The Na\textsuperscript{+}/H\textsuperscript{+} exchanger isoform 2 is the predominant NHE isoform in murine colonic crypts and its lack causes NHE3 upregulation


The Na\textsuperscript{+}/H\textsuperscript{+} exchanger isoform 2 is the predominant NHE isoform in murine colonic crypts and its lack causes NHE3 upregulation. Am J Physiol Gastrointest Liver Physiol 287: G125–G133, 2004. First published February 12, 2004; 10.1152/ajpgi.00332.2003.—The Na\textsuperscript{+}/H\textsuperscript{+} exchanger isoform NHE2 is highly expressed in the intestinal tract, but its physiological role has remained obscure. The aim of this study was to define its expression, location, and regulatory properties in murine colon and to look for the compensatory changes in NHE2 (−/−) colon that allow normal histology and absorptive function. To this end, we measured murine proximal colonic surface and crypt cell NHE1, NHE2, and NHE3 expression levels, transport rates in response to acid, hyperosmolarity and cAMP in murine proximal colonic crypts, as well as changes in transcript levels and acid-activated NHE activity in NHE2 (−/−) crypts. We found that NHE2 was expressed most abundantly in crypts, NHE1 equally in crypts and surface cells, and NHE3 much stronger in surface cells. NHE2, like NHE1, was activated by low intracellular pH (pHi), hyperosmolarity, and cAMP, whereas NHE3 was activated only by low pH. Crypts isolated from NHE2 (−/−) mice displayed increased acid-activated NHE1- and NHE3-attributable Na\textsuperscript{+}/H\textsuperscript{+} exchange activity, no change in NHE1 expression, and NHE3 expression levels twice as high as in normal littermates. No change in cellular ultrastructure was found in NHE2 (−/−) colon. Our results demonstrate high NHE2 expression in the crypts and suggest a role for NHE2 in cryptal pHi and volume homeostasis.

NHE2 knockout mice; pH regulation; colon; ion transport

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Materials. HOE-642 was prepared by Aventis (Frankfurt, Germany), bovine serum albumin (BSA; cell culture grade) was from Paesel und Lorei (Frankfurt, Germany) or from Merck (Darmstadt, Germany) at tissue culture grade or the highest grade available.

Animal breeding and genotyping. The NHE2 knockout mouse strain had been generated and characterized in the laboratories of Shull and colleagues (29). The animals were raised in the animal facilities of the University of Tübingen and the Medical School of Hannover and PCR-genotyped as previously described (31) using the primers outlined in Table 1 for detection of the wild-type and mutant sequence.

Preparation of colonic crypts and surface cells. After the mice were killed by CO\textsubscript{2}-narcosis and cervical dislocation, crypts and surface cells were isolated from an everted segment of proximal colon exactly as previously described (2, 3), using a combination of Ca\textsuperscript{2+}-chelation (solution composition (in mM): 127 NaCl, 5 KCl, 1 MgCl\textsubscript{2}, 5 Na-pyruvate, 10 HEPES, 5 EDTA, 1% BSA, pH 7.4) and mechanical vibration. Surface epithelial sheet fragments and intact crypts

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were harvested by low-speed centrifugation, and their viability was tested by trypan blue exclusion. This preparation results in the isolation of intact crypts that possess all the cell types along the crypt length, as well as isolated surface cells or surface cell sheets. The protocols for raising and killing the mice were approved by the local committee for the welfare of animals.

Quantitative RT-PCR in relation to 18S rRNA. Homologous primers for murine NHE1–3 were deduced from published sequence information (see Table 1). The identity of the PCR fragments was confirmed by restriction analysis or cloning and sequencing. RT-PCR was performed as described previously (2). As an internal control, an 18S rRNA fragment was amplified with the use of primers supplied by Ambion (Austin, TX). Briefly, the relationship of the amplification efficiency of the gene of interest v. 18S rRNA during the exponential phase of the reaction was calculated after semilogarithmic plotting of the integrated optical density (OD) values of the digitized bands against the cycle number (Fig. 1 A). To adjust the measured values to the expression levels of the NHE isoforms, the OD values of the different PCR products were corrected according to their length. {

\[ \text{pH} - \text{measurements. pH}_i \text{ was measured fluorometrically after loading crypts or surface cells for 30 min with 5 m\text{M} \text{BCECF in buffer A (in mM: 120 NaCl, 14 HEPES, 7 Tris, 3 K}_2\text{PO}_4, 2 K_2\text{HPO}_4, 1.2 MgSO}_4, 1.2 Ca^{2+}-\text{gluconate, 20 glucose, pH 7.4), fixing them between a glass coverslip and a 0.3 m\text{M polycarbonate membrane (Osmotics, Minnetonka, MN) in a custom-made perfusion chamber on the stage of an inverted microscope (Nikon Diaphot TMD, Nikon, Düsseldorf, Germany), and perfusing with different buffers following the appropriate protocol [buffer A, buffer B (40 m\text{M NaCl were replaced by NH}_4\text{Cl}), buffer C [containing 120 m\text{M tetramethylammonium chloride (TMA-Cl) instead of NaCl], buffer D (containing 120 mM sodium gluconate instead of NaCl), buffer E [containing 40 m\text{M (NH}_4\text{)}_2\text{SO}_4 and 80 mM sodium gluconate instead of NaCl], and buffer F (containing 120 mM TMA-hydroxide and 120 mM gluconic acid lactone instead of NaCl)]. Hyperosmolar solution contained 100 mM mannitol in addition. Calibration and determination of the intracellular buffering power ($\beta_i$) was performed as described previously (2); $\beta_i$ curves (see Fig. 2 B) were neither different between crypts and surface cells nor between cells from NHE2 (+/+) and NHE2 (-/-) mice.

Light and transmission electron microscopy. Immediately after removal, samples of the proximal colon from NHE2 (+/+) and NHE2 (-/-) mice and their normal littermates for histological and morphometric analysis were postfixed for 90 min at room temperature in 2% OsO$_4$ in the same buffered cacodylate/HCl buffer, cut into smaller pieces and stored in the intermediate toluol, and finally embedded in epoxy resin (Serva, Heidelberg, Germany). Semithin sections were stained with 1% Epon resin (Serva, Heidelberg, Germany), and perfusion with different buffers following the appropriate protocol {buffer A, buffer B (40 mM NaCl were replaced by NH$_4$Cl), buffer C [containing 120 mM tetramethylammonium chloride (TMA-Cl) instead of NaCl], buffer D (containing 120 mM sodium gluconate instead of NaCl), buffer E [containing 40 mM (NH$_4$)$_2$SO$_4$ and 80 mM sodium gluconate instead of NaCl], and buffer F (containing 120 mM TMA-hydroxide and 120 mM gluconic acid lactone instead of NaCl]). Hyperosmolar solution contained 100 mM mannitol in addition. Calibration and determination of the intracellular buffering power ($\beta_i$) was performed as described previously (2); $\beta_i$ curves (see Fig. 2 B) were neither different between crypts and surface cells nor between cells from NHE2 (+/+) and NHE2 (-/-) mice.

RESULTS

Relative expression levels of NHE1–3 mRNA in murine colonic surface cells and crypts. NHE1–3 expression levels were measured in isolated crypts and surface cells using a quantitative PCR protocol. These experiments revealed an even distribution of NHE1 mRNA expression in crypt vs. surface cells (Fig. 1B), significantly higher NHE2 mRNA expression levels in colonic crypts than in surface cells (Fig. 1C), and much higher surface cell than crypt expression for NHE3 mRNA (Fig. 1D).

Inhibition curve for HOE-642 on acid-activated Na$^+$/H$^+$ exchange. Because existing inhibition curves for HOE-642 consisted of 22Na$^+$ flux experiments with low extracellular sodium (22, 28), we performed a dose-response study for the inhibition of HOE-642 on acid-activated Na$^+$/H$^+$ exchange under 120 mM extracellular Na$^+$. (Fig. 2D). The rationale of the experiments was the following. If the chosen concentration

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**Table 1. Primers used for genotyping and the semiquantitative PCR experiments**

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence</th>
<th>PCR Product</th>
<th>Annealing</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHE2-wt Forward</td>
<td>CATCTCTATCACAAATGCGCCAACTGCTTGT</td>
<td>450 bp</td>
<td>60°C, 30 s</td>
<td></td>
</tr>
</tbody>
</table>
NHE2 IN MURINE COLON

Fig. 1. NHE1, NHE2, and NHE3 (NHE1–3) mRNA expression levels in colonic crypts and surface cells. A: representative amplification curve illustrating the RT-PCR technique. NHE1–3 and 18S rRNA PCR products were amplified, and the virtual relationship optical density integrated (ODI) of the gene of interest vs. ODI of 18S RNA was calculated every 2 cycles after semilogarithmic plotting of the values against the cycle number. After correction for the different length of the PCR products, this value represents the expression level of the gene of interest. B–D: relative expression levels of NHE1–3 in murine colonic crypts (solid bars) and surface cells (open bars). There is no significant difference (ns) in the NHE1 expression levels between crypts and surface cells. NHE2 mRNA was significantly higher in crypts (0.033 ± 0.009) than in surface cells (0.011 ± 0.0004, P < 0.05). As expected, NHE3 was more abundantly expressed in surface cells (0.034 ± 0.004) than in crypts (0.008 ± 0.001, P < 0.05); n = 4–6 animals in each group.

of 1 μM HOE-642, found to be selective for NHE1 in 22Na+ flux experiments (28), was indeed fully sufficient for NHE1 inhibition, but not high enough to inhibit NHE2, then a shift of the concentration to 0.7 or 3 μM should not cause a change in the percent Na+/H+ exchange inhibition. This was indeed the case. The same applied for 50 μM HOE-642 for NHE2 inhibition. These results demonstrate that the chosen concentrations of HOE-642 and 5-(N,N-dimethyl) amiloride (DMA) are good discriminators of NHE1–3 activity. Most importantly, they also demonstrate that NHE2 is either located basolaterally in colonic crypts or, if apically, is reached by the inhibitors in the crypt lumen. These conclusions are strengthened by the experiments performed in the NHE2 (−/−) crypts.

Differential activation of NHE1–3 by intracellular acidification in crypt and surface cells. Total Na+/H+ exchange rate in crypts was 18.7 ± 1.7 mM/min, and NHE1-selective inhibition with 1 μM HOE-642 reduced this rate by 40% (Fig. 3, A and B). Additional NHE2 inhibition with 50 μM HOE-642 by another 48%, and 700 μM DMA resulted in complete inhibition (Fig. 3B). In surface cells, the total acid extrusion rate (Fig. 3C) was 9.31 ± 0.29. The NHE1 and the NHE1 plus NHE2 selective concentrations of HOE-642 each blocked 20% of the acid extrusion rate; the remaining 55% could only be blocked by 700 μM DMA (Fig. 3D). Thus the major part of pH recovery from an acid load is mediated by NHE2 in colonic crypts and by NHE3 in surface cells.

Cl−-dependent Na+/H+ exchange is not functionally detectable in murine colon. A Cl−-dependent Na+/H+ exchanger has been functionally (24) and molecularly (26) identified in rat colonic epithelium. To assess its potential presence in murine colon, we performed pH recovery experiments in the absence of Cl− (Fig. 3E). Cells were washed several times with buffer D and perfused with this buffer for 15 min to achieve intracellular Cl− depletion. Using the same experimental protocol as above (replacing buffer A for buffer D, buffer B for buffer E, and buffer C for buffer F), acid-activated proton efflux rates were not significantly different from the Cl−-containing control. This argues against the presence of Cl−-dependent Na+/H+ exchange in murine crypts.

An increase in intracellular cAMP activates NHE1 and NHE2 in colonic crypts. Next, we measured the effect of a rise in intracellular cAMP on the acid-activated Na+/H+ exchange rate (Fig. 3F). Forskolin (10−5 M) stimulation increased acid-activated Na+/H+ exchange, and this activation was partially preserved in the presence of NHE1 inhibition and blocked by DMA, suggesting that a rise in cAMP stimulated both NHE1 and NHE2, either directly or via crypt shrinkage after stimulation of anion secretion. This demonstrates that NHE2 is an unlikely candidate for the residual EIPA-sensitive Na+ resorption mechanism found in the small intestine of NHE3 (−/−) mice, because this mechanism was inhibited by cAMP (5).

Hyperosmolarity activates only NHE1 and NHE2 in both crypt and surface cells. Hyperosmolarity caused an activation of colonic Na+/H+ exchange (Fig. 4, A and B). In crypts, inhibition of NHE1 by 1 μM HOE-642 reduced this Na+/H+ exchange rate by 40% and NHE1 and NHE2 inhibition by >90% (Fig. 4B). In surface cells (Fig. 4, C and D), NHE1 inhibition reduced the rate by 60% and additional NHE2 inhibition by >95%. Blocking NHE3 by DMA had no further effect (Fig. 4D), consistent with the inhibition of NHE3 by shrinkage, as found by others (18, 23). After Na+/H+ exchange inhibition by DMA, concomitant Cl−/OH− exchange activation is unmasked and causes a slight intracellular acidification.

Na+/H+ exchange in colonic crypts of NHE2 (−/−) mice. To investigate potential compensatory mechanisms, we studied pH recovery in colonic crypts of NHE2 (−/−) mice (Fig. 5A).

In crypts with similar pHi after the ammonium prepulse, initial proton/base flux rates under control conditions were not significantly different between crypts from NHE2 (+/+ and NHE2 (−/−) mice (19.66 ± 1.48 vs. 20.53 ± 1.19 mM/min). Inhibition of NHE1 reduced the Na+/H+ exchange rate by 60% compared with 50% in normal crypts. As expected, additional NHE2 inhibition did not cause any further decrease in the flux rates. Blocking all NHE isoforms with 700 μM DMA virtually inhibited the remaining 33.5% flux compared with 9.5% in normal crypts.

What could be the compensatory mechanisms that allow normal pH recovery in NHE2 (−/−) crypts? One such mechanism is a higher percentage of NHE1-mediated Na+/H+ exchange. However, we also found a higher HOE-642-insen-
significant higher in the former (Fig. 5, fi), levels were not different, and NHE3 mRNA levels were
/ H11001
copy of semithin sections from colon specimens of NHE2
upregulated in NHE2 defi cient colonic crypts. It appears that cryptal NHE3 expression seen in normal mice is
upregulated in NHE2 deficient colonic crypts.

Light and transmission electron microscopy. Light microscopy of semithin sections from colon specimens of NHE2
(+/+) (Fig. 6, A and B) and NHE2 (-/-) mice (Fig. 6, C and D) showed a regular colon mucosa, and no differences could be
detected between the two groups of animals. In both, the mucosa surface bordering the lumen and the upper parts of the
crypts were predominantly covered by simple columnar epithelial cells, the principal cells or absorptive cells. Large
numbers of mucus-producing goblet cells could be especially detected in deeper crypt regions. In addition, occasional en-
teroendocrine cells could be seen within the epithelial lining. Transmission electron microscopy of colon specimens de-

derived from NHE2 (-/-) mice were indistinguishable from the control specimens (not shown). The absorptive cells were
slender and elongated with a large number of mitochondria, little rough endoplasmic reticulum, and only moderately de-
veloped Golgi fields. Occasionally multivesicular bodies could be detected. The cells were closely neighbored, and intercell-
ular spaces were not detectable. At their luminal borders, they were attached by junctional complexes, which apparently re-
vealed a regular morphological appearance. The apical plasma membrane was elaborated into straight, unbranched microvilli
and covered by a glycocalix. Goblet cells were occasionally at
the surface intermingled with absorptive cells. However, they
were greater in number in the crypts with the bulk located in
the deeper regions. The theca of the goblet cells was tightly
packed with mucus granules. Well-developed Golgi fields were
detected between the theca and the nucleus, and a prominent
endoplasmic reticulum was predominantly found lateral to the
nucleus.

Morphometric analysis. In control animals morphometric analysis revealed a minimal crypt depth of ~67.4 μm and a
maximum depth of 147.5 μm. In NHE2 knockout mice the values ranged between 83.6 and 127.8 μm. However, relative
frequency distribution of the values (Fig. 6E) showed that in
both groups of animals the bulk of the crypts were approxi-
ately 100–125 μm in depth, and statistical analysis revealed
no significant differences between the datasets.

DISCUSSION

The present study was performed to investigate the expres-

sion and function of the three NHE isoforms expressed in
murine colon, with special emphasis on the location and
physiological function of NHE2. NHE1 was evenly expressed
in crypt and surface cells, NHE2 predominantly in the cryptal
region and NHE3 in surface cells. The relative expression
levels paralleled the respective participation of the isoforms in
pH recovery from an acid load. In contrast, hyperosmolarity and cAMP activated NHE1 and NHE2, but not NHE3. Colonic
crypts from NHE2-deficient mice display complete compensa-
tion of acid-activated NHE2 exchange activity by NHE1 and
by a DMA- but not HOE-642-sensitive Na\(^{+}/H\(^{+}\)) exchanger, most likely NHE3. Interestingly, NHE1 expression levels were not different in NHE2 (−/+ ) and (+/+ ) crypts, whereas NHE3 levels were twofold increased.

**NHE localization.** A number of studies have localized NHEs in the colonic epithelium (12, 16). Whereas all studies agreed on the predominantly surface cell localization of NHE3 (4, 16), NHE2 localization studies yielded controversial results (16, 33). In situ hybridization experiments in rat intestinal epithelium have shown a predominant signal in the villus or surface cell region and a relatively weak signal in the crypts (6), whereas an even distribution was found in human colon (12). Our method is very sensitive for measuring relative differences in expression levels of the same mRNA species in different samples (surface cells vs. crypts in this case) and therefore support the recent report by Chu et al. (9) of the presence of NHE2 in the colonic crypt region. They further demonstrate that NHE2 is the NHE isoform with the highest expression level in that area of the colonic epithelium.

**Contribution of NHE1−3 to acid-, hyperosmolarity-, and forskolin-induced Na\(^{+}/H\(^{+}\)) exchange.** Given the fact that different half-lives of NHE1−3 in the plasma membrane have been described (7), expression levels may not correlate with functional relevance. We therefore monitored Na\(^{+}/H\(^{+}\)) exchange activity by fluorometric measurements with BCECF (2, 3) and pharmacologically differentiated the activities of the NHE1 and NHE2 isoforms by using HOE-642, which has been shown to sequentially block NHE1−2 in a dose-dependent manner with a smaller overlap between the isoforms than other inhibitors (28), and assessed additional NHE3 activity by inhibiting it with 700 μM DMA (21). This approach is only valid if no other Na\(^{+}/H\(^{+}\)) exchangers are contributing to acid-,

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**Fig. 3.** NHE1−3 activation by low pH in murine crypts and surface cells, in the presence (A−D) and absence (E) of Cl\(^{-}\) and during cAMP-dependent stimulation (F). Representative pH, traces (A and C) and acid-activated proton flux rates (B and D) in the absence and presence of selective NHE inhibitors, in murine colonic crypts (A and B) and surface cells (C and D). Inhibitors were added 5 min before Na\(^{+}\) readdition. In crypts, 1 μM HOE-642 (HOE1) reduced acid-activated Na\(^{+}/H\(^{+}\)) exchange rates from 18.7 ± 1.7 to 10.4 ± 0.98 mM/min (P < 0.01), 50 μM HOE-642 (HOE50) to 1.98 ± 0.24 mM/min (P < 0.01), and 700 μM DMA to 0.22 ± 0.38 mM/min (P < 0.05). In surface cells, 1 μM HOE-642 reduced acid-activated Na\(^{+}/H\(^{+}\)) exchange rate from 9.3 ± 0.29 to 7.62 ± 0.47 mM/min (P < 0.05), 50 μM HOE-642 to 5.84 ± 0.43 mM/min (P < 0.01), and 700 μM DMA strongly reduced the rate to 0.81 ± 0.15 mM/min (P < 0.01; n = 6–8 crypts from 4–6 mice in each group). E: when crypts were Cl\(^{-}\)-depleted and the experiments performed in the absence Cl\(^{-}\). (see results for details), no significant difference in the acid extrusion rates either under control conditions or after inhibition with 1 and 50 μM HOE642 or DMA was noted, indicating that the measured Na\(^{+}/H\(^{+}\)) exchange activities are entirely Cl\(^{-}\)-independent (n = 4–6 crypts from 3–5 mice in each group). F: total Na\(^{+}/H\(^{+}\)) exchange activity increased in colonic crypts on a rise in intracellular cAMP (10−8 M forskolin, 17.42 ± 0.44 vs. 22.98 ± 2.27 mM/min, P < 0.05), and this activation was preserved during NHE1 inhibition with 1 μM HOE-642 (8.19 ± 0.47 vs. 10.64 ± 0.74, P < 0.05), whereas 50 μM HOE-642 and 700 μM DMA abolished this effect (n = 5–6 crypts from 4–5 mice in each group). This demonstrates NHE1 and NHE2 activation by cAMP.
hyperosmolarity-, and forskolin-activated \(Na^{+}/H^{+}\) exchange in colonic crypts. Because this is the critical issue in our experiments, we have performed a number of experiments to ascertain the fact that we really do differentiate between NHE1, -2, and -3. We have performed concentration-response curves, which clearly show that the chosen HOE-642 concentrations do not have an overlapping effect on NHE1 and NHE2 or on NHE2 and NHE3 (or some other NHE) under our experimental conditions. By using NHE2-deficient mice, we also ascertained that the NHE2-inhibitory concentration of HOE-642 does not inhibit something else. We ruled out the presence of NHE4 (Rossmann, unpublished observation) and of a Cl\(^{-}\)-dependent, amiloride-sensitive \(Na^{+}/H^{+}\) exchanger as found in the rat distal colon (24, 26). Because we measure

![Fig. 4. NHE1 and NHE2 activation of \(Na^{+}/H^{+}\) exchange by hyperosmolarity. A and C: representative pH traces with NHE1 inhibition using 1 \(\mu M\) HOE-642 and subsequent perfusion with hypotonic solution. B and D: 400 mosmol increased total \(Na^{+}/H^{+}\) exchange rate by 0.97 ± 0.08 mM/min in crypts and by 0.59 ± 0.03 mM/min in surface cells. Selective NHE1 inhibition reduced this rate by 40% (0.48 ± 0.06 mM/min, \(P < 0.01\)) in crypts and 83% (0.10 ± 0.02 mM/min, \(P < 0.05\)) in surface cells. Additional NHE2 inhibition completely prevented the hyperosmolarity-induced increase in \(Na^{+}/H^{+}\) exchange rate. As expected, additional NHE3 inhibition with DMA did not significantly alter the flux rates (\(n = 4–7\) crypts from 4–6 mice in each group; HOE1, 1 \(\mu M\) HOE-642; HOE50, 50 \(\mu M\) HOE-642).

![Fig. 5. Acid-activated NHE1 and NHE3 activity in crypts of NHE2 knockout mice (A) and NHE1 (B) and NHE3 (C) expression levels in normal and NHE2-deficient crypts. A: acid-activated \(Na^{+}/H^{+}\) exchange rates in colonic crypts from NHE2 (-/-) mice were similar as in normal crypts (19.66 ± 1.48 vs. 20.53 ± 1.19 mM/min). HOE-642 (1 \(\mu M\)) reduced this rate by 60% (8.12 ± 1.26 mM, \(P < 0.01\)) compared with 50% in NHE1 (+/+), HOE-642 (50 \(\mu M\)) inhibited \(Na^{+}/H^{+}\) exchange in NHE2 (+/+) mice only. Blocking all NHE isoforms with DMA virtually inhibited the remaining 33.5% (0.58 ± 0.3 mM, \(P < 0.01\)) in NHE2 (-/-) crypts compared with 9.5% in NHE2 (+/+) crypts (\(n = 5–7\) crypts from 3–4 mice in each group). B and C: NHE1 expression levels were equal, whereas NHE3 was significantly more abundant in NHE2 knockout than in normal crypts (0.00087 ± 0.00018 vs. 0.0014 ± 0.0014, \(P < 0.05\)) (3 mice, triplicates for each mouse).]
Na⁺-dependent changes in pHᵢ to assess NHE activity and not ²²Na⁺ uptake, the presence of Na⁺ conductances does not interfere with our measurements.

Of interest was the fact that Cl⁻ removal did not seem to affect Na⁺/H⁺ exchange rates at the pHᵢ that we acidified the colonic crypts to. Aharonovitz et al. (1) had recently shown that in NHE1–3-transfected AP1 cells (NHE-deficient Chinese hamster ovary cells), Cl⁻ replacement by nitrate or isethionate caused a decrease in the exchange rates of all three isoforms. On the other hand, other groups had found maximal Na⁺/H⁺ exchange rates to be independent of Cl⁻ in membrane preparations (e.g., Ref. 19). The reason for the discrepancy is not clear, but one possibility is that not all Cl⁻ replacements are inert. Another one is that the volume changes caused by Cl⁻ removal affect different cell types differently, and a third is that coupled Cl⁻/anion exchange may be necessary for intracellular gradient dissipation in some cells.

When heterologously expressed in PS120 fibroblasts, NHE1–3 proteins have different half-lives in the plasma membrane, as recently reported by Cavet et al. (7). The half-life of NHE1 was almost twice as long as that of NHE3, and NHE2 had the shortest. When we assume that NHE1–3 in native cells have similar differences in their half-lives [which needs not be the case, as PS120 fibroblasts have been found to lack important PDZ-domain adapter proteins for these transporters (36) which may influence localization of these NHEs in the membrane], this would explain why NHE1 expression levels, which are only one-third to one-fourth of those found for NHE2, are associated with a similar acid-activated transport activity. In addition, activation of NHE3 by pHᵢ has been found to be slower than for NHE1 and NHE2, possibly explaining a relatively minor role in cryptal pHᵢ regulation when the other NHEs are highly active (14). Taking all this into account, there is a surprisingly good correlation between the relative expres-

Fig. 6. Semithin sections of colon mucosa from NHE2 (+/+ ) (A and B) and NHE2 (−/−) (C and D) mice and relative frequency distribution of crypt depth (E). In A–D, regular simple columnar epithelial lining of the surface and the crypts can be noted. Toward the bottom of the crypts, goblet cells increase in number (bars, 20 μm). E: analysis of crypt depth showed similar results in both groups of animals; the largest number of values for the crypt length lies between 97 and 127 μm. Significant differences are not evident.
sion levels of the different NHEs and their respective activity in acid-activated Na\(^+/\)H\(^+\) exchange in surface and crypts. In summary, our findings suggest that the determination of the NHE2-mediated part of Na\(^+/\)H\(^+\) exchange is reliable using the pharmacological tools that we have.

With this approach, we found similarities between NHE2 and NHE1, in that they are both activated by low pH, hyperosmolarity, and cAMP. This has been observed for NHE1 and, more so, NHE2, heterologously expressed in the Na\(^+/\)H\(^+\)-deficient Chinese hamster ovary cell line AP1 (1, 18), and for NHE2 in LLC-PK\(_1\) cells (8), whereas an inhibition of NHE2 by hyperosmolarity has been described in PS120 cells (23) and by cAMP in the Caco-2 subclone C2bbe (5). Another recent publication studied the ability of NHE1-, NHE2-, and NHE3-transfected PS120 cells to perform a regulatory volume increase after hyperosmolar shrinkage (34). They found that only NHE1, not NHE2 and NHE3, could mediate this effect and that a point mutation in the first extracellular loop renders NHE2 sensitive to volume changes. This resembles the situation found for the small intestinal sodium channel hNaiC, where a point mutation in an extracellular domain results in an enormous increase in channel activity and amiloride sensitivity (27), suggesting, in the author’s opinion, the existence of an unknown physiological regulator of this channel. The reason(s) for these discrepancies are completely unclear at present and may be cell-type related. For example, NHE1 is activated by cAMP in a number of cell types and not affected in others. When transfected into OK cells, it is sorted to the basolateral membrane, but inhibited by cAMP, just like the endogenous amiloride-resistant apical Na\(^+/\)H\(^+\) exchanger (15). A similar situation may exist for NHE2 expressed in different tissues or species.

The high distal ileal and colonic expression levels and the apical location suggested a possible involvement in electroneutral NaCl absorption, similar to NHE3 (35), and several studies are in accordance with this hypothesis (11, 35). However, NHE2-deficient intestine displays neither gross morphological changes nor an obvious absorptive defect (29). Additional disruption of NHE2 in NHE3-deficient mice does not lead to any worsening of the diarrheal state (20), and NHE2 (-/-) mice were found to have similar small intestinal sodium absorption rates as controls (13). Gawaiens et al. (13) did, however, find a residual highly EIPA-sensitive Na\(^+\) absorptive rate in NHE3 (-/-) jejunum, which was abolished by forskolin stimulation. The stimulatory rather than inhibitory effect of cAMP on NHE2 (found in this study) makes it unlikely that NHE2 is the residual Na\(^+\) absorptive mechanism in NHE3-deficient intestine.

To get further insight into the physiological role of colonic NHE2, we studied potential pH\(_i\)-regulatory defects in NHE2 (-/-) crypts, as well as the compensatory mechanisms that may prevent them. Acid extrusion rates after intracellular acidification were normal in NHE2-deficient crypts, the part of pH\(_i\) recovery apparently mediated by NHE1 was larger, and interestingly, there was clearly also a higher percentage of non-NHE1 (and non-NHE2), DMA-sensitive Na\(^+/\)H\(^+\) exchange activity. We therefore measured NHE3 expression levels in NHE2 (+/+) and (-/-) crypts and indeed found significantly higher NHE3 expression levels but unchanged NHE1 expression levels in NHE2 (-/-) crypts. Obviously, NHE1 is not operating at its maximal capacity during pH\(_i\) recovery from pH\(_i\) ~ 6.4 and can increase transport rate without a need for an increase in transcriptional activity. The interesting question is, why is NHE3 upregulated in NHE2-deficient colon? When NHE2 is heterologously expressed in acid-suicide-selected LLC-PK\(_1\) cells, endogenous NHE3 expression is inhibited, and this precedes functional NHE2 expression (32). So this would suggest that some function of NHE2, possibly control of local Na\(^+\) concentration, or something else, influences NHE3 expression in a reciprocal fashion. It is, of course, tempting to speculate that this upregulation of NHE3 may compensate an Na\(^+\)-absorptive defect in NHE2 (---) colon. Due to restricted breeding facilities for NHE2 (---) mice and the difficulty of selectively blocking NHE3 in intact mucosa, we have not been able to perform sufficient experiments to rule in or out an increase in NHE3-mediated Na\(^+\) absorption in NHE2 (---) murine colon.

In summary, our data suggest that NHE2 is strongly expressed in murine colonic crypts where it is activated by low pH\(_i\), hyperosmolarity, and an increase in intracellular cAMP levels. In the absence of NHE2 expression, other NHEs compensate for NHE2-mediated pH\(_i\) recovery in NHE2-deficient crypts, and both NHE3 expression and participation in pH\(_i\) recovery are upregulated. Further studies will have to clarify the exact membrane location and provide detailed insight into the cellular functions of NHE2 in secretory epithelia.

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