2 days prior to study.

Na^+/H^+-Antiporter Activity. To measure Na^+/H^+-antiporter activity, cells were grown on glass coverslips, and cell pH was measured by using the pH-sensitive dye BCECF and calibrated with K^+/nigericin as described (4, 11). Na^+/H^+-antiporter activity was assayed as the initial rate of Na^+-dependent intracellular pH (pH_i) increase (dpH_i/dt) following an acid load in the absence of CO_2/HCO_3^- (12). Buffer capacity, measured as described (12), was not affected by any of the experimental maneuvers (data not shown); therefore, Na^+/H^+-antiporter activity is expressed as dpH_i/dt.

RNA Blotting. RNA was extracted by using a modification of the method of Chirgwin *et al.* (13) as described (8). Total

Abbreviations: NHE, Na^+/H^+ exchanger; PMA, phorbol 12-myristate 13-acetate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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RNA was size-fractionated by agarose-formaldehyde gel electrophoresis and was transferred to nylon filters (GeneScreen-Plus, New England Nuclear). Radiolabeled probes were synthesized from the appropriate cDNA by the random hexamer method with the following inserts: full-length c-fos (14), full-length OKP NHE-3 (7), and full-length rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH; ref. 15). Prehybridization, hybridization, washing, and exposure to film were performed as described (8).

c-src Assay. To examine the effect of acid on c-src activity, medium was changed 2 hr prior to the experiment to insure a pH of 7.4. At the time of experiment, cells were incubated at pH 7.4 or 7.0. Cells were then washed three times with phosphate-buffered saline (PBS) at 4°C, lysed with radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl/50 mM Tris, pH 7.4/50 mM β-glycerophosphate/50 mM NaF/1 mM sodium orthovanadate/2.5 mM EDTA/5 mM EGTA/0.5 mM dithiothreitol/1% Triton X-100/0.5% sodium deoxycholate/0.1% SDS/5 μg each of aprotinin and leupeptin per ml/2 μg of pepstatin per ml), scraped with a rubber policeman, incubated for 45 min at 4°C with rocking, and centrifuged at 2300 × *g* for 15 min at 2°C (Beckman Optima TLX, TLA-100.3 fixed-angle rotor). The supernatant was diluted to 1 mg of protein per ml (Bradford method, Bio-Rad), and 300 μl was incubated with 2 μg of anti-src monoclonal antibody 327 for 1.5 hr on a rocker at 4°C. Fifteen microliters of protein G-agarose was then added, and the mixture was incubated for 1 hr. Agarose beads were pelleted at 10,000 × *g* for 30 sec at 4°C and washed with RIPA buffer 4 times and then twice with 150 mM NaCl/20 mM sodium Hepes, pH 7.4. The pellet was then suspended in 500 μl of the NaCl solution, divided into three aliquots, and pelleted.

c-src activity was measured as enolase kinase activity. Two aliquots of the pellet were heated to 25°C for 1.5 min and then suspended in 10 μl of reaction buffer {150 mM NaCl/20 mM sodium Hepes (pH 7.4)/30 mM $MgCl_2$/0.01 mM ATP/0.3 mg of enolase per ml/5 μCi (185 kBq) of [γ-^{32}P]ATP} at 25°C for 5 min with rotation. The reaction was stopped by addition of 10 μl of 2× SDS loading buffer (1× = 5 mM Tris·HCl, pH 6.8/1% SDS/10% glycerol/1% 2-mercaptoethanol), and the sample was boiled for 10 min. The sample was then size-fractionated by SDS/PAGE on a 7.5% gel, and the gel was stained with Coomassie blue, dried, and exposed to film. To determine the total amount of c-src in the precipitate, c-src abundance was measured on one of the aliquots by immunoblot (Western blot). The precipitate was suspended in SDS loading buffer, boiled for 10 min, subjected to SDS/PAGE on 7.5% gels, and electrophoretically transferred to nitrocellulose. After blocking with 5% powdered milk and 0.05% Tween 20 in TBS (0.9% NaCl/20 mM Tris, pH 7.4) for 2 hr, blots were probed with a 1:500 dilution of rabbit anti-src antibody (SRC 2) in TBS containing 2% (vol/vol) bovine serum albumin and 0.05% Tween 20 for 1 hr. Blots were washed twice in TBS containing 5% powdered milk and 0.05% Tween 20 for 15 min each and twice in PBS containing 0.5% Tween 20 for 15 min before incubation with a 1:2500 dilution of horseradish peroxidase-labeled anti-rabbit IgG in PBS containing 0.5% Tween 20 for 1 hr; bands were visualized by enhanced chemiluminescence. Enolase kinase activity, measured on two aliquots, was quantitated by densitometry; results were averaged and corrected for differences in c-src abundance. Results are reported as means ± SEM.

RESULTS

Acid Activation of NHE-3 Is Not Mediated by Protein Kinase C. Inhibition of protein kinase C prevents acid activation of NHE-1 (16). To determine the role of protein kinase C in acid activation of NHE-3, OKP cells, which express an apical membrane Na^+/H^+ antiporter encoded by *NHE-3* (7), were exposed to 40 μM H7, a protein kinase C/protein kinase A inhibitor (17). This concentration was used because it inhibited PMA-induced increases in c-fos mRNA in OKP cells (Fig. 1 *Upper*). Incubation of OKP cells in acid medium for 24 hr increased Na^+/H^+-antiporter activity by 31% in the absence of H7 and by 33% in the presence of H7 (Fig. 1 *Lower*).

Herbimycin A Inhibits Acid-Induced Activation of NHE-3. To determine whether the effect of acid on NHE-3 activity was mediated by tyrosine kinase pathways, cells were pretreated with 1 μM herbimycin A. Incubation in acid medium for 24 hr caused a 31% increase in Na^+/H^+-antiporter activity, which was completely inhibited in cells treated with herbimycin (Fig. 2). However, herbimycin A decreased basal Na^+/H^+-antiporter activity, which either could indicate tyrosine kinase regulation of basal activity or could represent a nonspecific effect. To differentiate between these possibilities, we examined the effect of herbimycin A on dexamethasone-induced stimulation of Na^+/H^+-antiporter activity, which likely is independent of tyrosine kinase pathways. Treatment with 0.1 μM dexamethasone for 24 hr caused a 31% increase in Na^+/H^+-antiporter activity (Fig. 3). Herbimycin A again caused a decrease in basal Na^+/H^+-antiporter activity but did not inhibit dexamethasone-induced activation of the Na^+/H^+ antiporter.

Incubation of MCT cells in acid medium causes an increase in c-src activity (9). Therefore, we examined whether c-src was activated by acid in OKP cells and whether this effect was inhibited by herbimycin A. Incubation in acid medium for 1.5 min increased c-src activity by 64%; there was no effect in the presence of herbimycin A (Fig. 4). Thus, herbimycin A inhibits acid-induced activation of c-src and NHE-3 but does not inhibit dexamethasone-induced activation of NHE-3.

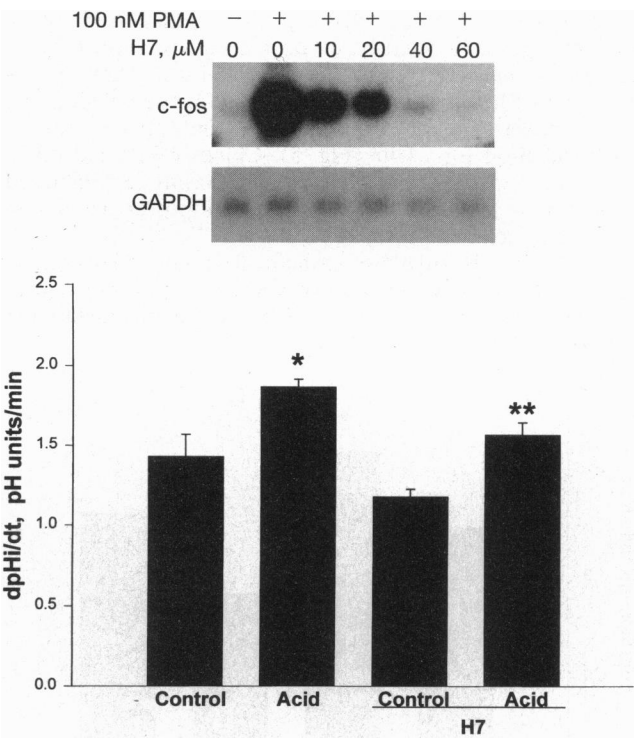

FIG. 1. Acid activation of the Na^+/H^+ antiporter is not inhibited by H7. (*Upper*) Northern blot showing the effect of H7 on PMA-induced c-fos expression. Cells were treated with 100 nM PMA for 30 min in the presence of various concentrations of H7. (*Lower*) Effect of 24 hr of incubation in control (pH 7.4) or acid (pH 7.0) medium on Na^+/H^+-antiporter activity in the absence or presence of 40 μM H7. Na^+/H^+-antiporter activity is plotted as dpH_i/dt, the initial rate of increase in cell pH upon Na addition. *, $P < 0.05$ vs. control; **, $P < 0.005$ vs. control; $n = 7$ for control, control/H7, and acid/H7; $n = 5$ for acid.

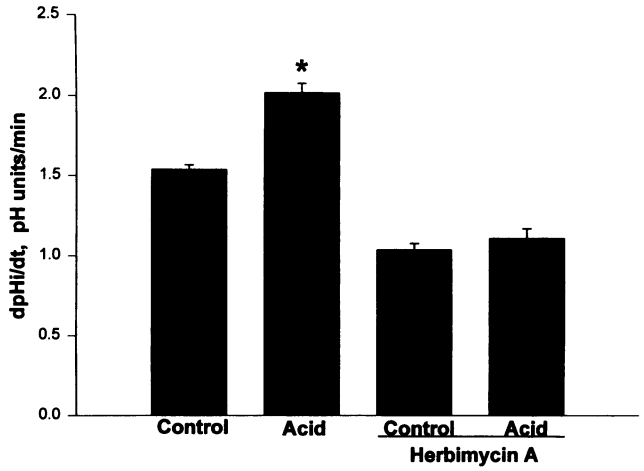

FIG. 2. Herbimycin A inhibits acid activation of the Na^+/H^+ antiporter. OKP cells were incubated in control or acid medium for 24 hr in the absence or presence of 1 μM herbimycin A. *, $P < 0.001$ vs. control; $n = 14$ for control and acid/herbimycin A, 16 for control/herbimycin A, and 13 for acid.

Overexpression of csk Inhibits Acid-Induced Activation of NHE-3. csk (*c*-terminal *s*rc *k*inase) phosphorylates tyrosine–527 of c-src and the equivalent tyrosine of other members of the src family of nonreceptor protein-tyrosine kinases, a process that serves as a physiologic inhibitor of these kinases (10, 18–23). Overexpression of csk was utilized to examine the effect of inhibiting src family nonreceptor protein-tyrosine kinases on acid-induced activation of the Na^+/H^+ antiporter. Three clones stably transfected with chicken csk, csk10, csk18, and csk23, were selected based on the highest expression of chicken csk mRNA by RNA blot. Pooled cells stably transfected with a neomycin-resistance gene alone demonstrated a 50% increase in Na^+/H^+-antiporter activity in response to acid incubation for 24 hr (Fig. 5). Clones csk10 and csk23 showed no response to acid, whereas csk18 cells demonstrated a small 18% stimulation of Na^+/H^+-antiporter activity in response to acid.

As a control, dexamethasone-induced stimulation of NHE-3 activity was examined in the two csk clones that were unresponsive to acid, csk10 and csk23. Fig. 6 shows that incubation

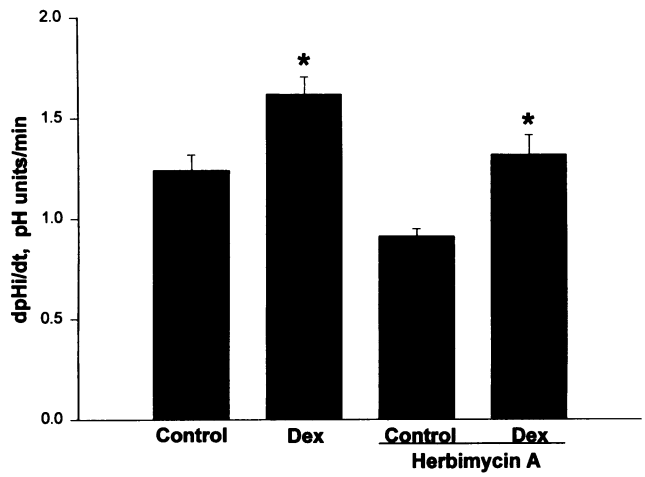

FIG. 3. Herbimycin A does not inhibit glucocorticoid-induced activation of the Na^+/H^+ antiporter. OKP cells were incubated in vehicle (Control) or 0.1 μM dexamethasone (Dex) for 24 hr in the absence or presence of 1 μM herbimycin A. *, $P < 0.05$ vs. control; $n = 6$ for control and dexamethasone, and $n = 7$ for control/herbimycin A and dexamethasone/herbimycin A.

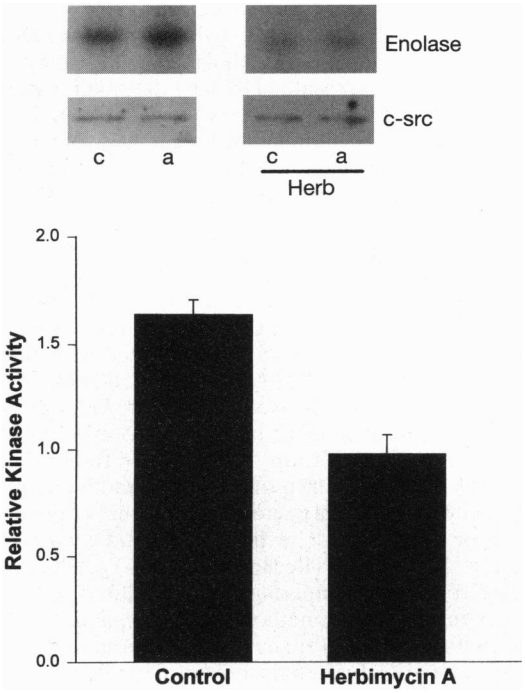

FIG. 4. Herbimycin A inhibits acid-induced activation of c-src. OKP cells were incubated in control or acid medium for 1.5 min in the absence (Left) or presence (Right) of 1 μM herbimycin A (Herb). c-src activity was measured as enolase kinase activity following immunoprecipitation. (*Upper*) Typical experiment showing enolase phosphorylation and c-src abundance. Lanes: c, control; a, acid. (*Lower*) Summary of four experiments. c-src activity is expressed as the ratio of activity in cells exposed to pH 7.0 versus 7.4.

with 0.1 μM dexamethasone for 24 hr increased Na^+/H^+-antiporter activity in both csk10 and csk23 cells. Thus, the observed inhibition is specific for acid.

Incubation of OKP cells in acid medium for 24 hr leads to a 2-fold increase in NHE-3 mRNA abundance (7). We next examined whether overexpression of csk inhibited this response. Incubation of neomycin-resistant cells in acid medium for 24 hr caused a 2-fold increase in NHE-3 mRNA (Fig. 7).

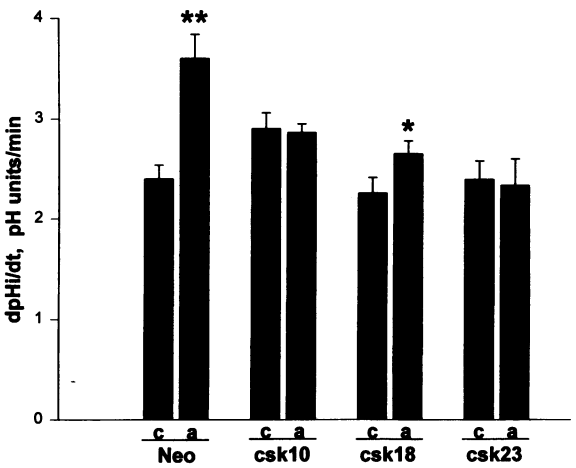

FIG. 5. Overexpression of csk inhibits acid activation of the Na^+/H^+ antiporter. Na^+/H^+-antiporter activity is plotted in pooled cells transfected with the neomycin-resistance vector alone (Neo) or in three clones overexpressing csk. Cells were incubated in control (bars c) or acid (bars a) medium for 24 hr. *, $P < 0.05$ vs. control; **, $P < 0.001$ vs. control; n experiments with control and acid medium, respectively, are 8 and 9 for neo, 13 and 14 for csk10, 14 and 15 for csk18, and 13 and 13 for csk23.

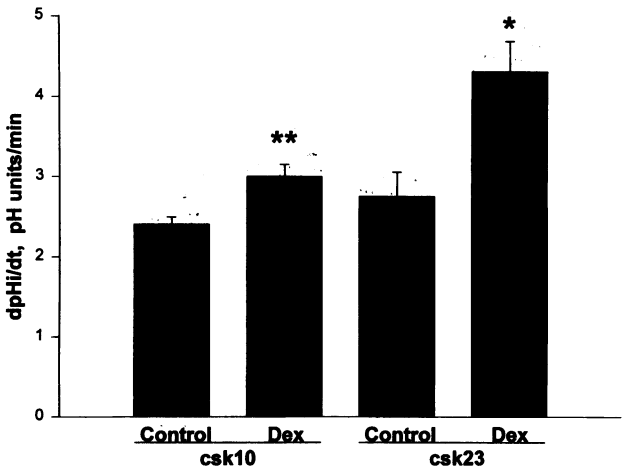

FIG. 6. Overexpression of csk does not inhibit glucocorticoid-induced activation of the Na^+/H^+ antiporter. Cells were incubated with 0.1 μM dexamethasone (Dex) or vehicle (Control) for 24 hr. Na^+/H^+-antiporter activity is plotted in two clonal cell lines overexpressing csk. *, $P < 0.05$ vs. control; **; $P < 0.01$ vs. control; n incubations with vehicle or dexamethasone, respectively, are 6 and 6 for csk10 and 4 and 5 for csk23.

This response was inhibited in all three clonal cell lines overexpressing csk, although there remained a tendency for a small effect of acid.

We next examined whether overexpression of csk inhibited acid activation of c-src. In preliminary studies, c-src activation by acid was intact in the csk-overexpressing clones at 1.5 min. Since the response to acid was a chronic response, csk-overexpressing cells could have an impaired response at later time points. Therefore, cells were incubated in control or acid

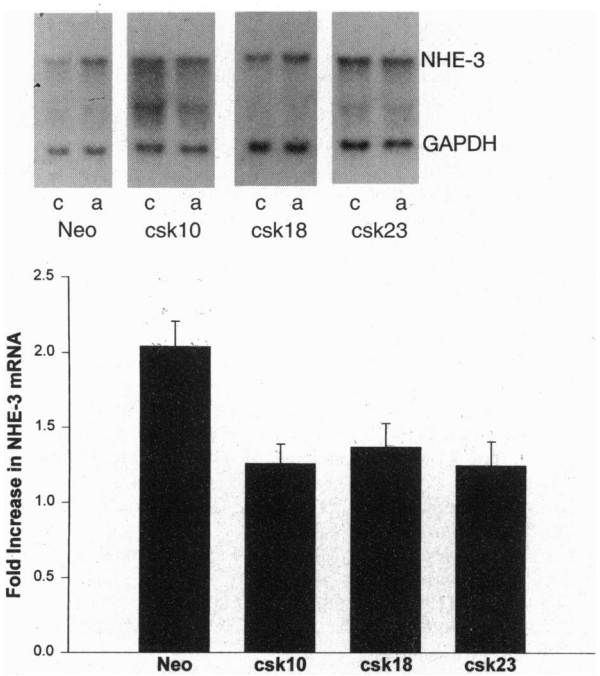

FIG. 7. Overexpression of csk inhibits acid-induced increases in NHE-3 mRNA abundance. The effect of 24 hr of incubation in acid (pH 7.0) medium on NHE-3 mRNA abundance was examined in pooled neomycin-resistant cells and in three clonal cell lines overexpressing csk. (*Upper*) Typical experiment. Lanes: c, control; a, acid. (*Lower*) Pooled results. The acid-induced increase in NHE-3 mRNA abundance normalized for GAPDH mRNA abundance is plotted. n = 8 (Neo) and 5 (csk10, csk18, and csk23).

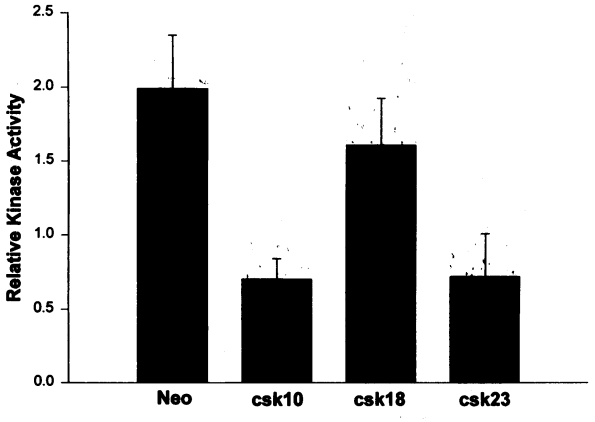

FIG. 8. Overexpression of csk inhibits acid activation of c-src. Pooled neomycin-resistant cells and three clonal cell lines overexpressing csk were incubated in control or acid medium for 10 min. c-src activity was measured as enolase kinase activity and is expressed as the ratio of activity in acid versus control cells. Data summarize three experiments.

medium (pH 7.4 vs. 7.0) for 10 min, and c-src activity was measured. In pooled cells transfected with the neomycin-resistance gene alone, acid increased c-src activity by 99% (Fig. 8). By contrast, there was a tendency for acid to decrease c-src activity in csk10 and csk23 cells. In csk18 cells acid incubation increased c-src activity by 61%. The less complete inhibition of c-src activation by acid in csk18 cells corresponds to the partial stimulation of NHE-3 by acid in these cells (Fig. 5).

DISCUSSION

Chronic metabolic acidosis causes a series of adaptations in cellular function that all tend to return blood pH to normal values. In the renal proximal tube, these adaptations include increases in the activities of the apical membrane Na^+/H^+ antiporter and Na^+ citrate cotransporter, the basolateral membrane $Na^+/HCO_3^-/CO_3^{2-}$ cotransporter, and a number of enzymes responsible for ammonia and citrate metabolism (1–3, 24). In addition, chronic metabolic acidosis causes enhanced renal growth, enhanced muscular protein catabolism, bone dissolution, and adaptations in the renal cortical collecting duct that modulate relative rates of H^+ and HCO_3^- secretion (24). The specific mechanisms by which acidosis brings about these diverse cellular effects have not been elucidated.

NHE-1 is a ubiquitous Na^+/H^+ antiporter that subserves housekeeping functions such as cell pH regulation and regulation of growth (25). NHE-3 is believed to encode the proximal tubule apical membrane Na^+/H^+ antiporter that mediates transepithelial H^+/HCO_3^- transport, based on the observations that (*i*) NHE-3 protein is localized to the proximal tubule apical membrane by immunohistochemistry; (*ii*) NHE-3 mRNA is expressed predominantly in renal cortex and small intestine; (*iii*) NHE-3 is amiloride resistant, similar to the proximal tubule apical membrane Na^+/H^+ antiporter; (*iv*) NHE-3 mRNA abundance and apical membrane Na^+/H^+ antiporter activity are increased by glucocorticoids; and (*v*) the ontogeny of apical membrane Na^+/H^+ antiporter activity parallels that of NHE-3 protein and mRNA abundance (26–33). In cultured proximal tubule cells, incubation in acid medium for 24–48 hr leads to increases in NHE-1 and NHE-3 activity and mRNA abundance (4–7).

Incubation of MCT cells in acid medium leads to transcriptional activation of immediate early genes, including c-*fos*, c-*jun*, *junB*, and *egr-1*, and increases in protein-tyrosine phosphorylation (8, 34). Immediate early gene activation is not blocked by inhibition of protein kinase C but is prevented by inhibition of tyrosine kinase pathways (8). In the present

studies, herbimycin A, a tyrosine kinase inhibitor, inhibited acid activation of NHE-3 activity. This effect was shown to be specific in that dexamethasone-induced increases in NHE-3 activity were not inhibited. H7, a protein kinase C/protein kinase A inhibitor (17), had no effect on acid-induced activation of NHE-3. The concentration used, 40 μM, is well above the K_i for protein kinase C and was shown to inhibit phorbol ester-induced activation of c-fos. Thus, these studies suggest that acid activation of NHE-3, similar to acid activation of immediate early gene expression, is mediated by tyrosine kinase pathways. By contrast, acid activation of NHE-1 is mediated by protein kinase C (16).

Decreases in intracellular pH activate c-src, a nonreceptor protein-tyrosine kinase, in MCT (9) and OKP cells. Herbimycin A inhibited acid activation of c-src. To examine the role of the src family nonreceptor protein-tyrosine kinases, we overexpressed csk in OKP cells. csk phosphorylates tyrosine-527 of c-src (10, 18) and equivalent tyrosines in other members of the src family (19–21). This serves the physiologic role of maintaining these nonreceptor protein-tyrosine kinases inactive (22, 23). Overexpression of csk in three clones inhibited acid-induced activation of NHE-3 to an extent that paralleled inhibition of acid-induced activation of c-src. Once again, the effect was shown to be specific in that overexpression of csk did not inhibit dexamethasone-induced increases in NHE-3 activity. Acid-induced increases in NHE-3 mRNA abundance were also inhibited.

These studies show that acid-induced activation of the src family nonreceptor protein-tyrosine kinases is required for acid-induced activation of NHE-3. However, the specific role played by these kinases is not yet clear. One possible role is to increase immediate early gene expression, which secondarily results in NHE-3 activation. In that immediate early gene activation rarely defines specificity of a process (35), activation of a src family kinase may also be required at a second step for increases in NHE-3. Lastly, it may be possible that activation of c-src and immediate early genes by acid is permissive for other mechanisms to activate NHE-3. This may be analogous to activation of these pathways by ultraviolet irradiation (36). In addition, acid-induced activation of src family nonreceptor protein-tyrosine kinases may play an important role in acid-induced regulation of other proximal tubule functions or in effects of acidosis in other cells.

The family of src-related nonreceptor protein-tyrosine kinases includes nine members. c-src and c-yes expression have been demonstrated in the proximal tubule (37, 38). The present studies do not distinguish which of these kinases are key in acid-induced regulation. An important role for c-src is suggested in that c-src activity is increased by decreases in intracellular pH of <0.1 pH units (9). However, it is possible that other members of the src family are also activated by acid and can also effect increases in NHE-3 activity. Redundancy among src family nonreceptor protein-tyrosine kinases has been suggested by the pausity of phenotypic abnormalities found in animals in which individual src family genes have been disrupted (39, 40).

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1. Cohn, D. E., Klahr, S. & Hammerman, M. R. (1983) *Am. J. Physiol.* **245,** F217–F222.
2. Tsai, C. J., Ives, H. E., Alpern, R. J., Yee, V. J., Warnock, D. G. & Rector, F. C., Jr. (1984) *Am. J. Physiol.* **147,** F339–F343.
3. Kinsella, J., Cujdik, T. & Sacktor, B. (1984) *J. Biol. Chem.* **259,** 13224–13227.
4. Horie, S., Moe, O., Tejedor, A. & Alpern, R. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4742–4745.
5. Igarashi, P., Freed, M. I., Ganz, M. B. & Reilly, R. F. (1992) *Am. J. Physiol.* **263,** F83–F88.
6. Moe, O. W., Miller, R. T., Horie, S., Cano, A., Preisig, P. A. & Alpern, R. J. (1991) *J. Clin. Invest.* **88,** 1703–1708.
7. Amemiya, M., Yamaji, Y., Cano, A., Moe, O. W. & Alpern, R. J. (1995) *Am. J. Physiol.*, in press.
8. Yamaji, Y., Moe, O. W., Miller, R. T. & Alpern, R. J. (1994) *J. Clin. Invest.* **94,** 1297–1303.
9. Yamaji, Y., Cano, A., Miller, R. T., Moe, O. W. & Alpern, R. J. (1994) *J. Am. Soc. Nephrol.* **5,** 266 (abstr.).
10. Sabe, H., Knudsen, B., Okada, M., Nada, S., Nakagawa, H. & Hanafusa, H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2190–2194.
11. Alpern, R. J. (1985) *J. Gen. Physiol.* **86,** 613–636.
12. Cano, A., Preisig, P. & Alpern, R. J. (1993) *J. Clin. Invest.* **92,** 1632–1638.
13. Chirgwin, J. J., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
14. Curran, T., Gordon, M. B., Rubino, K. L. & Sambucetti, L. C. (1987) *Oncogene* **2,** 79–84.
15. Fort, P., Marty, L., Piechaezyk, M., El Sabrouty, S., Dani, C., Jeanteur, P. & Blanchard, J. M. (1985) *Nucleic Acids Res.* **13,** 1431–1442.
16. Horie, S., Moe, O., Yamaji, Y., Cano, A., Miller, R. T. & Alpern, R. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5236–5240.
17. Hidaka, H. & Kobayashi, R. (1992) *Annu. Rev. Pharmacol. Toxicol.* **32,** 377–397.
18. Nada, S., Okada, M., MacAuley, A., Cooper, J. A. & Nakagawa, H. (1991) *Nature (London)* **351,** 69–72.
19. Okada, M., Nada, S., Yamanashi, Y., Yamamoto, T. & Nakagawa, H. (1991) *J. Biol. Chem.* **266,** 24249–24252.
20. Bergman, M., Mustelin, T., Oetken, C., Partanen, J., Flint, N. A., Amrein, K. E., Autero, M., Burn, P. & Alitalo, K. (1992) *EMBO J.* **11,** 2919–2924.
21. Ruzzene, M., James, P., Brunati, A. M., Donella-Deana, A. & Pinna, L. A. (1994) *J. Biol. Chem.* **269,** 15885–15891.
22. Imamoto, A. & Soriano, P. (1993) *Cell* **73,** 1117–1124.
23. Nada, S., Yagi, T., Takeda, H., Tokunaga, T., Nakagawa, H., Ikawa, Y., Okada, M. & Aizawa, S. (1993) *Cell* **73,** 1125–1135.
24. Alpern, R. J. (1995) *Kidney Int.* **47,** 1205–1215.
25. Sardet, C., Fafournoux, P. & Pouyssegur, J. (1991) *J. Biol. Chem.* **266,** 19166–19171.
26. Biemesderfer, D., Pizzonia, J., Exner, M., Reilly, R., Igarashi, P. & Aronson, P. S. (1993) *Am. J. Physiol.* **265,** F736–F742.
27. Tse, C. M., Brant, S. R., Walker, M. S., Pouyssegur, J. & Donowitz, M. (1992) *J. Biol. Chem.* **267,** 9340–9346.
28. Orlowski, J., Kandasamy, R. A. & Shull, G. E. (1992) *J. Biol. Chem.* **267,** 9331–9339.
29. Orlowski, J. (1993) *J. Biol. Chem.* **268,** 16369–16377.
30. Tse, C. M., Levine, S. A., Yun, C. H. C., Brant, S. R., Pouyssegur, J., Montrose, M. H. & Donowitz, M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9110–9114.
31. Baum, M., Moe, O. W., Gentry, D. L. & Alpern, R. J. (1994) *Am. J. Physiol.* **267,** F437–F442.
32. Yun, C. H. C., Gurubhagavatula, S., Levine, S. A., Montgomery, J. L. M., Brant, S. R., Cohen, M. E., Cragoe, E. J., Jr., Pouyssegur, J., Tse, C. M. & Donowitz, M. (1993) *J. Biol. Chem.* **268,** 206–211.
33. Baum, M., Biemesderfer, D., Gentry, D. & Aronson, P. (1994) *J. Am. Soc. Nephrol.* **5,** 247 (abstr.).
34. Yamaji, Y., Moe, O. W., Miller, R. T. & Alpern, R. J. (1993) *J. Am. Soc. Nephrol.* **4,** 506 (abstr.).
35. Chao, M. V. (1992) *Cell* **68,** 995–997.
36. Devary, Y., Gottlieb, R. A., Smeal, T. & Karin, M. (1992) *Cell* **71,** 1081–1091.
37. Sukegawa, J., Akatsuka, T., Sugawara, I., Mori, S., Yamamoto, T. & Toyoshima, K. (1990) *Oncogene* **5,** 611–614.
38. Chow, L. H. & Nord, E. P. (1993) *J. Am. Soc. Nephrol.* **3,** 484 (abstr.).
39. Soriano, P., Montgomery, C., Geske, R. & Bradley, A. (1991) *Cell* **64,** 693–702.
40. Stein, P. L., Vogel, H. & Soriano, P. (1994) *Genes Dev.* **8,** 1999–2007.