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The effect of lethal acid stress on Na^+/H^+ exchanger isoforms in cultured inner medullary collecting duct cells: deletion of NHE-2 and over expression of NHE-1

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Abstract

Cultured inner medullary collecting duct (mIMCD-3) cells express Na^+/H^+ exchanger isoforms NHE-2 and NHE-1 (Soleimani et al. (1994) J. Biol. Chem. 269, 27973–27978). In the present studies we examined the effect of lethal acid stress on Na^+/H^+ exchanger activity and isoform expression in mIMCD-3 cells. mIMCD-3 cells were incubated for 10 min with 20 mM ammonium, and exposed to an ammonium-free acidic solution (pH 6.0) for 120 min. Thereafter, cells were recovered and grown in normal culture media. The surviving clones were isolated and subjected to two additional cycles of acid stress. A mutant clone was isolated and characterized for Na^+/H^+ exchange activity and isoform expression. The mutant mIMCD-3 clone demonstrated significant over-expression of Na^+/H^+ exchange activity as assessed by acid-stimulated ^{22}Na influx (11.56 nmol/mg protein in mutant vs. 4.06 nmol/mg in parent cells, $P < 0.001$, $n = 4$) and sodium-dependent pH_i recovery from an acid load (0.55 pH/min in mutant vs. 0.28 pH/min in parent cells, $P < 0.01$, $n = 6$). A dose-response inhibition of the exchanger showed that the mutant cells were very sensitive to dimethylamiloride (IC_{50} 158 nM in mutant vs. 889 nM in parent mIMCD-3 cells, $P < 0.001$). To compare the Na^+/H^+ exchanger isoforms in mutant and parent mIMCD-3 cells, poly(A)$^+$ RNA was isolated from each group and probed with radiolabeled NHE-1 or NHE-2 cDNA. The expression of NHE-1 mRNA was increased by ~ 100% in mutant cells. The NHE-2 mRNA, on the other hand, was found to be absent in mutant mIMCD-3 cells. Examination of the regulatory mechanisms of the Na^+/H^+ exchanger isoforms in parent mIMCD-3 cells, which express NHE-2 and NHE-1, and mutant mIMCD-3 cells, which only express NHE-1, would be helpful in elucidating the roles of NHE-2 and NHE-1 in inner medullary collecting duct cells.

Keywords: Acid stress; Sodium ion–proton exchanger; Isoform; NHE-1; NHE-2; (mIMCD-3 cell)

1. Introduction

The Na^+/H^+ exchanger is a ubiquitous transporter present in the plasma membranes of nearly all mammalian cells [1–3]. It plays a key role in several vital functions including pH regulation, cell volume homeostasis, and cell proliferation [1–3]. Recent molecular cloning experiments have identified the existence of four Na^+/H^+ exchanger isoforms designated as NHE-1, NHE-2, NHE-3, and NHE-4 [4–8]. NHE-1 has been shown to be involved with cell pH regulation while NHE-3 mediates vectorial transport of bicarbonate. The role and regulation of two of these isoforms, NHE-2 and NHE-4, is less well understood.

Cultured inner medullary collecting duct (mIMCD-3) cells express NHE-2 and NHE-1 isoforms on their basolateral membranes [9]. Studies with mIMCD-3 cells indicate that NHE-2 and NHE-1 are regulated by different mechanisms. NHE-1 expression increased in acidosis while NHE-2 expression increased in high osmolality [9]. Thus, NHE-1 appears to be involved in cell pH regulation while NHE-2 may mediate volume regulation [9]. Still, the precise function of these isoforms has yet to be determined.

Isolation of clones deficient in or over expressing the sensitive Na^+/H^+ exchanger (NHE-1) has been employed

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to study the role of this isoform in several cell functions including pH and growth regulation [10,11]. To select for NHE-1 deficient clones, cells are exposed to Na-free acidic medium. Cells deficient in NHE-1 survive. To select for cells that overexpress NHE-1 cells are acid loaded then exposed to a Na-free acid medium. Only cells that upregulate their NHE-1 survive the intracellular acidosis. Studies in mIMCD-3 cells showed that NHE-2 mRNA decreased, whereas NHE-1 mRNA increased in acidosis [9]. Therefore, we used the NHE overexpression strategy to select mIMCD-3 cells overexpressing their Na^+/H^+ exchanger. Based on adaptive responses of NHE-2 and NHE-1 in acidosis [9], we hypothesized that surviving cells would overexpress their NHE-1 and possibly underexpress NHE-2. The cells that survived the NHE overexpression protocol were isolated, grown, and subjected to two additional cycles of lethal acid stress. The resulting strain was found to overexpress NHE-1 and to be totally deficient in NHE-2. Here we describe the function, kinetics, and isoform expression of the Na^+/H^+ exchanger in this inner medullary collecting duct cell strain.

2. Materials and methods

2.1. Cell culture

Inner medullary collecting duct (mIMCD-3) cells were grown in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (DMEM/F12) supplemented with 10% fetal bovine serum, 50 U/ml penicillin, and 50 μg/ml streptomycin. Cultured cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The medium was replaced every other day.

2.2. Mutagenesis

Actively proliferative, sub-confluent cultures were treated for 16 h with ethylmethylsulfonic acid (EMS) at 500 μg/ml. The cells were rinsed twice with phosphate buffered saline and grown in regular medium before subjected to lethal acid stress. The cells were then subjected to a modified protocol of lethal acid stress [11] as described below.

2.3. Selection of mutants by lethal acid stress

Following treatment with EMS, IMCD-3 cells were grown to confluence, trypsinized, and centrifuged at room temperature. The cells were resuspended for 10 min at 37°C in an ammonium-containing solution that consisted of 20 mM NH_4Cl, 120 mM TMA-Cl, 5 mM glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 2.5 mM K_2HPO_4, and 5 mM Hepes-Tris, pH 7.40. This procedure results in ammonium loading of the cells. The cells were then pelleted and incubated for 30 min in a solution that consisted of 130 mM TMA-Cl, 5 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM glucose, 20 mM Hepes-Tris, pH 5.5. This step results in acid loading secondary to passive diffusion of NH_3 from the cell. Thereafter, the cells were pelleted, washed, and incubated for 120 min at 37°C in a solution that consisted of 125 mM choline chloride, 5 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, and 20 mM of Mes (2-*N*-morpholinoethanesulfonic acid) at pH 6.0. The cells were then centrifuged, recovered, and seeded to culture-grade plastic dishes in DMEM/F12 medium (pH 7.4) for 10 days. The cells were trypsinized and subjected while in suspension to two more rounds of lethal acid stress. The cells were subcultured and passaged at very high dilutions (1:1000) to isolate individual colonies. A number of individual colonies were isolated with cloning cylinder then collected and subcultured. One strain (NHE2d) was studied in details for Na^+/H^+ exchanger activity and isoform expression.

2.4. Measurement of the Na^+/H^+ exchanger activity

$^{22}Na^+$ influx

Uptake of radiolabelled sodium by parent and mutant IMCD-3 cells grown in 24 well plastic plates was measured as previously described [9,12]. Cells were washed three times with a Na-free buffer consisting of 140 mM chloride salt of NMDG (*N*-methyl-D-glucamine), 4 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 7 mM Hepes, pH 7.4 (solution A). The cells were then incubated in an ammonium-containing solution consisting of 110 mM chloride salt of NMDG, 30 mM NH_4Cl, 4 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 7 mM Hepes, pH 7.4 (solution B) for 10 min. Thereafter, the ammonium-containing solution was replaced with uptake solution (solution A) containing 2 mM $^{22}NaCl$. The $^{22}Na^+$ uptake reaction was stopped after 4 min using four rapid washes with ice-cold saline. Cell-associated radioactivity was extracted with 1 ml of 1 M sodium hydroxide and counted by scintillation spectroscopy.

Intracellular pH measurement

The intracellular pH of parent and mutant IMCD cells grown on coverslips was measured with the use of pH-sensitive dye 2′,7′-biscarboxyethyl-5(and-6) carboxyfluorescein (BCECF) as employed [9]. The cells were incubated in the presence of 5 μM BCECF for 15 min in a solution consisting of 140 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 7 mM Hepes, pH 7.4 (solution C). The fluorescence of BCECF was monitored in a thermoregulated (37°C) double excitation beam spectrofluorometer (PTI double-beam fluorometer, Delta Scan I, Photon Tech. Int., South Brunswick, NJ). A calibration curve was generated using the KCl/nigericin technic. The fluorescence ratio at excitation wavelengths of 500 and 450 nm was utilized to determine intracellular pH values in the experimental groups by comparison to the calibra-

tion curve. Acid loading of IMCD cells grown on coverslips was achieved via NH_4 pulse using an NH_4-containing solution (solution B). Cell acidification was induced by replacing NH_4Cl solution with a sodium-free solution (solution A). Following intracellular acidosis, the initial rate of pH_i recovery was monitored in the presence of a sodium-containing solution (solution C).

2.5. Isolation of total and poly(A)$^+$ RNA

Total cellular RNA was extracted from the cells by the method of Chomczynski and Sacchi [13]. Cells were scraped off the plates with homogenizing buffer consisting of 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol. Protein was extracted by phenol/chloroform (1:1) and RNA was precipitated by isopropanol [20]. Poly(A)$^+$ RNA was extracted by passing total cellular RNA twice through Oligo dT cellulose spin columns (5′Prime-3′Prime, Inc., Boulder, CO). Total and poly(A)$^+$ RNA were quantitated spectrophotometrically at 260 nm.

2.6. Northern hybridization

Poly(A)$^+$ RNA samples, 10–15 μg/lane, were loaded and fractionated on 1% agarose-formaldehyde gels and transferred to nylon membranes by capillary diffusion [14]. The membranes were baked at 80°C for 2 h. The integrity of RNA preparations and the extent of RNA transfer was estimated by viewing the ethidium bromide stained gels and membranes. The membranes were prehybridized for 6 h at 42°C in 50% formamide, 5 × SSPE, 5 × Denhart's solution, 0.5% SDS, and 200 μg of denatured salmon sperm DNA/ml. The hybridization was carried out overnight at 42°C in the presence of $(30–50) \cdot 10^6$ cpm ^{32}P-labelled DNA probe for NHE-1, NHE-2, or β-actin. The cDNAs were labeled with ^{32}P-labeled deoxynucleotides using Random Primed DNA labelling kit (5′Prime-3′Prime, Inc.). Following hybridization, the membranes were washed twice in 2 × SSC, 0.5% SDS for 30 min at room temperature, and once for 30 min at 60°C in 0.1 × SSC, 0.5% SDS. For NHE-2 the membranes were given an additional wash at 50°C for 30 min in 2 × SSC, 0.5% SDS. Membranes were exposed to Kodak X-Omat films at −70°C with intensifying screens for 72–120 h. For NHE-1 and NHE-2, the *Pst*I-*Pst*I fragment (nucleotides 478–1850) and the *Ava*I-*Ava*I fragment (nucleotides 174–2032) were used for hybridization, respectively.

2.7. [^{3}H]Thymidine incorporation

DNA synthesis was measured by assaying the incorporation of [*methyl*-^{3}H]thymidine into parent or mutant mIMCD-3 cells as described [15]. Cells were plated in 60 mm plastic dishes at a density of 10^5 cells per dish, cultured for 18 h, and exposed to [*methyl*-^{3}H]thymidine (5 μCi/ml). Labelled thymidine uptake was subsequently assayed at 6 and 24 h. Each plate was rinsed twice with ice-cold saline. The cells were trypsinized, recovered, and centrifuged. The pellet was suspended in 5.0 ml of ice-cold 10% TCA and incubated for 10 min on ice. The precipitate was collected on 2.5 cm Millipore type HA filters that had been soaked in 10% cold TCA. The filters were washed twice with ice-cold 10% TCA. The counts were measured by scintillation spectroscopy.

2.8. Population doubling time

Population doubling times for parent mIMCD-3 and NHE2d cells were calculated by a standard method [16]. Exponentially growing cells were used to prepare multiple replicate cultures at known seeding density in DMEM/F12 medium containing 28 mM $NaHCO_3$, 10 mM Hepes, 50 U/ml penicillin and 10% fetal bovine serum. After 96 h the cells were trypsinized and recounted to measure the increase in cell number. The number of generations (n) during 96 h was calculated according to the formula, $n = 3.32(\log X_2 - \log X_1)/96$ h, where X_1 and X_2 are the number of cells at time 0 and 96 h, respectively. The population doubling time was calculated as $1/n$.

2.9. DNA and protein measurements

Protein concentration was determined by the bicinchoninic acid (BCA) method according to the manufacturer's protocol (Pierce Company, Rockford, IL). DNA concentration was measured according to established methods [15]

2.10. Statistical analysis

The data are expressed as means ± S.E. Statistical analysis was determined using analysis of variance or ANOVA where appropriate. $P < 0.05$ was considered statistically significant.

2.11. Materials

^{22}Na and ^{32}P were purchased from New England Nuclear (Boston, MA). Dimethylamiloride (DMA) and Nitrocellulose filters were obtained from Sigma (St. Louis, MO). BCECF and nigericin were purchased from Molecular Probes (Eugene, OR).

3. Results

3.1. Na^+/H^+ exchange activity

^{22}Na influx

Na^+/H^+ exchange activity in mIMCD-3 and NHE2d cells was determined by $^{22}Na^+$ influx assay method. Cells

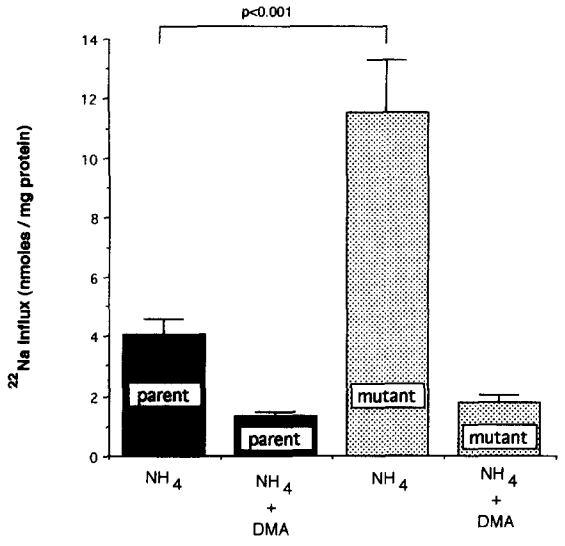

Fig. 1. Acid-stimulated $^{22}Na^+$ influx in mutant and parent mIMCD-3 cells grown on plastic dish. $^{22}Na^+$ (2 mM) uptake was measured at 4 min ± 0.5 mM DMA according to Section 2. Values represent means ± S.E. for four experiments performed in triplicates.

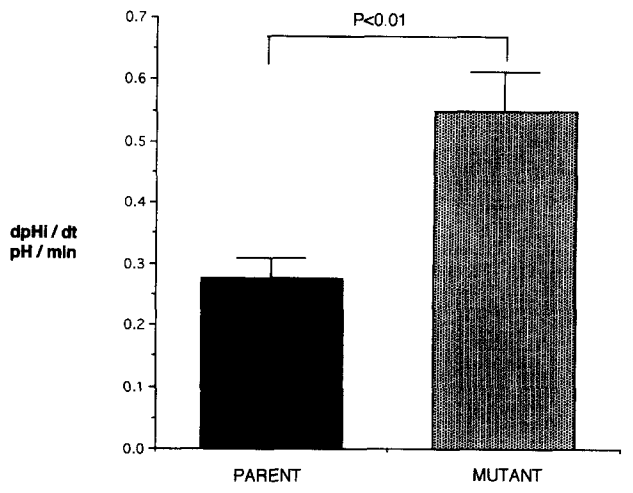

Fig. 3. Sodium-dependent pH_i recovery from an acid load in mutant and parent mIMCD-3 cells. Mutant or parent mIMCD-3 cells were grown to confluence on coverslips, pulsed with NH_4Cl, and assayed for Na^+/H^+ exchange activity as described in Section 2. Values represent means ± S.E. for six cover slips.

were acid loaded by ammonium prepulse and influx of $^{22}Na^+$ was assayed at 4 min in the presence or absence of 0.5 mM dimethylamiloride(DMA). As illustrated in Fig. 1, acid-stimulated, DMA-sensitive $^{22}Na^+$ uptake was significantly increased in NHE2d cells compared to the parent strain ($P < 0.001$).

Intracellular pH measurement

The pH sensitive dye, BCECF, was used to compare Na^+/H^+ exchanger activities in parent and NHE2d cells. Cells were grown to confluence on coverslips and assayed for Na-dependent pH_i recovery from an acid load. Fig. 2 shows a representative pH tracing from each strain. As illustrated, Na-dependent pH_i recovery was significantly increased in mutant cells as compared to parent cells. The results of six separate experiments are summarized in Fig. 3. As shown, the initial pH_i recovery from an acid load (dpH_i/dt) was 0.28 ± 0.03 pH/min in parent and 0.55 ± 0.06 pH/min in mutant mIMCD-3 cells, $P < 0.01$. Steady-state pH_i was 7.38 ± 0.06 in mutant cells and 7.31 ± 0.05 in parent cells ($P > 0.05$, $n = 6$ coverslips for each group). Following acid loading with NH_4Cl, intracellular pH decreased to 6.49 ± 0.07 in parent cells and 6.44 ± 0.06 in mutant cells ($P > 0.05$, $n = 6$ coverslips for each group). The cytosolic buffering capacity in parent and mutant cells were comparable (19.5 ± 1.9 in parent and 18.7 ± 1.8 mmol/l × pH in mutant cells, $P > 0.05$, $n = 4$).

Sensitivity of the Na^+/H^+ exchanger to inhibition by dimethylamiloride (DMA)

In these series of experiments we examined the sensitivity of the Na^+/H^+ exchanger to inhibition by the amiloride analog, DMA. Cells were grown to confluence in 24-well plates and assayed for Na^+/H^+ exchanger activity in the presence of increasing concentrations of DMA. A dose-response curve (Fig. 4) indicates that the sensitivity of the Na^+/H^+ exchanger in NHE2d cells was significantly increased compared to parent mIMCD-3 cells. The 50% inhibitory concentration (IC_{50}) for dimethylamiloride was 889 nM in the parent and 158 nM in the mutant cells ($P < 0.01$, $n = 3$).

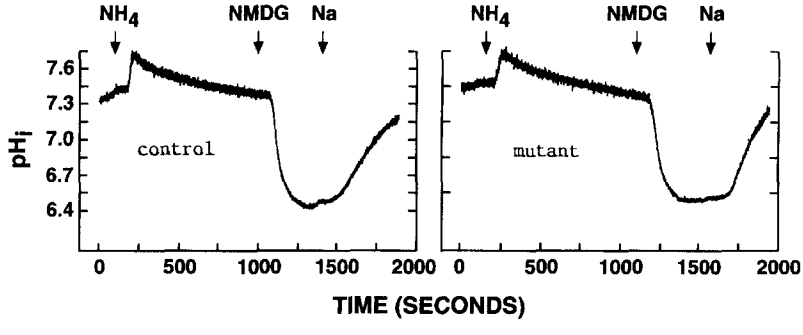

Fig. 2. Sodium-dependent pH_i recovery in parent or mutant mIMCD-3 cells using pH-sensitive dye BCECF (representative tracings). Cells were grown on cover slips, pulsed with ammonium, and assayed for Na^+/H^+ exchanger activity as sodium-dependent H^+ extrusion.

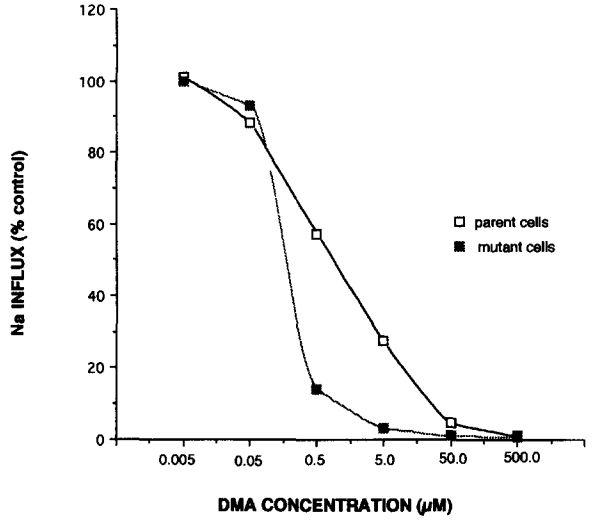

Fig. 4. Dimethylamiloride dose response inhibition of acid-stimulated ^{22}Na influx in mutant and parent mIMCD-3 cells. Values represent means ± S.E. for three experiments performed in triplicates.

Northern hybridization

Mutant cells exhibited overexpression of Na^+/H^+ exchanger activity as compared to the parent strain (Figs. 1–4). These data further indicate increased sensitivity of the Na^+/H^+ exchanger to the amiloride analog, DMA. mIMCD-3 parent cells express Na^+/H^+ exchanger isoforms NHE-2 and NHE-1 and are relatively resistant to inhibition by the amiloride analog, DMA [9]. The relative resistance of the Na^+/H^+ exchanger to DMA indicates that NHE-2 is the dominant isoform under baseline conditions in parent mIMCD-3 cells. The increased sensitivity of the exchanger to DMA in mutant cells indicates that NHE-1 is the dominant isoform in this cell line. Accordingly, parent and mutant cells were assessed to determine if lethal acid stress had affected the expression of NHE isoforms. Cells were grown to confluence in 100 mm dishes. Poly(A)$^+$ RNA was isolated from each group, size fractionated, transferred to a nylon membrane, and probed with radiolabeled NHE-1 or NHE-2 cDNA. A representative experiment is shown in Fig. 5a. As demonstrated, the expression of NHE-1 mRNA increased by ~ 100% in mutant cells as compared to parent cells. The membranes were stripped of radioactivity and probed for NHE-2 mRNA. Fig. 5b demonstrates that NHE-2 mRNA was absent in the mutant cells. The expression of β-actin mRNA in parent and mutant cells was not affected (Fig. 5a and b). Three separate Northern blots were performed on parent and mutant mIMCD-3 cells and the results invariably showed overexpression of NHE-1 and deletion of NHE-2 mRNA in NHE2d cells.

Growth studies

In the next series of experiments, the growth of the NHE2d cells were compared to the parent cells. Toward this end, we measured the population doubling time and thymidine uptake in both cell lines. The average doubling time for the NHE2d and the parent cells was 16.7 ± 0.2 h and 30.6 ± 0.5 h, respectively ($P < 0.01$). The results indicate that the number of NHE2d cells was increasing at a faster rate than the parent cells.

The rate of DNA synthesis was measured in both cell lines by measuring thymidine incorporation at various time intervals. Cells were plated in 60 mm plastic dishes at a density of 10^5/dish and exposed to [*methyl*-^{3}H]thymidine 18 h later. The [*methyl*-^{3}H]thymidine uptake was assayed

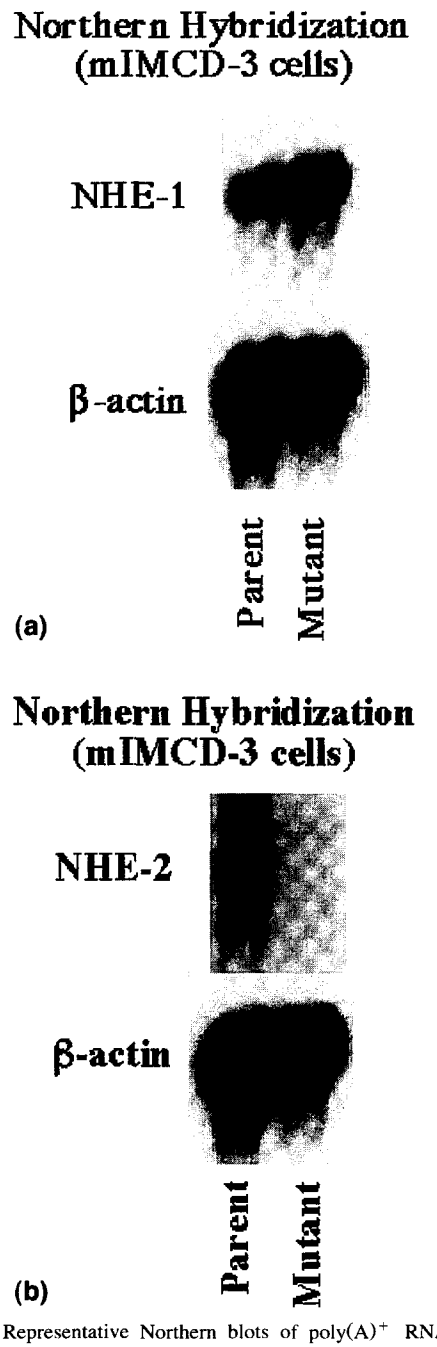

Fig. 5. (a) Representative Northern blots of poly(A)$^+$ RNA showing NHE-1 and β-actin transcript levels in parent or mutant mIMCD-3 cells. (b) Representative Northern blots of poly(A)$^+$ RNA showing NHE-2 and β-actin transcript levels in parent or mutant mIMCD-3 cells.

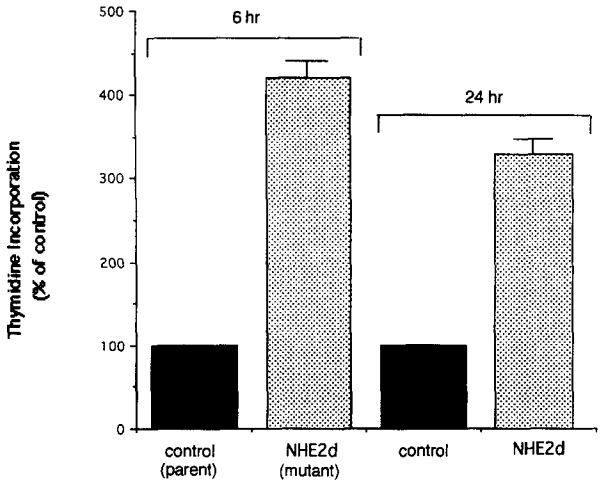

Fig. 6. [^{3}H]Thymidine incorporation in parent or mutant mIMCD-3 cells. Parent or mutant mIMCD-3 cells were seeded at known density and assayed for the rate of DNA synthesis by [^{3}H]thymidine incorporation according to Section 2. Values represent means ± S.E. for three experiments performed in quatriplicates.

at 6 and 24 h after addition of radiolabelled thymidine. As shown in Fig. 6, the thymidine incorporation was significantly higher in NHE2d cells at both 6 and 24 h ($P < 0.001$ for both time points compared to parent cells). In separate series of experiments, cells were seeded in a similar manner and exposed to [*methyl*-^{3}H]thymidine 1 h later. The incorporation of radiolabelled thymidine by cultured cells was assayed at 4 h. These results demonstrated that the NHE2d cells had increased thymidine incorporation as compared to parent cells (239% ± 13.1 in NHE2d cells as compared to 100% in parent cells, $P < 0.01$, $n = 4$ separate experiments).

4. Discussion

The Na^+/H^+ exchanger plays a key role in cell pH regulation by extrusion of acid across cell membranes [1–3]. The extrusion of hydrogen is coupled to reabsorption of sodium, a process that influences cell volume regulation [1–3]. There have been four structurally related NHE isoforms identified to date [4–8]. Recent studies have demonstrated distinct patterns of cellular distribution and subcellular localization for NHE isoforms in renal cells [9,12,17–19]. While all NHE isoforms are understood to subserve the same basic function, that is to exchange intracellular acid for extracellular sodium, the distinct localization of NHE isoforms in renal cells may suggest that these isoforms are differentially regulated. With regard to NHE-3, recent studies indicate that this isoform is expressed in the luminal membranes of the kidney proximal tubule [18,19] and the medullary thick ascending limb of Henle [20]. Its luminal location is consistent with a role for vectorial Na^+ and HCO_3^- transport and/or luminal acidification. In contrast, NHE-1 appears to be located in the basolateral membranes of most nephron segments [17]. Its basolateral membrane location is consistent with a 'housekeeping role', most likely related to cell pH regulation in these cells. NHE-2 appears to be predominantly expressed in the medulla [8]. Recent studies indicate that NHE-2 is expressed in inner medullary collecting ducts [9] and proximal tubules [21]. Functional studies have localized NHE-2 to the basolateral membranes of the inner medullary collecting duct [9] and the luminal membranes of the proximal tubules cells [21]. These results suggest that membrane localization of NHE-2 may be cell type or nephron segment specific, that is, its luminal or basolateral membrane localization is dependent upon the intracellular processing pathways. NHE-4 appears, by in situ hybridization, to be predominantly expressed in the inner medulla [20,22]. The role, subcellular localization, or functional regulation of NHE-4 is not known.

Cultured inner medullary collecting duct(mIMCD-3) cells express two Na^+/H^+ exchanger isoforms, NHE-1 and NHE-2, on their basolateral membrane [9]. The results of the current investigations indicate that the expression of NHE isoforms in IMCD cells is altered significantly in response to lethal acidosis. Subjecting IMCD cells to lethal acid stress resulted in selection of cells with Na^+/H^+ exchanger overexpression. This is presumably to fight the lethal cell acidosis. The kinetics of this upregulation were consistent with an increase in both affinity (decreased K_m) and capacity (increased V_{max}) of the transporter for Na(K_m and V_{max} were 17.8 mM and 168 nmol/min per mg protein in parent cells and 7.9 mM and 358 nmol/min per mg protein in mutant cells). Northern hybridization experiments demonstrated that NHE-1 expression increased significantly (Fig. 5). These results are consistent with a major role for NHE-1 in cell pH regulation. IMCD cells expressing NHE-2 did not survive the lethal acid stress. We have studied four separate clones of mIMCD-3 cells following exposure to lethal acid stress. All four clones demonstrated increased acid-stimulated ^{22}Na influx (3.8 ± 0.4-fold over parent cells) and increased dimethylamiloride (DMA) sensitivity (the IC_{50} for Na^+/H^+ exchange inhibition by DMA was 138 ± 26 nM in four separate surviving clones). The increased DMA sensitivity indicates that these clones predominantly express NHE-1 (which is highly sensitive to DMA) and are deficient in NHE-2 (which is significantly resistant to DMA). The parent mIMCD-3 cells invariably showed relative resistance to DMA indicating expression of NHE-2. The increased acid-stimulated ^{22}Na influx indicates that all selected clones are overexpressing their Na^+/H^+ exchanger (NHE-1). Further studies are needed to determine if all surviving clones demonstrate similar NHE properties. Functional and transcriptional upregulation of NHE-1 in mutant cells suggest that NHE-1 adaptation is essential for mIMCD-3 cell survival in lethal acid media; much the same way as in fibroblast cells [11]. As such, cells not expressing NHE-1 (i.e., expressing NHE-2) may not survive the lethal acid stress.

The results of the experiments shown in Figs. 1–5 suggest that NHE-2 may be involved in functions other than cell pH regulation. Chronic exposure of mIMCD-3 cells to hypertonic medium increased Na^+/H^+ exchanger activity via increased NHE-2 mRNA expression [9], suggesting that NHE-2 may be involved in volume regulation. With regard to deletion of NHE-2 and overexpression of NHE-1 in mutant mIMCD-3 (NHE2d) cells, it is plausible that the original cultured cell line is comprised of several distinct cell types and that each cell type expresses only one NHE isoform (i.e., NHE-1 or NHE-2). Accordingly, each cell type might have different surviving potential in response to lethal acid stress.

The mutant mIMCD-3 cells demonstrate increased proliferative rate as assessed by population doubling time and radiolabelled thymidine incorporation. The reason for this increased growth in response to lethal acid stress is not known. While induction of some cell growth factors in response to acidosis can not be ruled out, possibility of acidosis-induced SV40 T antigen-derived growth in this cell line is present. It is equally plausible that lethal acid stress could have suppressed the expression of some growth inhibiting factors, resulting in increased cell growth in mutant cells. Several studies have demonstrated that increased Na^+/H^+ exchange activity is associated with increased DNA synthesis [23,24]. It has been postulated that increased Na^+/H^+ exchange activity, by raising pH_i, plays a permissive role in increased DNA synthesis [23,24]. This, however, may be an unlikely mechanism since preincubation with DMA, 1 μM/l, failed to prevent increased DNA synthesis (data not shown).

Long-term exposure of mIMCD-3 cells to hypertonic medium resulted in decreased NHE-1 mRNA expression, indicating that this isoform is not involved in chronic osmolality-induced Na^+/H^+ exchange activation [9]. The results of experiments with cultured vascular smooth muscle (VSM) cells, which express NHE-1, have shown that long-term high osmolality activates the Na^+/H^+ exchanger [25]. Taken together, this suggests that chronic osmolality-induced down regulation of NHE-1 in IMCD cells could be tissue specific. The Na^+/H^+ exchanger isoform NHE-1, which is the only isoform in vascular smooth muscle cells, demonstrated increased activity in long-term hypertonicity [24], indicating that it was involved in cell volume regulation. It is plausible that either NHE-1 or NHE-2 could regulate cell volume homeostasis if each was the only isoform present in IMCD cells.

In conclusion, mIMCD-3 cells were subjected to lethal acid stress and studied for Na^+/H^+ exchanger activity and isoform expression. A mutant clone that is deficient in NHE-2 and overexpresses NHE-1 was isolated. Comparison of NHE adaptation in the parent and mutant mIMCD-3 strains in pathophysiologic conditions, i.e., acidosis, ADH administration, hypertonicity, or cAMP would significantly increase our understanding about the precise role or function of NHE-1 or NHE-2 in inner medulla.

Acknowledgements

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