Differential localization of colonic H\(^+\)-K\(^+\)-ATPase isoforms in surface and crypt cells

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Rajendran, Vazhaikkurichi M., Satish K. Singh, John Geibel, and Henry J. Binder. Differential localization of colonic H\(^+\)-K\(^+\)-ATPase isoforms in surface and crypt cells. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G424–G429, 1998.—Two distinct colonic H\(^+\)-K\(^+\)-adenosinetriphosphatase (H\(^+\)-K\(^+\)-ATPase) isoforms can be identified in part on the basis of their sensitivity to ouabain. The colonic H\(^+\)-K\(^+\)-ATPase \(\alpha\)-subunit (HK\(\alpha\)) was recently cloned, and its message and protein are present in surface (and the upper 20% of crypt) cells in the rat distal colon. These studies were performed to establish the spatial distribution of the ouabain-sensitive and ouabain-insensitive components of both H\(^+\)-K\(^+\)-ATPase activity in apical membranes prepared from surface and crypt cells and K\(^+\)-dependent intracellular pH (pH\(_i\)) recovery from an acid load both in isolated perfused colonic crypts and in surface epithelial cells. Whereas H\(^+\)-K\(^+\)-ATPase activity in apical membranes from surface cells was 46% ouabain sensitive, its activity in crypt apical membranes was 96% ouabain sensitive. Similarly, K\(^+\)-dependent pH\(_i\) recovery in isolated crypts was completely ouabain sensitive, whereas in surface cells K\(^+\)-dependent pH\(_i\) recovery was insensitive to ouabain. These studies provide compelling evidence that HK\(\alpha\) encodes the colonic ouabain-insensitive H\(^+\)-K\(^+\)-ATPase and that a colonic ouabain-sensitive H\(^+\)-K\(^+\)-ATPase isoform is present in colonic crypts and remains to be cloned and identified.

\(K^+\)-dependent intracellular pH regulation; rat colon; H\(^+\)-K\(^+\)-adenosinetriphosphatase

ACTIVE ABSORPTION of \(K^+\) is a novel function of the mammalian distal colon and is likely energized by an apical membrane H\(^+\)-K\(^+\)-adenosinetriphosphatase (H\(^+\)-K\(^+\)-ATPase) (1). The \(\alpha\)-subunit of the colonic H\(^+\)-K\(^+\)-ATPase (HK\(\alpha\)) has recently been cloned and sequenced (7, 11) and is a member of a gene family of P-type ATPases that also includes Na\(^+\)-K\(^+\)-ATPase and gastric H\(^+\)-K\(^+\)-ATPase. Given the 50–60% shared homology between these ATPases, it is not surprising that the pharmacological properties of the colonic H\(^+\)-K\(^+\)-ATPase overlap those of the ouabain-sensitive, omeprazole/SCH-28080-insensitive Na\(^+\)-K\(^+\)-ATPase and the ouabain-insensitive, omeprazole/SCH-28080-sensitive gastric H\(^+\)-K\(^+\)-ATPase.

Physiological studies of active \(K^+\) absorption in the rat distal colon and pharmacological characterization of colonic H\(^+\)-K\(^+\)-ATPase provide compelling evidence for two different colonic H\(^+\)-K\(^+\)-ATPases (9, 15), one that is insensitive to ouabain and stimulated by aldosterone and a second that is sensitive to ouabain but nonresponsive to aldosterone. Which of these two \(\alpha\)-subunits is encoded by HK\(\alpha\) cDNA is not known.

Expression studies of HK\(\alpha\) have provided important information regarding its segmental and spatial distribution but have yielded conflicting data regarding its sensitivity to ouabain (5, 6, 9, 13). First, in situ hybridization studies localized HK\(\alpha\) message to the surface (and the upper 20% of crypt) cells of the distal colon (11). Localization of the HK\(\alpha\) message to the proximal colon of normal animals has not been reported. Second, antibodies raised to a fusion protein developed from a segment of HK\(\alpha\) cDNA identified a protein only in the apical membrane of surface cells of the distal colon (13). Third, infection of Sf9 cells with baculovirus transfected with HK\(\alpha\) cDNA resulted in expression of ouabain-insensitive H\(^+\)-K\(^+\)-ATPase activity (13). This activity in Sf9 cells was completely inhibited by orthovanadate and was inhibited 18% by SCH-28080. H\(^+\)-K\(^+\)-ATPase activity in apical membranes isolated from epithelial cells of rat distal colon, which are predominantly surface cells, is 100% vanadate sensitive, 55% ouabain insensitive, and 18% SCH-28080 sensitive (13); it was therefore concluded that HK\(\alpha\) cDNA most likely encoded the ouabain-insensitive isoform (13). Fourth, in contrast to these studies, expression studies of HK\(\alpha\) cRNA in Xenopus oocytes provide evidence of both ouabain-sensitive and relatively ouabain-insensitive \(^{32}P\) uptake (5, 6).

We have recently developed methods to study specific functions of surface and crypt cells independently (16). This present study was therefore designed to determine the ouabain sensitivity of two different colonicocyte functions, apical membrane H\(^+\)-K\(^+\)-ATPase activity and K\(^+\)-dependent recovery of intracellular pH (pH\(_i\)) from an acid load both in surface and in crypt cells. These results establish that these two functions in crypt cells are completely ouabain sensitive. Because HK\(\alpha\) message and protein are localized to surface cells but not crypt cells, these studies provide compelling evidence that HK\(\alpha\) message encodes the ouabain-insensitive H\(^+\)-K\(^+\)-ATPase \(\alpha\)-subunit isoform in surface cells of rat distal colon.

METHODS

H\(^+\)-K\(^+\)-ATPase Determinations

Apical membrane preparation. Apical membranes from surface cells and from crypt cells were prepared from the distal colon of normal male Sprague-Dawley rats (200 g), as described previously (17). In brief, surface cells and crypt glands were isolated by diverlag cation chelation technique using EDTA and utilized for apical membrane preparation (16). Surface cells and crypt glands were homogenized with Omni Mix (Waterbury, CT) at 2,500 and 4,000 rpm, respectively. Brush-border caps were isolated by Percoll gradient (10%) centrifugation. Apical membranes were purified by disrupting the brush-border caps by homogenization (Polys...
tron, Westbury, NY) and centrifugation (Sorvall RC5B, SS34 rotor, 10,999 rpm, 15 min). Apical membranes used for enzyme assays were resuspended in 50 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer (pH 7.4) containing 250 mM sucrose. Resuspended membranes were stored at −70°C until further use. Purity of the surface cell apical membrane was assessed by 10- to 12-fold enrichment of H^+-K^-ATPase activity (9), whereas crypt cell apical membrane purity was assessed by the presence of a Cl^-dependent Na^+/H^+ exchange activity (18).

Enzyme assay. H^+-K^+-ATPase activity was measured by the method of Forbush (10) as described previously (9). In brief, 500 μl reaction mixture (50 mM Tris-HCl, 5 mM MgCl2, 20 mM KCl, 5 mM ATP, and 10 μg protein) was incubated for 30 min at 37°C. The reaction was arrested by adding 1 ml of ice-cold stop solution (3% acetic acid, 1% sodium dodecyl sulfate, and 0.5% ammonium molybdate in 0.5 N HCl), and the color was developed by adding 1.5 ml of 1% sodium arsenate in 5% acetic acid. Mg^2+-ATPase activity was measured in the absence of KCl. H^+-K^+-ATPase activity was measured in the presence of both Mg^2+ and K^+. H^+-K^-ATPase activity was calculated by subtracting the Mg^2+-ATPase activity from the activity obtained in the presence of both Mg^2+ and K^+. H^+-K^-ATPase activity was also measured in the presence of 1 mM ouabain. H^+-K^-ATPase activity measured in the presence of ouabain represents ouabain-insensitive H^+-K^-ATPase activity. Ouabain-sensitive H^+-K^-ATPase activity was calculated by subtracting H^+-K^-ATPase activity in the presence of ouabain from H^+-K^-ATPase activity in the absence of ouabain. ATPase activity is presented as micromoles P_i liberated per milligram protein per minute. Results are presented as means ± SE of three different membrane preparations; eight animals were used for each preparation. Protein was measured by the method of Lowry et al. (14).

K^+-Dependent pH_i Determinations

Measurement of pH_i in isolated crypts. pH_i of individual colonic cells of isolated perfused crypts was determined as previously described (16). Briefly, individual crypts were isolated using a hand dissection technique and perfused in vitro as previously described (15). After isolation, the individual crypts were transferred to a thermostatically controlled chamber mounted on the stage of an inverted microscope. After transfer the crypts were double cannulated using two glass coverslips with a volume of ~100 μM per side and was mounted on the stage of a Zeiss IM-35 inverted microscope, equipped for epi-illumination (20). Luminal and basolateral (i.e., "bath") solutions were switched via computer-controlled five-way valves with zero dead space. Chamber temperature was monitored and maintained at 37°C by prewarming the bath solution with a flow rate of ~3 ml/min.

Surface cells were loaded with the pH-sensitive dye BCECF (18) by switching the mucosal solution to an N-2-hydroxysulfopiperazone-N'-2-ethanesulfonic acid (HEPES)-Ringer solution containing 10 μM BCECF-AM and then temporarily halting flow for ~15 min. Reinitiating mucosal flow after dye loading minimized extracellular accumulation of dye. The dye uptake pattern was restricted to the surface epithelial cell, similar to that described in other studies (8,12). At the end of each experiment, the absence of both mucosal-to-serosal leak and surface cell viability was confirmed with trypan blue (Sigma, St. Louis, MO). Experiments in which cells failed to exclude trypan blue were not used (~1% of experiments in which BCECF was initially loaded).

Solutions. The HEPES-Ringer solution contained (in mM) 125 NaCl, 5 KCl, 1.0 CaCl_2, 1.2 MgSO_4, 2 NaH_2PO_4, 10.2 glucose, and 32 HEPES. KCl replaced NaCl (and vice versa) in selected experiments. The pH of all solutions was 7.40 at 37°C unless otherwise indicated. High-K^+/-nigericin calibration solution contained (in mM) 10 μM nigericin, 105 KCl, 1.0 CaCl_2, 1.2 MgSO_4, 2 H_2PO_4, 10.5 glucose, 32.2 HEPES, and 46.4 N-methyl-D-glucamine (NMDG); pH was adjusted with HCl or NMDG. All solutions were equilibrated with air and adjusted to an osmolality of 300–310 mosmol/kg with NaCl or mannitol.

Optical system. A solid-state intensified TV system, with digital image acquisition and analysis, was used in these studies, as described previously (20). Briefly, fluorescent emission or intensity of emitted light (I) was monitored at 530 ± 20 nm while dye was alternately excited at 440 and 490 nm.

RESULTS

Distribution of H^+-K^-ATPase Isoform Activity in Surface and Crypt Cells

Surface cells. Both ouabain-sensitive and ouabain-insensitive H^+-K^-ATPase activities have previously...
been identified in apical membranes isolated predominantly from surface cells (9). HKα message and protein level were expressed only in surface (and the upper 20% of crypt) cells (11, 13). It is not known whether both ouabain-sensitive and ouabain-insensitive isoforms of H⁺-K⁺-ATPase are expressed solely in surface cells or in both surface and crypt cells; therefore, H⁺-K⁺-ATPase activity was assayed in apical membranes isolated from surface and crypt cells. As presented in Fig. 1, H⁺-K⁺-ATPase activity (94.0 ± 11.3 nmol Pi liberated·mg protein⁻¹·min⁻¹) in surface cell apical membrane was partially inhibited by 1 mM ouabain (50.9 ± 7.0 nmol·mg protein⁻¹·min⁻¹). The ouabain-insensitive fraction of H⁺-K⁺-ATPase activity was 54% of the total activity. These results are similar to those previously presented (9).

Crypt cells. H⁺-K⁺-ATPase activity was also measured in apical membranes isolated from crypt cells and is presented in Fig. 1. H⁺-K⁺-ATPase activity of crypt cell apical membranes was substantially lower than that of surface cell apical membranes (24.0 ± 4.2 vs. 94.0 ± 11.3 nmol·mg protein⁻¹·min⁻¹). In contrast to surface cell apical membranes, H⁺-K⁺-ATPase of crypt cell apical membranes was almost completely (96%) inhibited by 1 mM ouabain. These results indicate that the ouabain-insensitive H⁺-K⁺-ATPase isoform is expressed only in apical membranes of surface cells and that the ouabain-sensitive isoform is the only H⁺-K⁺-ATPase isoform expressed in apical membranes from crypt cells. Because ouabain-insensitive H⁺-K⁺-ATPase is present in surface but not in crypt cells, a spatial distribution identical to that of HKα message and protein (11, 13), it is likely that HKα encodes the ouabain-insensitive H⁺-K⁺-ATPase α-subunit.

Although these results demonstrate the presence of both ouabain-sensitive and ouabain-insensitive H⁺-K⁺-ATPase isoforms in surface cell apical membranes, it is possible that only the ouabain-insensitive isoform is present in surface cells and that the ouabain-sensitive H⁺-K⁺-ATPase activity identified in this surface cell apical membrane preparation represents contamination of this preparation by crypt cell apical membranes. Because the method to separate surface and crypt cells may result in some cross-contamination, additional studies were performed to determine K⁺-dependent regulation of pHᵢ in using video fluorescent microscopy. This method visually analyzes individual cells and should resolve whether the presence of both ouabain-sensitive and ouabain-insensitive H⁺-K⁺-ATPase activity in surface cell apical membranes represents partial contamination of the surface cell preparation with crypt cells.

K⁺-Dependent Regulation of pHᵢ

Crypt cells. To study K⁺-dependent regulation of pHᵢ in crypt cells, a recently developed preparation of isolated colonic crypts (16, 19) was used that permits separate perfusion of apical (lumen) and basolateral (bath) membranes. An acid load was induced by removing Na⁺ from both lumen and bath solutions while maintaining lumen K⁺ concentration at 5 mM. Figure 2 demonstrates that the addition of 20 mM K⁺ to the lumen solution increased pHᵢ to its baseline value. The presence of 0.5 mM ouabain before the readdition of 20 mM K⁺ completely prevented K⁺-dependent pHᵢ recovery (Fig. 3A). These studies demonstrate that K⁺-dependent pHᵢ recovery from an acid load in crypt cells is exclusively ouabain sensitive (Table 1) and is consistent with the presence of the ouabain-sensitive H⁺-K⁺-ATPase isoform in crypt cell apical membranes.

Surface cells. Apical K⁺-dependent acid extrusion was also studied in polarized surface colonocytes, using

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1 Use of an identical method to acid load both crypt and surface cells was not possible, as the luminal membrane of crypt cells is essentially impermeable to NH₄⁺ (20) and the chamber used to study surface cells does not permit ready access to the basolateral aspect. As a consequence, bilateral Na⁺ removal and an NH₄⁺ pump prepulse were used for acid loading in crypt and surface cells, respectively.
a newly developed preparation. After an NH₄Cl-induced acid load (2), pHᵢ recovered rapidly to resting pHᵢ values in the presence of both Na⁺ and K⁺. In the absence of both Na⁺ and K⁺, pHᵢ did not alkalinize toward resting pHᵢ values until either K⁺ or Na⁺ was returned to the mucosal solution. pHᵢ recovery was initiated after the addition of 5 mM mucosal K⁺, and further recovery was observed on increasing mucosal K⁺ concentration to 20 mM (Fig. 3B). Recovery in the presence of either 5 or 20 mM mucosal K⁺ was not inhibited by 1.0 mM mucosal ouabain (Fig. 3B and Table 1). Because HKα message and protein are localized to surface cells but not crypt cells, these studies of K⁺-dependent regulation of pHᵢ provide further support for the high probability that the functional activities encoded by HKα isoform are not ouabain sensitive.

Role of voltage. To exclude the possibility that K⁺-dependent recovery of pHᵢ in response to an acid load is a result of membrane depolarization and not apical membrane electroneutral H⁺/K⁺ exchange, experiments were performed with barium in both surface and crypt cells. Two separate sets of experiments were performed. In one series of experiments the effect of luminal 5 mM barium on basal pHᵢ was determined. Barium will depolarize the apical membrane, so if K⁺-dependent recovery of pHᵢ were a result of membrane depolarization, the addition of barium should have caused an intracellular alkalization. Table 2 demonstrates that the luminal addition of barium to either surface or crypt cells did not alter basal pHᵢ.

In a second series of studies, barium was added to the luminal solution before the induction of an acid load. The acid load was induced in surface cells by a prepulse of NH₃/NH₄Cl and in crypt cells by the removal of luminal and bath Na⁺. Table 2 demonstrates that barium failed to alter the recovery of pHᵢ in response to an acid load in either surface or crypt cells. These two sets of experiments exclude the possibility that voltage as a result of K⁺-induced apical membrane depolarization is responsible for the observed pHᵢ recovery from an acid load that is observed after the addition of K⁺ (Figs. 2 and 3).

Table 1. K⁺-dependent pHᵢ recovery from acid load in surface and crypt cells

<table>
<thead>
<tr>
<th>Surface cells</th>
<th>dPHᵢ/dt</th>
<th>n</th>
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<tbody>
<tr>
<td>Control</td>
<td>22.1 ± 4.6</td>
<td>11</td>
</tr>
<tr>
<td>+ Ouabain</td>
<td>24.7 ± 5.6</td>
<td>12</td>
</tr>
<tr>
<td>Crypt cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28.2 ± 9.0</td>
<td>25</td>
</tr>
<tr>
<td>+ Ouabain</td>
<td>21.3 ± 3.0</td>
<td>25</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = number of surface cells studied from 5 animals for control studies and 4 animals for ouabain studies, and number of crypt cells studied from 5 animals for both control and ouabain studies. Intracellular pH (pHᵢ) recovery rates from an acid load after an increase in K⁺ concentration from 5 to 20 mM. All recovery rates were calculated from an initial pHᵢ of 6.7. dPHᵢ/dt units are × 10⁻⁴ pH units/s. Concentration of ouabain was 0.5 mM in crypt cell studies and 1.0 mM in surface cell studies.

Table 2. Effect of barium on basal steady-state pHᵢ and K⁺-dependent pHᵢ recovery in surface and crypt cells

<table>
<thead>
<tr>
<th></th>
<th>Basal pHᵢ*</th>
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<tbody>
<tr>
<td></td>
<td>pH units</td>
</tr>
<tr>
<td>Surface cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.43 ± 0.17</td>
</tr>
<tr>
<td>+ Barium</td>
<td>7.43 ± 0.18</td>
</tr>
<tr>
<td>Crypt cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.20 ± 0.15</td>
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<tr>
<td>+ Barium</td>
<td>7.17 ± 0.13</td>
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</tbody>
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<thead>
<tr>
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<th>K⁺-Dependent pHᵢ Recovery</th>
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<tr>
<td></td>
<td>dpHᵢ/dt</td>
</tr>
<tr>
<td>Surface cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.3 ± 7.6</td>
</tr>
<tr>
<td>+ Barium</td>
<td>11.6 ± 3.1</td>
</tr>
<tr>
<td>Crypt cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>27.3 ± 8.1</td>
</tr>
<tr>
<td>+ Barium</td>
<td>27.5 ± 6.2</td>
</tr>
</tbody>
</table>

Data are means ± SD; N = number of cells studied/number of animals. *Steady-state pHᵢ was determined before and after addition of 5 mM barium to the lumen solution of surface cells and 2 mM barium to the lumen solution of crypt cells. tPHᵢ recovery from an acid load after an increase in K⁺ concentration from 5 to 20 mM. All recovery rates were calculated from an initial pHᵢ of 6.85. dPHᵢ/dt units are × 10⁻⁴ pH units/s. In barium studies 5 mM barium was added to surface cells before addition of the NH₄Cl/NH₃ prepulse and 2 mM barium was added to crypt cells before removal of both lumen and bath Na⁺.
**DISCUSSION**

Physiological studies of active K\(^+\) absorption in the distal colon of the rat have revealed the presence of two distinct K\(^+\) transport processes (9, 15). In these studies of active K\(^+\) absorption, which were performed across isolated colonic mucosa under voltage-clamp conditions, K\(^+\) absorption consists of mucosal Na\(^+\)-sensitive and mucosal Na\(^+\)-insensitive components (15). Aldosterone stimulated the mucosal Na\(^+\)-sensitive, but not the mucosal Na\(^+\)-insensitive, component of active K\(^+\) absorption (15). The mucosal Na\(^+\)-sensitive component was also ouabain insensitive, whereas the mucosal Na\(^+\)-insensitive component was ouabain sensitive. In addition, two distinct H\(^+\)-K\(^+\)-ATPase fractions, ouabain sensitive and ouabain insensitive, were also identified in studies with apical membranes isolated from rat distal colon (9). Neither of these studies was designed, however, to identify the spatial localization of either the active K\(^+\) absorptive process or H\(^+\)-K\(^+\)-ATPase (9, 15).

In contrast, these present studies were planned to establish whether these different K\(^+\)-dependent processes were exclusively present in surface and/or in crypt epithelial cells. The results of these studies provide compelling evidence that a single K\(^+\)-dependent transport process is present in crypt epithelial cells. K\(^+\)-dependent pH\(_i\) recovery from an acid load in crypt cells was completely ouabain sensitive (Figs. 2 and 3A), whereas H\(^+\)-K\(^+\)-ATPase activity in apical membranes isolated solely from crypt cells was 96% inhibited by ouabain (Fig. 1). Thus it appears that there is but a single K\(^+\)-dependent transport process in crypt epithelial cells, one that is ouabain sensitive. These studies also suggest that it is likely that only one K\(^+\)-dependent pH\(_i\) transport process is present in surface epithelial cells and that, in contrast to that in crypt cells, this K\(^+\)-dependent pH\(_i\) transport process is ouabain insensitive (Fig. 3B).

The results of these physiological studies must be interpreted in relation to the recent cloning and localization of the colonic H\(^+\)-K\(^+\)-ATPase. HK\(_{\alpha}\) cDNA was cloned from rat colon and is a member of a gene family of related P-type ATPases (7, 11). Both HK\(_{\alpha}\) message and protein have been localized to surface (and to 20% of upper crypt) epithelial cells of the rat distal colon, and Western blot analysis and immunocytochemical studies have established that HK\(_{\alpha}\) protein is present in the apical membrane of surface cells (11, 13). Controversy exists regarding the ouabain sensitivity of the H\(^+\)-K\(^+\)-ATPase that is encoded by this HK\(_{\alpha}\) cDNA (5, 6, 13). The H\(^+\)-K\(^+\)-ATPase activity expressed in Sf9 cells by HK\(_{\alpha}\) cDNA was ouabain insensitive (13). In contrast, the ouabain sensitivity of \(^{86}\)Rb uptake after the injection of HK\(_{\alpha}\) cRNA into Xenopus oocytes depended on the specific \(\beta\)-subunit cRNA that was co-injected. Coinjection of an amphibian bladder \(\beta\)-subunit resulted in 100% ouabain-sensitive \(^{86}\)Rb uptake (6), whereas the ouabain-sensitive fraction of \(^{86}\)Rb uptake by oocytes co-injected with either gastric H\(^+\)-K\(^+\)-ATPase \(\beta\)-subunit cRNA or Na\(^+\)-K\(^+\)-ATPase \(\beta\)-subunit cRNA was less than 50% of total \(^{86}\)Rb uptake (5).

Although the ouabain sensitivity of H\(^+\)-K\(^+\)-ATPase activity and that of K\(^+\)-dependent pH\(_i\) recovery in crypt cells manifest excellent concordance, there was modest dissociation of their inhibition by ouabain in surface cells. Because pH\(_i\) recovery in surface cells was measured by video fluorescent microscopy in individual cells, ouabain insensitivity of pH\(_i\) recovery undoubtedly represents a surface cell function. In contrast, it is not unlikely that the apical membranes isolated from surface cells are partially contaminated with ouabain-sensitive H\(^+\)-K\(^+\)-ATPase activity from crypt apical membranes. This suggestion is based on our recent demonstration (16) that Cl\(^-\)-dependent Na\(^+\)/H\(^+\) exchange is the sole Na\(^+\)/H\(^+\) exchange in crypt apical membrane vesicles, whereas in surface cell apical membrane vesicles Cl\(^-\)-dependent exchange Cl\(^-\)-dependent exchange in crypt apical membrane vesicles is but a single K\(^+\) movement.

In these studies an intracellular acid load was induced either by the removal of lumen and bath Na\(^+\) in crypt cells or by an NH\(_3\)/NH\(_4\)Cl prepulse in surface cells. We established in both surface and crypt cells that in the absence of lumen Na\(^+\), recovery from an acid load required lumen K\(^+\). Although this represents the first identification of a K\(^+\)-dependent recovery from an acid load in small or large intestinal epithelia, we do not as yet know whether this represents an important control mechanism to regulate pH\(_i\) in colonocytes or is merely an expression of H\(^+\)/K\(^+\) exchange that mediates transepithelial K\(^+\) movement.

The results of these present studies, combined with the recent localization of HK\(_{\alpha}\) message and protein to surface epithelial cells (11, 13), provide compelling evidence that HK\(_{\alpha}\) cDNA encodes a protein that is ouabain insensitive and that the ouabain-sensitive H\(^+\)-K\(^+\)-ATPase isoform is both exclusively localized in crypt cells and encoded by a cDNA that has not as yet been cloned.

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