Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9110–9114, October 1993
Cell Biology

Functional characteristics of a cloned epithelial Na^+/H^+ exchanger (NHE3): Resistance to amiloride and inhibition by protein kinase C

CHUNG-MING TSE*†, SUSAN A. LEVINE*, C. H. CHRIS YUN*, STEVEN R. BRANT*, JACQUES POUYSSEGUR‡, MARSHALL H. MONTROSE*, AND MARK DONOWITZ*

*Departments of Medicine and Physiology, Gastroenterology Division, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205; and ‡Centre De Biochimie–Centre National de la Recherche Scientifique, Parc Valrose, 06108 Nice, France

Communicated by John W. Littlefield, May 18, 1993

ABSTRACT **We previously cloned an isoform Na^+/H^+ exchanger (NHE3), which was expressed only in intestine, kidney, and stomach. We show here the functional characteristics of NHE3 as a Na^+/H^+ exchanger by stably transfecting NHE3 cDNA into PS120 cells, a fibroblast cell line that lacks endogenous Na^+/H^+ exchangers. NHE3 was 39- and 160-fold more resistant to inhibition by amiloride and ethylisopropyl amiloride, respectively, than NHE1, the housekeeping Na^+/H^+ exchanger isoform. Although both exchangers were stimulated by serum, NHE3 was inhibited by phorbol 12-myristate 13-acetate (PMA), which stimulated NHE1. Mechanistically, serum and PMA stimulated NHE1 by an increase in the apparent affinity of the exchanger for intracellular H^+. In contrast, serum stimulated and PMA inhibited NHE3 by a V_{max} change. When NHE3 was stably expressed in Caco-2 cells, an intestinal epithelial cell line, NHE3 was functionally expressed in the apical membrane. Thus, NHE3 is a good candidate to be an epithelial brush border Na^+/H^+ exchanger. Furthermore, Na^+/H^+ exchangers can be rapidly regulated by mechanisms that change either the V_{max} or the affinity for intracellular H^+, depending on the Na^+/H^+ exchanger subtype.**

Mammalian Na^+/H^+ exchangers (NHEs) are plasma membrane proteins that catalyze the electroneutral exchange of Na^+ for H^+. Epithelial cells such as intestine and renal tubule cells have NHEs on both apical and basolateral membranes (1–4). Apical membrane Na^+/H^+ exchange is involved in Na^+ absorption and is distinguished from the basolateral housekeeping isoform as being relatively amiloride resistant (1–4) and being inhibited by protein kinases, including protein kinase C (4–7), although there have been some conflicting reports (8, 9).

Human NHE1 (10) is a glycoprotein of 815 amino acids and can be stimulated by growth factors. In intestinal and kidney epithelial cells, NHE1 was shown to localize to the basolateral membrane (11–13). The epithelial brush border NHE has not yet been identified. We and others recently cloned a cDNA encoding a putative NHE isoform, NHE3 (14, 15). NHE3 exhibits overall 41% amino acid identity with NHE1. Its message is expressed only in intestine, kidney, and stomach (14, 15), suggesting that NHE3 is an epithelial-specific NHE isoform. In this communication, we show that NHE3 has two functional characteristics that are unique to intestinal and renal epithelial brush border NHEs—i.e., amiloride resistance and protein kinase C inhibition (2–7). When NHE3 cDNA was stably expressed in Caco-2 cells, which endogenously have only a functional basolateral membrane NHE (NHE1) (16), NHE3 was functionally expressed in the apical membrane. These studies suggest that NHE3 is a candidate to be an epithelial brush border NHE isoform.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

Transfection. Full-length rabbit NHE3 cDNA (nucleotides −26 to +2537) was subcloned into pMAM-neo vector and stably transfected into PS120 cells by $CaPO_4$ precipitation (14). Transfected cells were selected by both G418 resistance and the acid suicide technique (11, 17, 18). A mixed cell population (called NHE3/PS120) was used for functional characterization. Similarly, NHE3 cDNA was transfected into Caco-2 cells at 30% confluency as described for PS120 cells. Transfectants were selected only by G418 resistance as Caco-2 cells have endogenous basolateral Na^+/H^+ exchange activity (16). A mixed cell population resistant to G418 (called NHE3/Caco-2) was used for analysis of functional apical Na^+/H^+ exchange activity. Both control (untransfected PS120 and Caco-2 cells) and transfected cells were maintained in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 25 mM $NaHCO_3$, 10 mM Hepes, penicillin (50 units/ml), streptomycin (50 μg/ml), and 10% fetal bovine serum (FBS) in a 5% CO_2/95% air incubator at 37°C. Caco-2 cell medium contained 1% nonessential amino acids.

Measurement of Na^+/H^+ Exchange. *Spectrofluorometry.* (*i*) PS120. Stably transfected NHE1/PS120 [rabbit NHE1 cDNA previously transfected into PS120 (11)] or NHE3/PS120 cells were grown to 70–80% confluent density on glass coverslips and serum starved for 17 h to arrest growth. At the start of the experiment, the culture medium was removed and the cells were washed with Na medium (130 mM NaCl/5 mM KCl/2 mM $CaCl_2$/1 mM $MgSO_4$/0.8 mM Na_2HPO_4/0.2 mM NaH_2PO_4/25 mM glucose/20 mM Hepes, pH 7.4). The cells were then incubated with the acetoxymethyl ester of 2′,7′-bis(2-carboxyethyl)-5-(and -6)carboxyfluorescein (BCECF) (5 μM) in Na medium for 60–90 min at 22°C and then washed three times with tetramethylammonium (TMA) medium (same as Na medium except equimolar NaCl was replaced with TMA chloride and NaOH was replaced with TMA hydroxide) to remove extracellular dye. The coverslip was mounted in a cuvette (19) and the cell surface was perfused at 37°C. Cells were pulsed with 30 mM NH_4Cl in TMA medium for 5–10 min, depending on the degree of acidification desired. Removal of NH_4Cl and perfusion with TMA chloride resulted in stable acidification of the cells. The cuvette was then perfused with Na medium, and pH recovery was measured. BCECF fluorescence was measured at 530 ± 10 nm in response to alternating excitation wavelengths of 500 ± 1 and 440 ± 1 nm. Autofluorescence-corrected fluorescence ratio values (500/440) were calculated every 3 s

Abbreviations: NHE, Na^+/H^+ exchanger; BCECF, 2′,7′-bis(2-carboxyethyl)-5-(and -6)carboxyfluorescein; TMA, tetramethylammonium; EIPA, ethylisopropylamiloride; PMA, phorbol 12-myristate 13-acetate; FBS, fetal bovine serum.

†To whom reprint requests should be addressed at: Gastroenterology Division, 918 Ross Research Building, The Johns Hopkins University, School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205.

during the experiment by a CompuAdd 286 computer. Each experiment was calibrated versus pH using the nigericin/high K method (20). (*ii*) Caco-2 cells. Caco-2 cells were seeded on Cyclopore membranes (Falcon; Becton Dickinson) glued over an aperture in a plastic coverslip and grown for 7–14 days (3–10 days postconfluence). The method of BCECF loading is as described above for PS120 cells. The coverslip was mounted in a cuvette designed to perfuse apical and basolateral surfaces independently (19). The cuvette was placed in an SPF-500C spectrofluorometer (SLM Aminco, Urbana, IL); each compartment was perfused at the same rate (1 ml/min) and thermostatted at 37°C. As described above for PS120 cells, Caco-2 cells were acidified by a NH_4Cl prepulse on both apical and basolateral membrane surfaces and pH recovery was monitored in response to Na^+ addition (130 mM) to either the apical or basolateral surfaces.

$^{22}Na^+$ uptake. NHE3/PS120 were grown to near confluence in 24-well plates. Cells were acidified with 40 mM NH_4Cl for 20 min. $^{22}Na^+$ influx was measured at 22°C by incubating acidified cells for 90 s with 2 mM $^{22}Na^+$, 1 mM ouabain, and various concentrations of amiloride or ethylisopropylamiloride (EIPA) as described (21). The $^{22}Na^+$ uptake into NHE3/PS120 was linear for at least 3 min (data not shown). EIPA-sensitive $^{22}Na^+$ influx was determined as the difference between $^{22}Na^+$ influx measured in the presence and absence of 300 μM EIPA, the former of which contributed <3% of the total $^{22}Na^+$ uptake into the cells.

RESULTS

Functional Characterization of NHE3. NHE3 message was found, by RNase protection assay, only in NHE3/PS120 cells and not in untransfected PS120 cells (data not shown). Fig. 1*A* shows a typical time course of Na^+-dependent (130 mM) pH_i recovery from an acid load measured at 37°C using NHE3/PS120 cells. Cells remained acidified at constant pH_i in TMA medium, but addition of Na medium initiated alkalinization. The latter could be inhibited 80–93% by addition of 3 mM amiloride, suggesting that NHE3 is a functional NHE. Addition of amiloride eliminated all Na^+-dependent pH_i changes ($dpH_i/dt = 0$) over the pH_i range 6.3–7.1, suggesting that Na^+/H^+ exchange was the only process contributing to Na^+-dependent pH_i recovery (in the absence of extracellular HCO_3^-) over this pH_i range. However, as shown in Fig. 1*A*, addition of amiloride at $pH_i > 7.1$ unmasked an acidification process activated at high pH_i values, as shown in rat mesangial cells (22). Further experiments were performed at pH_i values < 7.1 so that Na^+/H^+ exchange would be measured specifically.

Fig. 1*B* shows the concentration dependence of amiloride and EIPA inhibition of $^{22}Na^+$ influx into acid-loaded NHE3/PS120 cells. IC_{50} values for amiloride and EIPA are 39 and 8 μM, respectively. Since the K_m (Na^+) is 16 mM (data not shown), the K_i of NHE3 for amiloride and EIPA is extremely close to these IC_{50} values (23). These results contrast with those from NHE1, for which the K_i values for amiloride and EIPA are 1 μM and 50 nM, respectively (21, 24). Thus, compared to NHE1, NHE3 is 39-fold more resistant to amiloride and 160-fold more resistant to EIPA.

Regulation of NHE1 and NHE3 by Serum and Phorbol 12-Myristate 13-Acetate (PMA). In epithelial cells, protein kinase regulation of brush border Na^+/H^+ exchange is distinct from regulation of the basolateral isoform. In particular, protein kinase C inhibits only the brush border NHE (1, 4–7). Thus, regulation of NHE1 and NHE3 by addition of serum or PMA was studied. Initially, qualitative studies were performed by addition of serum or PMA at the steady-state pH_i as described (11). FBS caused intracellular alkalinization when added at the steady-state pH_i to NHE1/PS120 (Fig. 2*A*) or NHE3/PS120 (Fig. 2*B*). Similarly, when PMA (0.1 μM) was added to NHE1/PS120 (Fig. 2*C*) at the steady-state pH_i, it caused intracellular alkalinization. These results suggested that serum activated Na^+/H^+ exchange by both NHE1 and NHE3 and that PMA also activated NHE1 (1, 11, 25). In contrast, addition of PMA (0.1 μM) to NHE3/PS120 (Fig. 2*D*) at steady-state pH_i caused intracellular acidification, suggesting inhibition of Na^+/H^+ exchange and/or activation of the underlying acidification process (active at $pH_i > 7.1$) (22). However, PMA had no effect on this acidification process unmasked by amiloride and also did not cause cellular acidification at $pH_i < 7.1$ (data not shown). Thus, these results suggest a qualitative difference in regulation of NHE1 and NHE3 by PMA.

Based on these results, kinetic analyses of Na^+/H^+ exchange were performed to quantify effects specific to the exchangers and define mechanisms of transport regulation. The effects of serum on NHE1 and NHE3 were studied under

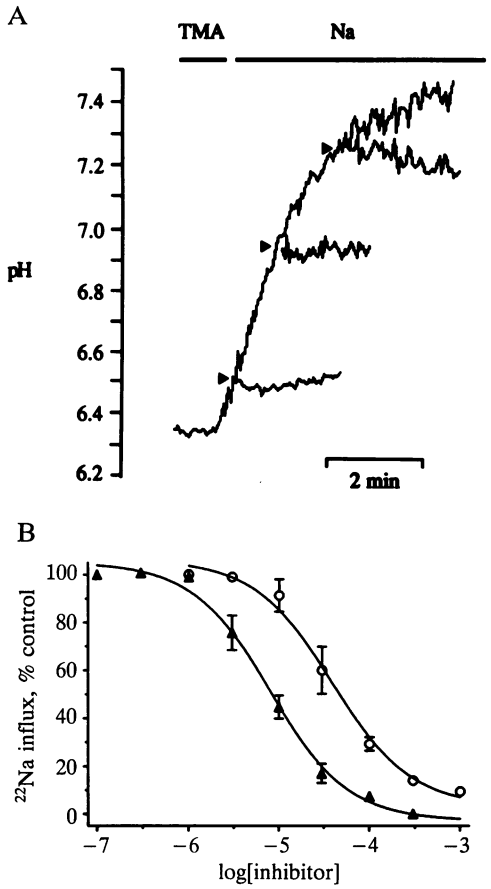

FIG. 1. Functional characterization of NHE3. (*A*) Inhibition of NHE3 by amiloride. NHE3/PS120 cells loaded with BCECF were studied spectrofluorometrically to measure intracellular pH (pH_i). This composite tracing from four representative experiments shows the effect of 3 mM amiloride added during the Na^+-dependent recovery from an acid load at pH 6.50, 6.90, and 7.25 (arrowheads indicate amiloride addition), compared with untreated control. Na^+/H^+ exchange was inhibited 80% at pH 6.50 and 93% at pH 6.90 in these experiments. Amiloride addition at pH 7.25 caused a decrease in pH_i, presumably due to unmasking of an underlying acidification process present at high pH_i. (*B*) Concentration–response curves for inhibition of $^{22}Na^+$ influx by amiloride and EIPA in NHE3/PS120. NHE3/PS120 were grown to near confluence in 24-well plates and $^{22}Na^+$ uptake into acid-loaded cells in the presence of various concentrations of amiloride (○) and EIPA (▲) was measured as described. Results are presented as means ± SEM of three experiments. Curves are nonlinear least-squares fits of the data assuming a single inhibitor binding site. From these curves, the IC_{50} values were calculated to be 8 and 39 μM for EIPA and amiloride, respectively.

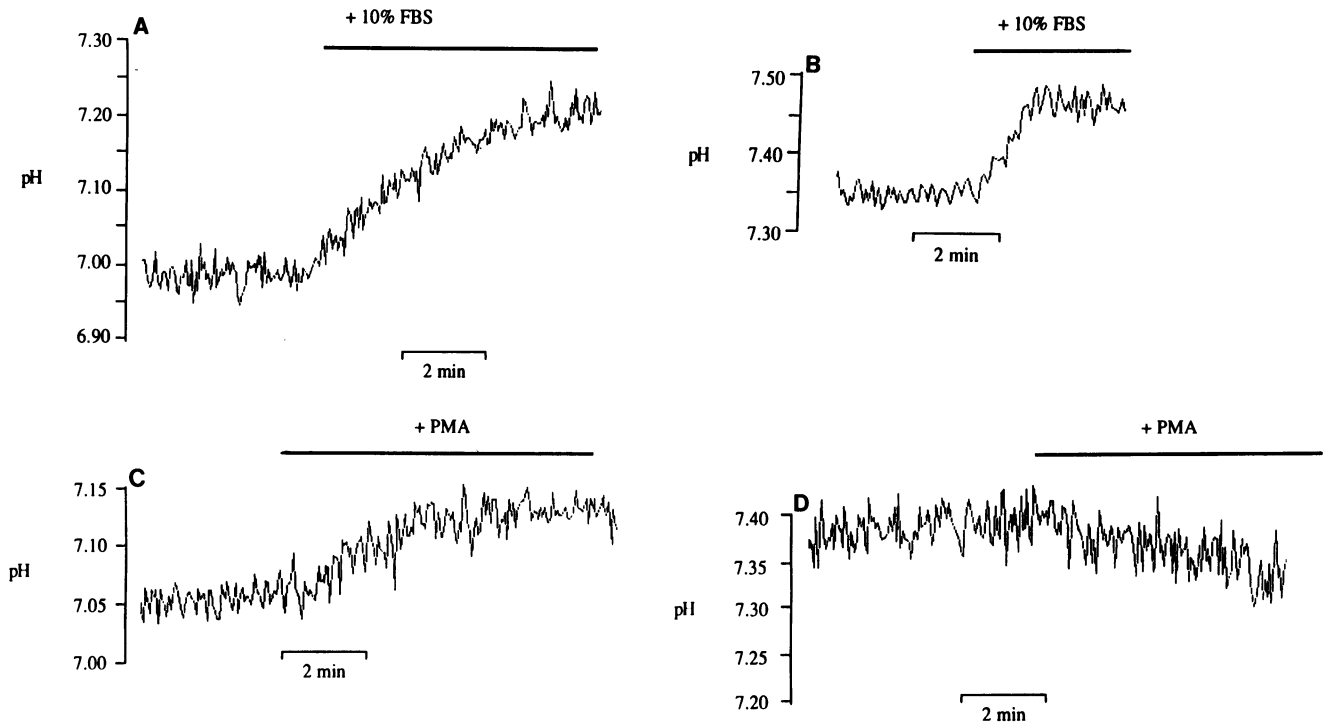

FIG. 2. Regulation of NHE1 and NHE3 by FBS or PMA. Experiments were performed with either NHE1/PS120 (*A* and *C*) or NHE3/PS120 (*B* and *D*). Tracings are representative of five experiments. Cells loaded with BCECF were acidified by a NH_4Cl prepulse (see Fig. 1) and then allowed to recover to steady-state pH_i in Na medium (130 mM). At the times indicated, perfusion was changed to Na medium containing either 10% FBS or 0.1 μM PMA. In appropriate experiments, calibration medium also contained 10% FBS to compensate for quenching due to the yellow color of the serum.

conditions where Na^+/H^+ exchange was the only mechanism responsible for the Na^+-dependent pH_i recovery (22)—i.e., $pH_i < 7.1$. Shown in Fig. 3*A* is the effect of 10% FBS on the initial rate of Na^+/H^+ exchange of NHE1. The Na^+/H^+ exchange rate had a non-Michaelis–Menten dependence on intracellular $[H^+]$ in both the presence and absence of serum with a Hill coefficient of 2.5 for intracellular H^+ (26). As also shown by others (e.g., see ref. 26), serum stimulated NHE1 activity by increasing the apparent affinity for intracellular H^+ $[K'(H^+)]$ without any significant change in V_{max}. Although FBS stimulated NHE3 in a qualitatively similar fashion to NHE1 (Fig. 2 *A* and *B*), a similar kinetic analysis showed that FBS stimulated NHE3 by increasing its V_{max} (increase of 110%) without significantly altering the apparent affinity for intracellular H^+ (Fig. 3*B*). The dependence of Na^+/H^+ exchange in NHE3/PS120 on intracellular $[H^+]$ also exhibited non-Michaelis–Menten kinetics, with a Hill coefficient of 2.4 in serum-stimulated cells and control cells. In contrast to the similar activation of both NHE1 and NHE3 by serum, they responded differently to PMA. While we previously showed that PMA stimulated NHE1 (11), PMA (0.1 μM) inhibited NHE3 (Figs. 2*D* and 3*C*). Kinetically, PMA inhibited NHE3 by decreasing its V_{max}, again with no significant change in the apparent affinity for intracellular H^+ (Fig. 3*C*). There was no change in the Hill coefficient between the control and PMA-inhibited cells. Since PMA inhibition of NHE3 could be reversed by 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H_7; 65 μM) (data not shown), the results suggest that protein kinase C is involved in this inhibitory pathway.

Apical Expression of NHE3 in Caco-2 Cells. To establish whether NHE3 can be functionally expressed in the apical domain in an epithelial cell, the NHE3/pMAM-neo construct was transfected into Caco-2 cells (11). Only the NHE3/Caco-2 cells, but not the control untransfected Caco-2 cells, express NHE3 message (data not shown). As shown in Fig. 4*A*, endogenous Na^+/H^+ exchange activity is restricted to the basolateral surface of untransfected Caco-2 cells (16). In contrast, NHE3/Caco-2 cells exhibited both apical and basolateral membrane Na^+/H^+ exchange activity (Fig. 4*B*). NHE3, which was expressed in the apical domain of Caco-2 cells, is also resistant to amiloride. Amiloride at 30 μM significantly inhibited Caco-2 basolateral Na^+/H^+ exchange; the basolateral amiloride effect was similar in NHE3/Caco-2 and untransfected Caco-2 cells [42% ± 6% inhibition of initial Na^+-dependent pH recovery rate ($n = 7$) in NHE3/Caco-2 vs. 44% ± 6% ($n = 3$) in untransfected Caco-2 cells]. In contrast, the apical NHE in NHE3/Caco-2 cells was not significantly inhibited by 30 μM amiloride (2% ± 9%; $n = 5$). These results suggest that when NHE3 is expressed in Caco-2 cells, it is functionally expressed in the apical domain of these intestinal epithelial cells and is amiloride resistant.

DISCUSSION

Although the identity of the epithelial apical NHEs is not known, three NHE isoforms (NHE2, NHE3, and NHE4), in addition to the basolateral housekeeping isoform NHE1, have recently been cloned and sequenced (14, 15, 27) with message of all three predominantly present in intestine and/or kidney (14, 15, 27). Antibodies to these isoforms are not as yet available. However, defining the pharmacological and physiological properties of these NHE isoforms can also indicate whether they have features consistent with being epithelial apical and/or basolateral NHEs.

In the present study, we demonstrated that NHE3 cDNA encodes a functional NHE. We also showed that NHE3, when expressed in either PS120 cells or Caco-2 cells, is resistant to amiloride and EIPA and is inhibited by protein kinase C. These two functional properties are unique to NHE3 and are consistent with the pharmacological and physiological properties of intestinal and renal apical NHEs (1–7). In contrast to NHE3, when NHE1 and NHE2 were expressed in PS120 cells, both were sensitive to amiloride inhibition, although NHE2 is 20-fold more resistant to EIPA than is NHE1 (24, 27). Furthermore, both NHE1 and NHE2

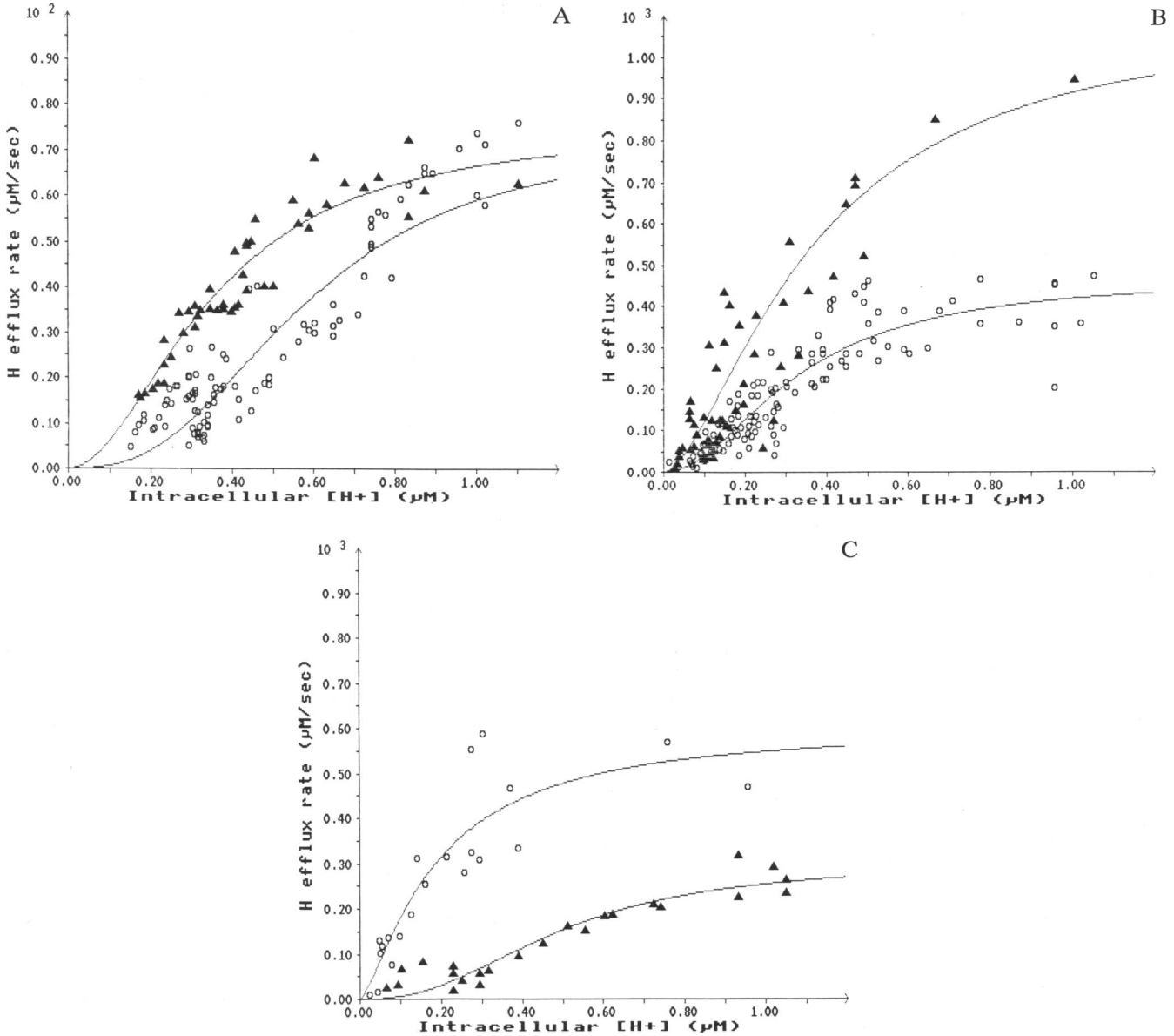

FIG. 3. Effect of FBS and phorbol ester on kinetics of Na^+/H^+ exchange mediated by NHE1 and NHE3. Cells loaded with BCECF were acidified to various pH_i values by changing the duration of the NH_4Cl prepulse; Na^+/H^+ exchange rates were calculated directly after exposure of acidified cells to Na medium. Initial rates of pH_i recovery were obtained by calculating the first-order derivative of the first 30–40 s of the pH_i recovery trace. Results are plotted against the initial H^+ concentration after acidification. Na^+/H^+ exchange rates (μM H^+ per s) were determined by multiplying the rate of Na^+-dependent alkalinization by the cellular buffering capacity at the same pH_i. Buffering capacity was determined in a separate series of experiments as described (11). Kinetic parameters (V_{max} and K') were determined by using a computer program (ENZFITTER; Biosoft, Cambridge, U.K.) that allowed fitting of data with the Hill equation ($v = V_{max}[S]^n/K'(H^+) + [S]^n$) where v, $[S]$, $K'(H^+)$, and n represent Na^+/H^+ exchange rate, intracellular H^+ concentration, apparent affinity for intracellular H^+, and the Hill coefficient, respectively. Lines in the plots are lines that were generated by the computer program using the Hill equation and were the best fits with the data. ○, Control NHE1 or NHE3; ▲, serum- or PMA-treated NHE1 or NHE3. (*A*) Stimulation of Na^+/H^+ exchange in NHE1/PS120 by serum. Exposure to 10% FBS was coincident with the addition of Na medium. Kinetic analysis showed that the increase in NHE1 activity caused by 10% FBS was due to an increased affinity for intracellular H^+, reflected by a decrease in $K'(H^+)$ from 0.37 ± 0.08 μM (control) to 0.13 ± 0.06 μM (serum-activated NHE1). There was no significant change in V_{max} (80 ± 4 μM/s and 75 ± 6 μM/s for control and serum-activated NHE1, respectively). (*B*) Stimulation of Na^+/H^+ exchange in NHE3/PS120 by serum. Kinetic analysis showed an increase in V_{max} from 482 ± 19 μM/s to 1010 ± 115 μM/s for control and serum-activated NHE3, respectively, without significant change in the apparent affinity for $K'(H^+)$ (0.11 ± 0.02 and 0.20 ± 0.08 μM for control and serum-activated NHE3, respectively). (*C*) Inhibition of Na^+/H^+ exchange in NHE3/PS120 cells by PMA. Cells were exposed to PMA (0.1 μM) for 5 min before acidification and PMA was also present in the Na medium during pH recovery, while control cells were perfused with Na medium without PMA. Kinetic analysis showed that PMA inhibited NHE3 by a decrease in V_{max} from 626 ± 62 μM/s (control) to 294 ± 26 μM/s (PMA-treated cells) without significant change in $K'(H^+)$ (0.13 ± 0.05 and 0.18 ± 0.10 μM for control and PMA-inhibited NHE3, respectively).

were stimulated by PMA (Fig. 2*C*; ref. 28). These results suggest that both pharmacological and physiological properties of NHE isoforms are intrinsic properties of the proteins. Furthermore, we showed that the cloned NHE isoforms can also be regulated by growth factors/protein kinases by a change in either V_{max} (NHE3) or the affinity for intracellular H^+ (NHE1), depending on the NHE subtype. In neither kinetic mechanism of regulation is there an apparent change in cooperativity of proton binding—i.e., similar Hill coefficients.

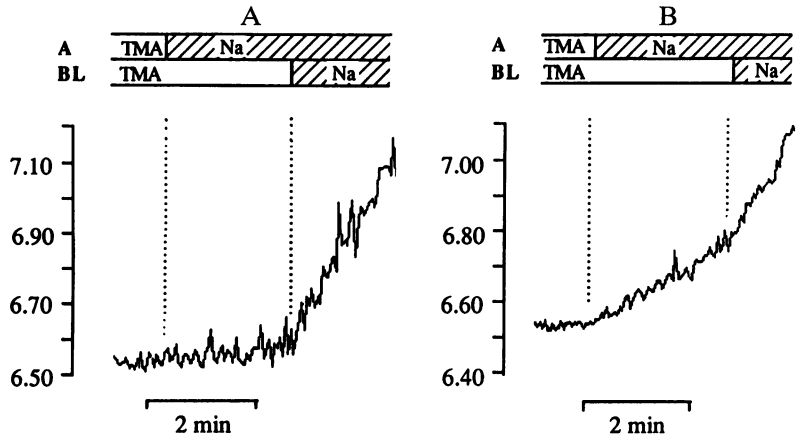

FIG. 4. Apical expression of NHE3 in Caco-2 cells. (*A*) Absence of functional Na^+/H^+ exchange activity in untransfected Caco-2 cells. Nontransfected Caco-2 cells have only basolateral Na^+/H^+ exchange, as demonstrated in this experiment in which Na^+ added to the apical surface (A) of acidified cells caused no change in pH_i, while addition of Na^+ to the basolateral surface (BL) resulted in prompt alkalinization. (*B*) Presence of both apical and basolateral Na^+/H^+ exchange activity in NHE3/Caco-2 cells. NHE3/Caco-2 cells have Na^+/H^+ exchange activity on both basolateral and apical surfaces. When Na^+ was added to the apical surface of acidified cells, there was a rapid increase in pH_i; subsequent addition of Na^+ to the basolateral surface caused a further rapid increase in pH_i.

In an attempt to determine whether NHE3 could be expressed in the apical domain of epithelial cells, NHE3 cDNA was stably transfected into Caco-2 cells and its functional activity was demonstrated on the apical membrane surface of the transfected cells. This result further suggests that NHE3 is a candidate to be the ileal and renal brush border NHE. The mechanism or signal that allows functional localization of NHE3 to the apical domain of Caco-2 cells is not known.

In conclusion, NHE3 is resistant to amiloride and EIPA and is inhibited by protein kinase C (14). Serum stimulates and PMA inhibits NHE3 by a mechanism that alters V_{max}, suggesting that growth factors regulate NHE3 by a mechanism that is distinct from that of NHE1. Changes in V_{max} are probably due to changes in the number of active transporters in the plasma membrane or in the turnover number of NHE3. Because serum stimulated NHE3 and PMA inhibited NHE3, it suggests that endogenous activity of NHE3 represents a balance in regulation by stimulatory and inhibitory pathways.

We thank Ms. Judith Hariton for editorial assistance. This work was supported in part by National Institutes of Health Grants R01DK26523, R01DK42457, R29DK43778, and P01DK44484; Institutional Training Grant T32DK07632; and National Research Service Awards F32DK08820 and F32DK08672.

1. Grinstein, S., Rutin, D. & Mason, M. J. (1989) *Biochim. Biophys. Acta* **988**, 73–97.
2. Haggerty, C., Agarwal, N., Reilly, R. F., Adelberg, E. A. & Slayman, C. W. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6797–6801.
3. Knickelbein, R. G., Aronson, P. S. & Dobbins, J. W. (1990) *Am. J. Physiol.* **259**, G802–G806.
4. Clark, J. D. & Limbird, L. L. (1991) *Am. J. Physiol.* **261**, C945–C953.
5. Rood, R. P., Emmer, E., Wesolek, J., McCullen, J., Husain, Z., Cohen, M. E., Braithwaite, R. S., Murer, H., Sharp, G. W. G. & Donowitz, M. (1988) *J. Clin. Invest.* **82**, 1091–1097.
6. Cohen, M. E., Wesolek, J., McCullen, J., Rys-Sikora, K., Pandol, S., Rood, R. P., Sharp, G. W. G. & Donowitz, M. (1991) *J. Clin. Invest.* **88**, 855–863.
7. Helmle-Kolb, C., Montrose, M. H., Stange, G. & Murer, H. (1990) *Pflügers Arch.* **415**, 461–470.
8. Weinman, E. J. & Shenolikar, S. (1986) *J. Membr. Biol.* **93**, 133–139.
9. Mellas, J. & Hammerman, M. R. (1986) *Am. J. Physiol.* **250**, F451–F459.
10. Sardet, C., Franchi, A. & Pouyssegur, J. (1989) *Cell* **56**, 271–280.
11. Tse, C. M., Ma, A. I., Yang, V. W., Watson, A. J. M., Levine, S., Montrose, M. H., Potter, J., Sardet, C., Pouyssegur, J. & Donowitz, M. (1991) *EMBO J.* **10**, 1957–1967.
12. Biemesderfer, D., Reilly, R. F., Exner, M., Igarashi, P. & Aronson, P. S. (1992) *Am. J. Physiol.* **263**, F833–F840.
13. Reilly, R. F., Hildebrant, F., Biemesderfer, D., Sardet, C., Pouyssegur, J., Aronson, P. S., Slayman, C. W. & Igarashi, P. (1991) *Am. J. Physiol.* **261**, F1088–F1094.
14. Tse, C. M., Brant, S. R., Walker, M. S., Pouyssegur, J. & Donowitz, M. (1992) *J. Biol. Chem.* **267**, 9340–9346.
15. Orlowski, J., Kandasamy, R. A. & Shull, G. E. (1992) *J. Biol. Chem.* **267**, 9331–9339.
16. Watson, A. J., Levine, S., Donowitz, M. & Montrose, M. M. (1991) *Am. J. Physiol.* **261**, G229–G238.
17. Pouyssegur, J., Franchi, A., L'Allemain, G. & Paris, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4833–4837.
18. Pouyssegur, J. (1985) *Trends Biochem. Sci.* **10**, 453–455.
19. Krayer-Pawlowska, D., Helmle-Kolb, C., Montrose, M. H., Krapf, R. & Murer, H. (1991) *J. Membr. Biol.* **120**, 173–183.
20. Thomas, J. A., Bushbaum, R. N., Zimniak, A. & Racker, E. (1979) *Biochemistry* **18**, 2230–2238.
21. Franchi, A., Cragoe, E. & Pouyssegur, J. (1986) *J. Biol. Chem.* **261**, 14614–14620.
22. Boyarsky, S., Ganz, M. B., Cragoe, E. J. & Boron, W. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5921–5924.
23. Segel, I. H. (1976) *Biochemical Calculations* (Wiley, New York), 2nd Ed.
24. Counillon, L., Franchi, A. & Pouyssegur, J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4508–4512.
25. Bianchini, L., Woodside, M., Sardet, C., Pouyssegur, J., Takai, A. & Grinstein, S. (1991) *J. Biol. Chem.* **266**, 15406–15413.
26. Wakabayashi, S., Fafournoux, P., Sardet, C. & Pouyssegur, J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2424–2428.
27. Tse, C. M., Levine, S. A., Yun, C. H. C., Montrose, M. H., Little, P. J., Pouyssegur, J. & Donowitz, M. (1993) *J. Biol. Chem.* **268**, 11917–11924.
28. Levine, S. A., Montrose, M. H., Tse, C. M. & Donowitz, M. (1993) *J. Biol. Chem.*, in press.