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Na^+-Dependent Recovery of Intracellular pH from Acid Loading in Mouse Colonic Crypt Cells

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Tsuchiya, Y., Hayashi, H. and Suzuki, Y. *Na^+-Dependent Recovery of Intracellular pH from Acid Loading in Mouse Colonic Crypt Cells.* Tohoku J. Exp. Med., 2001, **193** (1), 1-11 —— The membrane transport mechanism for regulating the intracellular pH value (pH_i) was investigated in mouse distal colon crypt cells. pH_i was measured by microfluorometry in an isolated crypt fragment loaded with the pH-sensitive fluoroprobe, 2′, 7′-bis-(2-carboxyethyl)-5-(6) carboxyfluorescein. The pH_i recovery process after acid loading induced by a 40 mM NH_4Cl prepulse was almost totally dependent on Na^+ in both the presence and absence of CO_2/HCO_3^- in the perfusion solution. In the CO_2/HCO_3^--free, HEPES-buffered solution, amiloride partially inhibited the pH_i recovery rate from acid loading with an ED_{50} value of 15 μM and maximum inhibition of 83%. In a CO_2/HCO_3^- solution, amiloride inhibited the pH_i recovery rate with an ED_{50} value of 18 μM, which was similar to that in the HEPES-buffered solution, while the rate of pH_i recovery remaining in the presence of the maximum effective concentration of amiloride was significantly larger than that in the HEPES-buffered solution. The Na^+-dependent pH_i recovery from the acid loading was significantly less (by 18%) in the presence of forskolin. These results suggest that the pH_i recovery from acid loading was mediated by 1) amiloride-sensitive Na^+/H^+ exchanger, 2) the amiloride-insensitive Na^+/H^+ exchanger, and 3) the Na^+- and HCO_3^--dependent acid extruder. The pH_i recovery could be inhibited by cAMP. —— NHE; Na^+/H^+ exchanger; Na^+/HCO_3^- cotransporter; BCECF; microfluorometry

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The intracellular pH level (pH_i) plays an important role in a variety of cellular processes, including cellular metabolism, protein synthesis, contractility, ion-channel conductivity, and cell-cycle control (Madshus 1988; Boron 1992). The pH_i regulation in epithelial tissue is also intimately linked to the transepithelial transport of various substrates (DeSoignie and Sellin 1994; Chu and Montrose 1997; Hayashi and Suzuki 1998).

The colon, the terminal part of the gastrointestinal tract, both secretes and absorbs electrolytes and fluid. The gland-like crypt is generally, but not exclusively, responsible for the secretory processes, as opposed to the surface epithelium which is mainly, but not exclusively, involved in absorptive processes (Singh et al. 1995). Previous studies by different approaches have suggested several Na^+-dependent and Na^+-independent acid and base transporters playing roles in the regulation of pH_i in mammalian crypt cells. Transport studies have demonstrated amiloride-sensitive, and amiloride-insensitive (Cl^--dependent) Na^+/H^+ exchange activities (Diener et al. 1993; Abrahamse et al. 1994; Teleky et al. 1994; Rajendran et al. 1995; Chu and Montraose 1997; Rajendran et al. 1999). In accordance with these findings, expression and localization studies have shown the presence of isoforms of the Na^+/H^+ exchanger, NHE1 and NHE2, in the crypt cells (Bookstein et al. 1994, 1997; Dudeja et al. 1996; Hoogerwerf et al. 1996). In addition, the Na^+/HCO_3^- cotransporter has been detected in the colonic mucosa, although the precise localization within the colonic mucosa remains to be determined (Rajendran et al. 1991; Romero et al. 1997; Abuladze et al. 1998; Ishibashi et al. 1998). Furthermore, the Cl^-/HCO_3^- (and Cl^-/OH^-) exchanger and K^+/H^+ exchanger have also been reported to be present in crypt cells (Singh et al. 1995; Ikuma et al. 1998; Rajenderan et al. 1998; Alper et al. 1999; Rajendran and Binder 1999). Finally, organic acid transporters have been suggested to be involved in the pH_i regulation of crypt cells (Chu and Montrose 1995, 1997).

In the present study, pH_i was measured by microfluorometry in an isolated crypt from the mouse distal colon which had been loaded with pH-sensitive dye. We particularly focused on the Na^+-dependent pH_i regulatory mechanisms because, although they appear to play a major part in pH_i regulation of the colonic crypt as discussed above, they had not been characterized in detail. To this end, we precisely examined the inhibitory effect of amiloride on the pH_i recovery from acid loading, and compared the amiloride effects in the presence and absence of CO_2/HCO_3^- in the bathing solution.

METHODS

Solutions

The HEPES-buffered standard solution contained (in mM) NaCl, 114; Na-gluconate, 21; K_2PO_4, 2.4; KH_2PO_4, 0.6; $CaCl_2$, 1.2; $MgCl_2$, 1.2; N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 10; and D-glucose 10. The pH value was adjusted to 7.4 with NaOH at 37°C, and was gassed with 100% O_2. The CO_2/HCO_3^--buffered standard solution contained (in mM) NaCl, 119; $NaHCO_3$, 21; K_2HPO_4, 2.4; KH_2PO_4, 0.6; $CaCl_2$, 1.2; and $MgCl_2$, 1.2. This solution was gassed with 95% O_2-5% CO_2 pH 7.4 at 37°C. In the ammonium-containing solution for NH_4Cl pulsing, 40 mM NH_4Cl was added in place of 40 mM NaCl. The Na^+-free solution was prepared by an equimolar substitution with N-methyl-D-glucamine and choline.

Microfluorometric recording of the pH_i level

The present experiments were performed under the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences as recommended by the Physiological Society of Japan. Male ddY mice (Japan SLC, Shizuoka) weighing between 35–40 g were acclimatized on a standard diet, with food and

water provided ad libitum. The animals were killed by dislocation of the cervical vertebrae. A segment of the distal colon was excised and opened along the mesenteric border. The serosa, submucosa and muscular layer were stripped away with fine forceps to obtain a mucosal preparation. This mucosal preparation was then cut into pieces of approximately 1 mm × 1 mm with a scalpel. These pieces were shaken gently in an oxygenated HEPES-buffered solution containing dispase (2.3 mg ml^{-1}) and collagenase (0.6 mg ml^{-1}) for 30 minutes at 37°C to obtain preparations containing crypt fragments. These preparations were then washed several times by centrifugation, before being resuspended in a HEPES-buffered solution containing 12 μM of 2′7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) for 5 minutes in the dark at room temperature (20–25°C). The BCECF-AM had first been dissolved to a concentration of 8 mM in dimethyl sulphoxide. After dye loading, the preparations were washed with the HEPES-buffered solution and submitted to microfluorometric pH_i measurement as described previously (Hayashi and Suzuki 1998). The preparations were seeded on a specially designed glass perfusion vessel, which had been coated with Cell-Tak cell adhesive, and the vessel was centrifuged at 200 × g for 5 minutes at room temperature to fix the preparations. The vessel was then placed on the stage of an inverted microscope (TMD, Nikon, Tokyo) equipped with a microscopic dual-wavelength fluorometer (CAM-230, Japan Spectroscopic, Tokyo), and perfused at 7 ml min^{-1} with the gas-equilibrated solution and maintained at 35–37°C by prewarming the solution. The whole area of a selected crypt fragment without a surface epithelial layer was illuminated alternately at 440 nm and 500 nm for 50 milliseconds by a chopper at 100 Hz, and the fluorescence was measured at 520–570 nm through a band-pass filter. The pH_i value was calculated from the mean fluorescence ratio (F_{500}/F_{440}) every 2 seconds. All these procedures were controlled by a computer (Macintosh LC) which was equipped with a data acquisition and analysis system (Lab View 2, National Instruments, Houston, TX, USA). Any autofluorescence from the crypt cells was found to be negligible. The pH_i level was calibrated in situ by the nigericin-K^+ method. Dye-loaded crypt cells were incubated with nigericin (20 μM) for 5 minutes, and then with a high-K^+ perfusion solution at various pH values (KCl, 130 mM; NaCl, 10 mM; $CaCl_2$, 1.5 mM; $MgCl_2$, 1.0 mM; HEPES or MES, 10 mM; the pH value was adjusted with Tris at 37°C) to determine the relationship between the fluorescence ratio (F_{500}/F_{440}) and pH_i value. Calibration curves were obtained over a pH range of 6–8. An acid load was imposed on the crypt cells by a 60-second pulse perfusion with 40 mM NH_4Cl. To assess the effects of inhibitors on the pH_i recovery from acid loading, three measurements were performed consecutively on each tissue sample, first in the absence of an inhibitor, second in the presence of an inhibitor, and then finally in the absence of an inhibitor again. The mean value of the pH_i recovery rate from the first and final measurements was taken as the control value and compared with the rate in the presence of the inhibitor for the same tissue sample. The result of the experiment was discarded when the pH_i recovery rate of the final control value was less than half of that of the first. The initial rate of pH_i recovery after acid loading was determined by taking data collected during the initial linear phase of pH_i recovery.

Chemicals

We obtained amiloride and nigericin from Sigma (St. Louis, MO, USA), BCECF-AM from Molecular Probes (Eugene, OR, USA), dispase from Godo Susei (Tokyo), collagenase from Yakult (Tokyo), and Cell-Tak from Becton Dickinson Labware (Bedford, MA, USA).

Data and statistical analyses

The relationship between the amiloride concentration and the inhibition of pH_i recovery rate was fitted to the Michaelis-Menten equation by using a non-linear least-squares algorithm (Ultra Fit, Biosoft, Cambridge, UK). Each experimental value is presented as the mean$\pm$S.E. (n=number of animals). Two-group comparisons were analyzed by an unpaired or paired Student's t-test. In all experiments, a p-value of <0.05 has been considered significant.

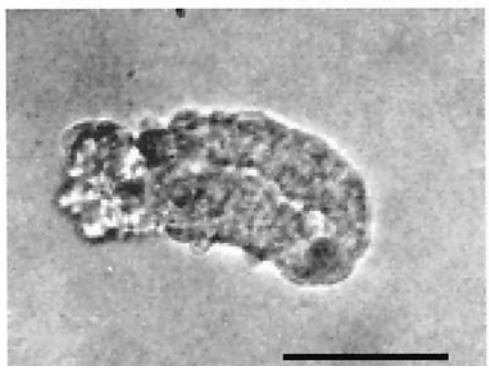

Fig. 1. Transmitted light image of an isolated crypt fragment loaded with 2′7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF).
This image was taken before the start of the NH_4Cl-pulse experiment. A crypt fragment containing surface epithelial cells was not used in the present study. Bar=50 μm.

RESULTS

Fig. 1 shows the transmitted light image of an isolated crypt fragment. It appears to retained its original shape. In the resting condition, the pH_i level of the crypt cells was 7.54 ± 0.08 ($n=24$) in the CO_2/HCO_3^--free, HEPES-buffered solution. When the crypt cells were perfused with the CO_2/HCO_3^--buffered solution, the resting pH_i level was 7.23 ± 0.05 ($n=33$), a value significantly lower than that in the HEPES-buffered solution.

We first examined the recovery of pH_i from acid loading in the CO_2/HCO_3^--free, HEPES-buffered solution so as to minimize the possible activities of the HCO_3^--dependent pH_i regulatory mechanism. An acid load was imposed on the cells by pulsed perfusion with 40 mM NH_4Cl. As shown in Fig. 2, upon replacing the perfusate with an Na^+-free solution, the pH_i level rapidly decreased with little recovery (nadir $pH_i=6.42\pm0.07$, $n=24$). However, when the perfusate was replaced with the Na^+-containing solution, the pH_i level rapidly returned to the resting level. Amiloride, a known inhibitor of the Na^+/H^+ exchanger, had no noticeable effect on the pH_i level under the Na^+-free condition after acid loading, while it attenuated the Na^+-dependent pH_i recovery rate (Fig. 2, center). The inhibitory effect of amiloride was largely reversed after removing

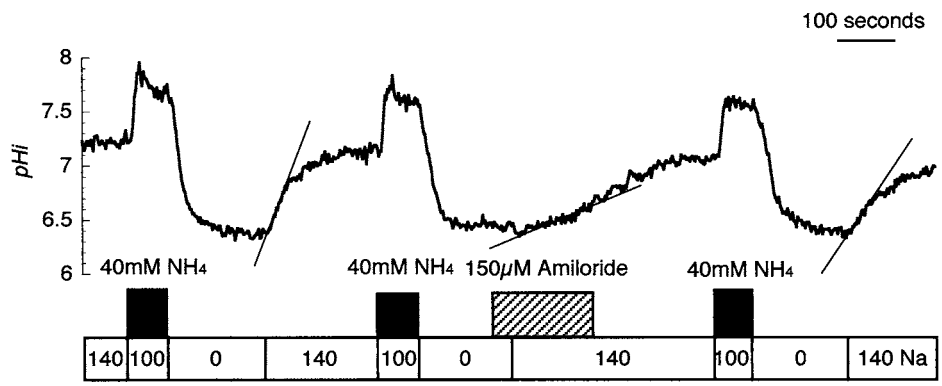

Fig. 2. Na^+-dependent pH_i recovery from acid loading imposed by pulsed NH_4Cl and the effect of amiloride on pH_i recovery in the HEPES-buffered solution.
The crypt cells were acid-loaded 3 times by being exposed to 40 mM NH_4Cl for 60 seconds. Lines indicate the initial rate of pH_i recovery. This is a representative trace.

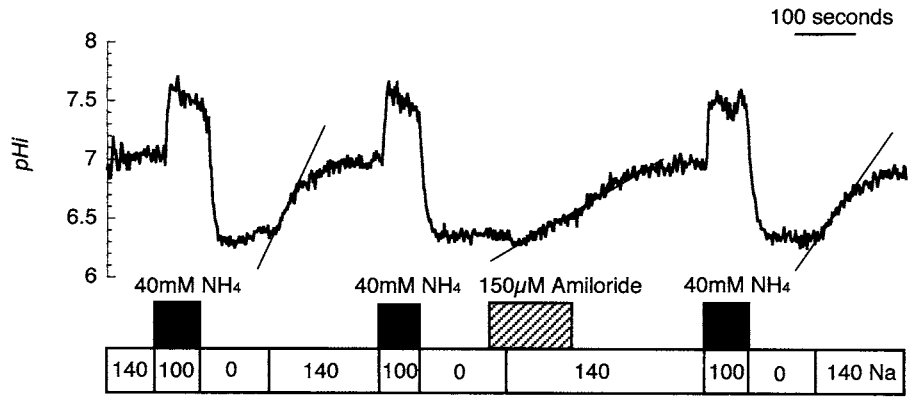

Fig. 3. Na^+-dependent pH_i recovery from acid loading imposed by pulsed NH_4Cl and the effect of amiloride on pH_i recovery in the CO_2/HCO_3^--buffered solution.
The crypt cells were acid-loaded 3 times by being exposed to 40 mM NH_4Cl for 60 seconds. Lines indicate the initial rate of pH_i recovery. This is a representative trace.

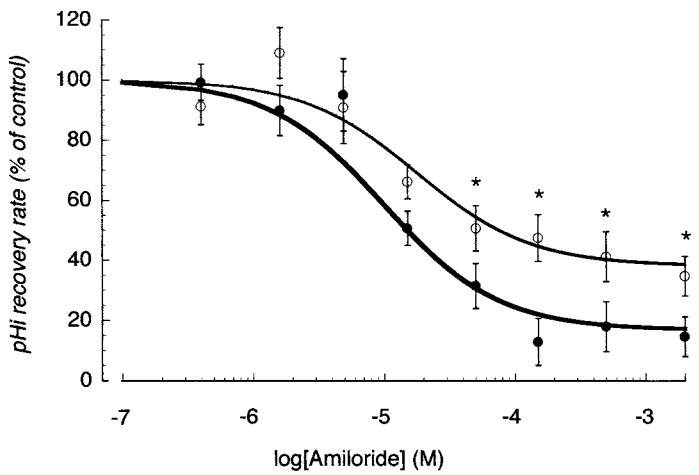

Fig. 4. Dose-response relationship for the inhibition of pH_i recovery by amiloride in the HEPES-buffered solution and the CO_2/HCO_3^--buffered solution.
The mean value of pH_i recovery rate from the first and third measurements (amiloride-free) was taken as the control value and compared with the rate in the presence of amiloride (percentage of the control) (Figs. 2 and 3). Closed circles show the values obtained with the HEPES-buffered solution and open circles show the values obtained with the CO_2/HCO_3^--buffered solution. The continuous curves were determined by fitting the data to the Michaelis-Menten. The number of animals was 3-6 for each concentration of amiloride.
*Significantly different from the HEPES-buffered solution ($p<0.05$).

amiloride from the perfusate. We also performed a similar experiment in the CO_2/HCO_3^--buffered solution (Fig. 3). Pulsed perfusion with 40 mM NH_4Cl and then with the Na^+-free solution caused a decrease in pH_i with little recovery. The nadir pH_i in the CO_2/HCO_3^- buffered solution was 6.23 ± 0.03 ($n=33$), which was significantly lower than that in HEPES-buffered solution. Amiloride had no noticeable effect on the pH_i level under the Na^+-free condition after acid loading, while it attenuated the Na^+-dependent pH_i recovery. Fig. 4 summarizes the result of the foregoing experiments with various concentrations of amiloride in both the presence and absence of CO_2/HCO_3^-. The inhibitory effect of amiloride would have reached its maximum at 0.15 mM because the pHi recovery rates with 0.15 and 2 mM amilor-

ide were not significantly different in either the absence or presence of HCO_3^-/CO_2. The dose-response curve for the inhibition of Na^+-dependent pH_i recovery by amiloride indicated a half-maximal concentration (IC_{50}) of 15 μM and a maximal inhibition of 83% in the CO_2/HCO_3^--free, HEPES-buffered solution. In the CO_2/HCO_3^--buffered solution, a half-maximal concentration (IC_{50}) of 18 μM and maximal inhibition of 62% were obtained. Thus, the IC_{50} value for amiloride inhibition was similar in the presence and absence of CO_2/HCO_3^-. On the other hand, the percentage of the pH_i recovery rate which was not inhibited by a saturating concentration of amiloride (0.15-2 mM) was significantly larger in the HCO_3^-/CO_2 solution than in the HEPES solution, suggesting the presence of an Na^+ and HCO_3^--dependent, but amiloride-insensitive, acid extrusion mechanism. To confirm the presence of such an Na^+- and HCO_3^--dependent acid extrusion mechanism, however, we need to compare the pH_i recovery rate at the same pH_i level, because the intrinsic buffer capacity of the cell is a function of pH_i level. However, in the present study, the pH_i level at which the pH_i recovery rate after acid loading was determined was slightly but significantly lower in the HCO_3^-/CO_2 solution than in the HEPES solution (see the nadir pH_i values already shown). To compromise these difficulties, we first compiled the results from the experiments with a saturating concentration of amiloride (0.15, 0.5 and 2 mM). We then excluded several results so that the pH_i recovery rate could be compared at the same pH_i level between the presence and absence of HCO_3^-/CO_2 ($pH_i = 6.33 \pm 0.08$ with a range of 6.05-6.75 for the HEPES solution and $pH_i = 6.28 \pm 0.06$ with a range of 5.95-6.58 for the HCO_3^-/CO_2 solution, $p = 0.60$. See the legend to Fig. 5 for details), the result being summarized in Fig. 5. The pH_i recovery rate that was not inhibited by a maximum concentration of amiloride was, indeed, significantly larger in the presence than

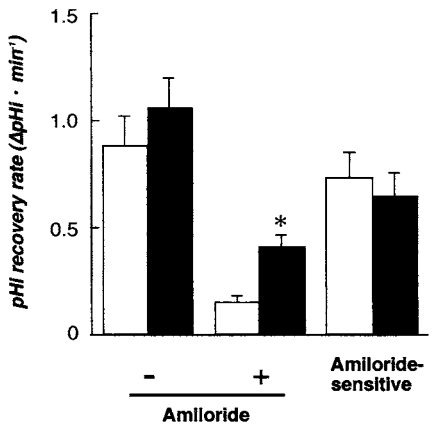

Fig. 5. Amiloride-sensitive and amiloride-insensitive components of pH_i recovery in the absence (HEPES) and presence of CO_2/HCO_3^-.
Open bar: HEPES. Closed bar: CO_2/HCO_3^-. The rate of pH_i recovery induced by adding Na^+ was determined in the absence (-) and presence (+; the amiloride-insensitive component) of a saturating concentration of amiloride (0.15-2 mM). The amiloride-sensitive components represent a difference between the presence and absence of amiloride. This result was extracted from the data shown Fig. 4. The data with 0.15, 0.5 and 2 mM amiloride were combined for each solution condition. We then excluded several data with a relatively high nadir pH_i value in the HEPES-buffered solution (3/12) and those with a relatively low nadir pH_i value in the HCO_3^-/CO_2 solution (2/14), while the mean value for the percentage inhibition was largely unchanged (from 84% to 83% in HEPES-buffered solution and from 58% to 61% in the HCO_3^-/CO_2-buffered solution). This resulted in the pH_i value at which the recovery rate was determined to be similar between these two solution conditions. $n = 9$ for the HEPES and $n = 12$ for the HCO_3^-/CO_2 solution. $^*p < 0.01$ compared with the HEPES case.

in the absence of HCO_3^-/CO_2. This difference in the pH_i recovery rate does somewhat underestimate the difference in the H^+ extrusion rate because, in the HCO_3^-/CO_2 solution, intracellular HCO_3^- may have additionally contributed to the intracellular buffer capacity (Boron 1992). Thus, it is likely that an HCO_3^--dependent acid extrusion mechanism that is not

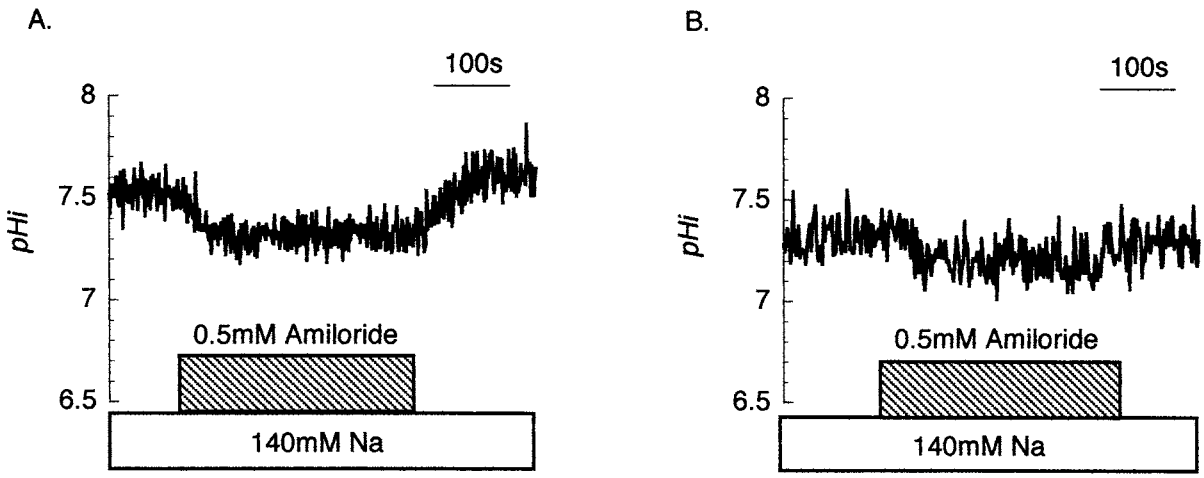

Fig. 6. Effects of 0.5 mM amiloride on the resting pH_i value. Representative traces in the HEPES-buffered solution (A) and the HCO_3^-/CO_2 solution (B) are presented.

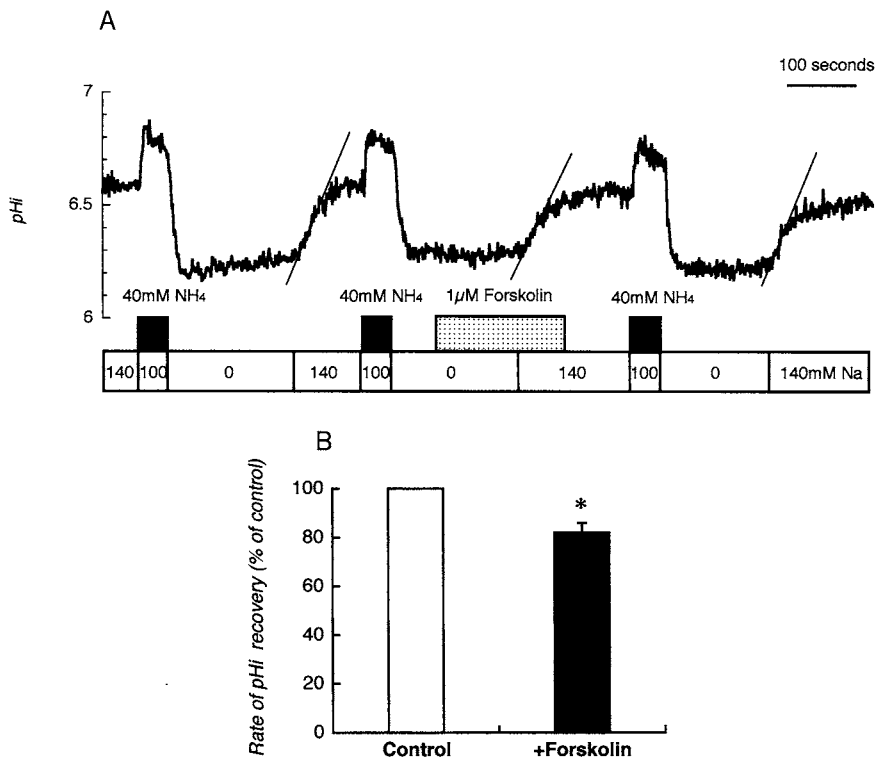

Fig. 7. Effects of forskolin on the Na^+-dependent pH_i recovery in the HCO_3^-/CO_2 solution. A) The crypt cells were acid-loaded three times. Lines indicate the initial rate of pH_i recovery. This is a representative trace from four similar results. B) The mean value of pH_i recovery rate from the first and third measurements (forskolin-free) was taken as the control value and compared with the rate in the presence of forskolin. $n=4$. *Compared with the group of control ($p<0.05$).

inhibited by a maximum concentration of amiloride was actually present.

We next examined the effect of amiloride on the resting pH_i level in both the HEPES-buffered and the HCO_3/CO_2-buffered solutions. As shown in Fig. 6, the pH_i level was

significantly decreased by 0.5 mM amiloride, the decrease being from 7.50 ± 0.16 to 7.37 ± 0.10 in the HEPES-buffered solution ($n=3$) and from 7.26×0.08 to 7.13 ± 0.11 in the HCO_3/CO_2-buffered solution ($n=7$).

Finally, we examined the effect of cAMP on the Na^+-dependent pH_i recovery after acid loading. As shown in Fig. 7, the addition of forskolin, an activator of adenylyl cyclase, had no noticeable effect on the pH_i level under the Na^+-free condition after acid loading, while it significantly attenuated the Na^+-dependent pH_i recovery rate ($18 \pm 4\%$ inhibition, $n=4$, Fig. 7B).

Discussion

Our aim was to identify acid-base transporters that are involved in the recovery of the pH_i level from acid loading in crypt cells of the mouse distal colon. The results show that (1) the recovery of pH_i from acid loading was largely dependent on extracellular Na^+; (2) amiloride partly inhibited the recovery of pH_i; (3) the rate of the amiloride-insensitive pH_i recovery was greater in the presence of CO_2/HCO_3^- than in its absence.

We demonstrated that the recovery of pH_i from acid loading by pulsed NH_4 was largely dependent on extracellular Na^+ and was inhibited by amiloride. These results suggest that an amiloride-sensitive Na^+-H^+ exchanger (NHE) was mainly responsible for the pH_i recovery (Noel and Pouyssegur 1995; Orlowski and Grinstein 1997; Wakabayashi et al. 1997). Previous studies that applied a similar method to that used here have also suggested the involvement of NHE in pH_i recovery from acid loading in crypt cells from various species (Diener et al. 1993; Abrahamse et al. 1994; Teleky et al. 1994; Chu and Montrose 1995). Multiple isoforms of the NHE family have been identified; of these, NHE1, NHE2 and NHE3 were expressed in the colonic mucosa (Orlowski et al. 1992; Wang et al. 1993; Bookstein et al. 1994; 1997 Cho et al. 1994; Hoogerwerf et al. 1996). The NHE isoforms differ in their sensitivity to amiloride: NHE1 and NHE2 are inhibited by amiloride with an IC_{50} value of 5–10 μM in the presence of a physiological concentration of Na^+, while NHE3 is relatively resistant to amiloride with an ED_{50} of 300–400 μM (Chambery et al. 1997; Schwark et al. 1998). We therefore determined, for the first time, the dose-inhibition relationship for amiloride over a wide range of amiloride concentration to identify the NHE isoforms responsible in the mouse crypt cells. The result shows that the initial pH_i recovery rate was inhibited by amiloride with an IC_{50} value of 15 μM in the CO_2/HCO_3^--free, HEPES-buffered solution, and with an IC_{50} value of 18 μM in the CO_2/HCO_3^--buffered solution. Therefore, both NHE1 and NHE2 could have been involved in the pH_i recovery process that was inhibited by a low concentration of amiloride. Tissue distribution studies have suggested that NHE1 is distributed in both surface and crypt cells, while NHE2 is present mainly in the surface epithelial cells, but to a lesser extent in the crypt cells of the colon (Bookstein et al. 1994, 1997; Dudeja et al. 1996; Hoogerwerf et al. 1996). It is, therefore, likely that the component of pH_i recovery that was inhibited by amiloride in the present crypt preparation was mediated mainly by NHE1, although it cannot be excluded that it was also partly mediated by NHE2. It is unlikely that NHE3 was also involved in the present pH_i recovery process, because the degree of inhibition was no different between 0.15 mM and 2 mM of amiloride in both the presence and absence of HCO_3^-/CO_2 in the bathing solution (Fig. 4). If NHE3, which can be inhibited by amiloride with an ED_{50} value of 300–400 μM (Chambery et al. 1997; Schwark et al. 1998), have had substantially contributed to the pH_i recovery, a greater inhibition of the pH_i recovery rate would have been apparent in the presence of 2 mM amiloride than of 0.15 mM amiloride. In addition, NHE3 has been shown to be mainly distributed in the surface epith-

elial cells of the colon (Bookstein et al. 1994, 1997; Cho et al. 1994; Hoogerwerf et al. 1996).

In the HCO_3^--free buffered solution, amiloride at a concentration of 2 mM did not completely inhibit the recovery of the pH_i level, although the nature of this Na^+-dependent, amiloride-resistent pH_i characteristic is not addressed here. A novel, Cl^--dependent Na^+/H^+-exchange activity has been suggested in the rat colon that is resistant to amiloride and mainly present in the crypt cells (Rajendran et al. 1995, 1999; Dudeja et al. 1996). Whether a similar Na^+/H^+ exchanger is also present in mouse crypt cells and responsible for the amiloride-resistant pH_i recovery remains to be elucidated.

In the presence of a maximum concentration of amiloride, the rate of pH_i recovery was larger in the HCO_3^--buffered solution than that observed in the HEPES-buffered solution (Fig. 5). The difference in the H^+ extrusion rate may even be larger than that, because the intracellular buffering capacity should be larger in the presence of HCO_3^-/CO_2 than its absence due to intracellular HCO_3^-, although, at a pH_i level of less than 6.5, the buffer capacity based on intracellular HCO_3 is small (generally less than 10% of the total buffer capacity) (Boron 1992; Hayashi and Suzuki 1998). Thus, the larger pH_i recovery rate under the HCO_3^-/CO_2-buffered conditions indicates that the acid extrusion (or alkaline loading) rate was actually larger under these condition than that under HCO_3^-/CO_2-free conditions. Therefore, an Na^+- and HCO_3^--dependent, but amiloride-insensitive pH_i recovery from acid loading is likely to be present in the mouse crypt cells. Similar pH_i measurements on human colonic crypt cells have demonstrated the involvement of Na^+-dependent, HCO_3^- transporters in the pH_i recovery from acid loading which was inhibited by a stilben-derivative anion transport inhibitor. The molecular identity of the Na^+- and HCO_3^--dependent, but amiloride-insensitive pH_i recovery mechanism was unknown, but could have been an Na^+-HCO_3^- cotransporter (NBC) or related protein (Romero and Boron 1999). A functional study on the membrane vesicle has reported the presence of a stilben-sensitive, electrogenic Na^+-HCO_3^- cotransporter in the basolateral membrane from the rat distal colon, although this transporter was also inhibited by amiloride (Rajendran et al. 1991). In addition, NBC mRNA has been detected in the large intestine (Romero et al. 1997; Abuladze et al. 1998; Ishibashi et al. 1998).

Forskolin inhibited the Na^+-dependent pH_i recovery rate, indicating that cAMP can downregulate the Na-dependent pH_i recovery mechanism. The inhibition of either Na^+/H^+ exchanger or $NaHCO_3$ cotransporter by cAMP could be a possible explanation for it. Certain members of NHE and NBC families have been reported to be inhibited by the cAMP-protein kinase A-signaling pathway in many cell types (Noel and Pouyssegur 1995; Wakabayashi 1997; Romero and Boron 1999; Soleimani and Burnham 2000). It has been shown in crypt cells that cAMP activates apical Cl^- channels as well as basolateral K^+ channels, thereby enhancing transepithelial Cl^- secretion (Greger 2000). The physiological role of the inhibition of Na^+-dependent pH_i recovery by cAMP for the cAMP-induced Cl^- secretion should be determined.

The colon has the unique opportunity for its luminal surface to be exposed to a high concentration of short-chain fatty acids which are avidly absorbed through the epithelial cells. Short-chain fatty acids have been shown to decrease the pH_i level in colonic crypt cells (Diener 1993; DeSoignie and Sellin 1994; Chu and Montrose 1995; Chu and Montrose 1997) as well as in a variety of other cell types. Thus, the acid extrusion mechanisms demonstrated here in crypt cells may well be important for maintaining short-chain fatty acid absorption. Na^+-dependent and amiloride-sensitive mechanism has previously been demonstrated in

colonic epithelial cells to be partly responsible for pH_i recovery after exposure to short-chain fatty acids (DeSoignie and Selline 1994; Chu and Montrose 1997). The results of the present study, in addition, have shown that the addition of 0.5 mM amiloride decreased the pH_i level under the resting condition in both the HCO_3^-/CO_2^- and HEPES-buffered solutions, suggesting that an amiloride-sensitive Na^+/H^+ exchanger was in operation to exclude metabolic acid production in crypt cells at least under the present experimental conditions.

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