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Evidence for NHE3-mediated Na transport in sheep and bovine forestomach

Imtiaz Rabbani,1,2,* Christiane Siegling-Vlitakis,1,* Bardhyl Noci,3 and Holger Martens1

1Institute of Veterinary Physiology, Free University of Berlin, Berlin, Germany; 2University of Veterinary & Animal Sciences Lahore, Pakistan; and 3Clinic for Ruminants and Swine, Free University of Berlin, Berlin, Germany

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Rabbani I, Siegling-Vlitakis C, Noci B, Martens H. Evidence for NHE3-mediated Na transport in sheep and bovine forestomach. Am J Physiol Regul Integr Comp Physiol 301: R313–R319, 2011. First published May 25, 2011; doi:10.1152/ajpregu.00580.2010.—Na absorption across the cornified, multilayered, and squamous rumen epithelium is mediated by electrogenic amiloride-insensitive transport and by electroneutral Na transport. High concentrations of amiloride (>100 μM) inhibit Na transport, indicating Na+/H+ (NHE) activity. The underlying NHE isoform for transepithelial Na absorption was characterized by mucosal application of the specific inhibitor HOE642 for NHE1 and S3226 for NHE3 in Ussing chamber studies with isolated epithelia from bovine and sheep forestomach. S3226 (1 μM; NHE3 inhibitor) abolished electroneutral Na transport under control conditions and also the short-chain fatty acid-induced increase of Na transport via NHE. However, HOE642 (30 μM; NHE1 inhibitor) did not change Na transport rates. NHE3 was immunohistochemically localized in membranes of the upper layers toward the lumen. Expression of NHE1 and NHE3 has been previously demonstrated by RT-PCR, and earlier experiments with isolated rumen epithelial cells have shown the activity of both NHE1 and NHE3. Obviously, both isoforms are involved in the regulation of intracellular pH, pHi. However, transepithelial Na transport is only mediated by apical uptake via NHE3 in connection with extrusion of Na by the basolaterally located Na-K-ATPase. The missing involvement of NHE1 in transepithelial Na transport suggests that the proposed “job sharing” in epithelia between these two isoforms probably also applies to forestomach epithelia: NHE3 for transepithelial transport and NHE1 for, among others, pH, and volume regulation.

Further characterization of Na transport has revealed that, in the absence of permeable anions, Jnet Na equals Isc (5). Isc and, hence, electroneutral Na transport, is abolished by serosal ouabain (17), is insensitive to mucosal amiloride (28), is modulated by divalent cations (22), and is mediated by a potential sensitive cation conductance in the apical membrane (20, 25).

Electroneutral Na transport is generally larger than the Isc and depends on the presence of CO2/HCO3− and Cl− in the buffer solution (5, 13, 28). Because Cl− is also actively absorbed (37, 39), coupled with Na (27), Chien and Stevens (5) have proposed a NaCl cotransport or alternatively parallel-working Na+/H+ (NHE) and Cl−/HCO3− exchangers. The proposed model of Na absorption via NHE has been corroborated by the stimulation of electroneutral Na transport by short-chain fatty acids (SCFA) (7, 15, 38), by its inhibition through mucosal amiloride (1 mM) (28, 38), and indirectly, with its inhibition by acetazolamide (10, 38). Obviously, two uptake mechanisms of Na (electroneural and electrogenic) exists in the luminal membrane of the rumen epithelium, and transepithelial transport of Na is accomplished by extrusion of Na via the Na-K-ATPase in deeper cell layers of the epithelium (16, 19, 26, 34).

Na+/H+ exchange was first described by Murer et al. (30), and currently, 10 isoforms (NHE1–10) have been described (1, 9, 21). NHE1 is ubiquitously expressed and, among others, is involved in the regulation of cell volume and pH (18). In epithelia, NHE1 is located in the basolateral membrane (6, 33) and is considered as a “housekeeper.” NHE3 is present in the apical membrane of many epithelia, such as the small and large intestine, gall bladder, and various parts of the kidney tubule (9).

The localization of NHE3 in the apical membrane and NHE1 in the basolateral membrane of polarized cells has led, in epithelia, to the generally accepted model of “job sharing” between these two NHE isoforms: NHE3 mediates transepithelial electroneutral Na transport (9), and NHE1 is involved in the regulation of pH and homeostasis of cell volume (33).

In a recent study, Graham et al. (16) have demonstrated the expression of mRNA of NHE1–3 and NHE8 by RT-PCR in bovine rumen epithelium. In contrast to the general assumption, Graham et al. showed with immunostaining that NHE1 is apically localized in the stratum granulosum of the multilayered squamous rumen epithelium. Graham et al. (16) also discuss the interaction of NHE1 with SCFA transport, as has been proposed by Gäbel and Sehested (14): the uptake of SCFA across the luminal membrane by nonionic diffusion, the release of protons, and the activation of NHE. Furthermore, the stimulation of NHE1 should lead to the generation of “local-

SODIUM IS THE MAJOR CATION in isotonic saliva of ruminants (2), with 1.2–1.5 mol Na being daily secreted by an adult sheep (8), which is flowing into the rumen together with 0.1–0.2 mol dietary Na. Some 50% of salivary Na and a corresponding amount of dietary Na is absorbed from the rumen against a concentration and electrical gradient (blood-side positive), indicating an active transport mechanism (8). Early in vitro studies with isolated rumen epithelium demonstrated a Na absorption (Jnet Na) in the mucosal-serosal direction under Ussing chamber conditions, confirming the assumption of active Na transport (39). Furthermore, Jnet Na is larger than the short circuit current (Isc), indicating electroneutral Na transport (39). This in vitro observation (Jnet Na > Isc) has been confirmed in all following studies with rumen from sheep (11), goats (5), cattle (5, 38), or deer (40).

* I. Rabbani and C. Siegling-Vlitakis contributed equally to this work.

Address for reprint requests and other correspondence: H. Martens, Dept. of Veterinary Physiology, Free Univ. of Berlin, Oertzenweg 19b, 14163 Berlin, Germany (e-mail: martens@vetmed.fu-berlin.de).
ized extracellular acidity to promote nonionic diffusional uptake of SCFA (16). As a consequence, the SCFA-dependent increase of transepithelial Na transport (15, 38) should be mediated by NHE1 in the luminal membrane, in contrast to the general model of the functions of NHE3 and NHE1 in epithelia (see above) and our previous findings concerning the inhibition of Na transport via NHE by amiloride (28).

NHE1 is inhibited by amiloride in the micromolar range (IC50 10.7 μM) (35), which is without an effect on Jms Na (mucosal to serosal sodium flux) in studies of isolated sheep rumen epithelium (28). Higher concentrations (1 mM) significantly reduce Jms and Jnet Na in sheep (28) and bovine rumen epithelium (38). Furthermore, the recovery of pH, in ruminal epithelial cells (REC) after an acid load by butyrate is significantly reduced by amiloride, by S3226 (inhibitor of NHE3), and by HOE642 or HOE694 (inhibitor of NHE1) (29, 36).

These data clearly indicate the physiological presence of NHE1 and NHE3 in rumen epithelium (29, 36), and the corresponding RT-PCR products with a high-sequence homology to human NHE3 and NHE1 have been detected (36); however, the specific functional relevance of the transepithelial Na transport and/or regulation of pH cannot be deduced unequivocally from our current knowledge. Therefore, we have decided to study Na transport in the forestomach epithelia of cattle and sheep with the aid of the more specific NHE blockers HOE642 (NHE1) and S3226 (NHE3). Most of the studies were performed with tissues from sheep because ruminal Na transport has been primarily studied in this species, and consequently, the knowledge about electrogenic and electroneutral Na transport is well established in this tissue (14–15, 25, 28–29, 36). Experiments with bovine epithelia were included from a comparative point of view. The inhibitors were applied at the mucosal side because the electroneutral absorption of Na should be primarily characterized.

MATERIAL AND METHODS

Animals. Three sheep with an age between 6 and 9 mo and a body weight of 30–40 kg were fed hay ad libitum for at least 3 wk before the experiment. The sheep had free access to tap water and a mixture of minerals (lack stone for sheep). Holstein bull calves were fed colostrum for 3 or 4 days postpartum and were then placed into individual pens in a climate-controlled room. From the 4th day, the calves were fed milk replacer (Denkamilk, Fa. Denkavit, Warendorf, Germany). Modified tips filled with KCl-agar were kindly supplied by Dr. J. Pünter (Sanofi-Aventis, Frankfurt, Germany). 22Na was purchased from Sigma-Aldrich (Munich, Germany), while the inhibitors of NHE1 (HOE642) and NHE3 (S3226) were kindly supplied by Dr. J. Pünter (Sanofi-Aventis, Frankfurt, Germany). All reagents used were of analytic grade.

Electrical measurement. Electrical measurements were continuously obtained from a computer-controlled voltage-clamp device (Mussler, Aachen, Germany). Modified tips filled with KCl-agar were positioned ~3 mm from each surface of the tissue and connected to Ag-AgCl electrodes for measurement of the transepithelial potential difference (PDt). Similar types of tips were inserted ~2 cm from the surface of the tissue for the application of current (Isc). The tissues were incubated under short-circuit conditions. Transepithelial conductance (Gt) was calculated by measuring the displacements in the potential difference (ΔPD), caused by the application of a bipolar pulse of 100 μA, 1-s duration, and any changes in its amplitude. Since the inhibitors were applied only on the mucosal side, for the interpretation of Isc, only m2 tissues were used.

Flux studies. Unidirectional mucosal (m) to serosal (s) and serosal (s) to mucosal (m) fluxes (Jm, Jms) of Na were determined on paired tissues from the same rumen under short-circuit conditions. The tissue conductance (Gt) of each tissue in a pair did not differ by more than 25%. Net transepithelial fluxes were calculated as the difference between the two oppositely directed unidirectional fluxes (Jnet = Jms − Jm). Usually, the flux rates were studied in 12 pairs of tissues from the same rumen simultaneously. When the electrical parameters have been stabilized (generally 30–40 min after mounting), the mucosal buffer was changed (SCFA and pH 6.4), and the tissues were short-circuited. Then, the inhibitors were added on the mucosal side of

Table 1. A general description for the experimental animals and buffer solution used in the study showing pH and the application of SCFA

<table>
<thead>
<tr>
<th>Species</th>
<th>Buffer Composition</th>
<th>Mucosal</th>
<th>Serosal</th>
<th>N/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf</td>
<td>+SCFA 80 mM, pH 6.4</td>
<td>SCFA, pH 7.4</td>
<td>3/35</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>+SCFA 80 mM, pH 6.4</td>
<td>SCFA, pH 7.4</td>
<td>3/32</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>−SCFA, pH 6.4</td>
<td>SCFA, pH 7.4</td>
<td>3/34</td>
<td></td>
</tr>
</tbody>
</table>

All animals and used buffer compositions were divided into four groups: 1) control, 2) 1 mM amiloride, 3) 30 μM NHE1 inhibitor (HOE 642), and 4) 1 μM NHE3 inhibitor (S3226). The inhibitors were only added to the mucosal side. The results are presented in Tables 2–4. SCFA, short-chain fatty acids. N is number of animals; n is number of paired tissues.
both $J_{ms}$ and $J_{sa}$ tissues, whereas one group was always kept as control (without inhibitor). $^{22}$Na (80 kBq) was added to the mucosal side of one tissue of each pair and to the serosal side of the other (“hot side”). Samples (1 ml) were taken from the unlabeled (“cold”) side after an equilibration period of half an hour at 30-min intervals for three flux periods. The flux rates were calculated for each flux period, and the mean was taken as the transport rate. Each sample was replaced by an equal volume of corresponding buffer solution. Samples (100 μl) from the labeled bathing solution (“hot side”) were taken before the first and after the last flux period for the calculation of the specific radioactivity. $^{22}$Na was assayed in scintillation liquid (Rotiszint, Roth-Karlsruhe, Germany) by using a β-counter (LKB Wallace-Perkin-Elmer; Überlingen, Germany).

Immunocytochemistry. Rumen tissue of sheep was washed and stripped as described above, and the epithelium was fixed in 4% neutral buffered formaldehyde. Rumen slices were dehydrated and embedded in paraffin. Tissue sections (3 μm thick) were mounted on SuperFrost Plus adhesive glass slides (Menzel-Gläser, Braunschweig, Germany), in paraffin. Tissue sections (3 μm thick) were mounted on SuperFrost Plus adhesive glass slides (Menzel-Gläser, Braunschweig, Germany), deparaffinized in Roti-Histol (T6640, Carl Roth, Karlsruhe, Germany), and rehydrated in isopropanol and graded ethanol. Endogenous peroxidase activity was blocked by 30-min incubation in 0.3% H$_2$O$_2$ in methanol. The slides were washed, and nonspecific deparaffinized in Roti-Histol (T6640, Carl Roth, Karlsruhe, Germany), and rehydrated in isopropanol and graded ethanol.

Notably, both in the presence and in the absence of SCFA, amiloride (1 mM), HOE642 (30 μM; (34)) failed to cause any significant inhibition of Na transport. It is important to mention here that the tissue conductance ($G_t$) among various groups did not change significantly (Table 2) and should be considered as a control parameter of tissue integrity during the time course of the experiment. The $I_{sc}$ of bovine rumen epithelium was not changed by the inhibitors (Table 2).

Effect of NHE inhibitors on Na transport across rumen epithelium of sheep. An identical design was chosen for corresponding studies with sheep rumen epithelium. We sought to determine the isoforms of NHE responsible for both SCFA-independent (Table 3) and SCFA-stimulated (Table 4) electroneutral Na transport (7, 15, 38). The NHE inhibitors applied on the mucosal side showed a similar response compared with the experiments with calves. In the absence of SCFA, amiloride, and the NHE1-specific inhibitor, HOE642 did not significantly change $J_{ms}$ and $J_{net}$ Na. Again, the NHE3 specific inhibitor, S3226, caused a significant decrease of $J_{net}$ Na from 3.14 ± 0.76 to 1.70 ± 0.43 μEq-cm$^{-2}$·h$^{-1}$. Despite a substantial, but nonsignificant, inhibition of $J_{net}$ Na, $I_{sc}$ was almost identical to $J_{net}$ Na (0.74 vs. 0.87 μEq-cm$^{-2}$·h$^{-1}$) in the presence of S3226 (Table 3).

The presence of SCFA caused approximately an 80 to 90% increase of $J_{ms}$ and $J_{net}$ Na and again amiloride or S3226 but not HOE642 caused a significant decrease of these flux rates, indicating that the stimulation of Na transport by SCFA represented the activation of NHE3. The magnitude of this inhibition was larger for S3226 than amiloride (Table 4).

Notably, both in the presence and in the absence of SCFA, $I_{sc}$, which represents electrogenic Na transport, was significantly reduced after the addition of S3226 (Tables 3 and 4) and by amiloride (not significant). However, this effect on $I_{sc}$ exhibited some delay, and a new steady state of $I_{sc}$ was
and stratum basale. Immunolocalization of NHE3. Immunocytochemistry demonstrated various intensities of staining in the rumen epithelium of the various animals. In two of three epithelia, the strongest intensity appeared in the stratum granulosum (luminal surface). In all epithelia, the intensity decreased from the stratum granulosum through to the stratum basale without any staining in the stratum corneum (Fig. 2A). One epithelium showed evenly distributed staining in the stratum granulosum, stratum spinosum, and stratum basale.

DISCUSSION

The data of the present study are in agreement with findings in many previous studies of Na transport in rumen epithelium: $J_{\text{ms}}$ Na in the mucosal-serosal direction, with $J_{\text{ms}}$ > $I_c$ (11, 15, 28, 36, 38–39), and stimulation of $J_{\text{ms}}$ Na by SCFA (7, 14–15, 38). Furthermore, the inhibition of Na transport by amiloride and S3226 supports the early conclusion that electroneutral Na transport is mediated via NHE (5, 28). The pronounced or missing effects of various inhibitors of NHE give further insight into the isoform of NHE that is probably involved.

Amiloride (1 mM) was simply used as a demonstration of the effects known from previous experiments with rumen epithelium and served as a further control within the framework of this study (28, 38). The NHE3 in bovine rumen epithelium seems to be less sensitive to amiloride, with Diermaes et al. (7) observing no effect of amiloride (1 mM) on Na transport. In the present study, amiloride reduced $J_{\text{ms}}$ Na in calf rumen (Table 2) by only 20% (not significant), and a similar (6%), but significant, decrease of $J_{\text{ms}}$ Na was noticed by Sehested et al. (38) in studies with bovine rumen.

SCFA stimulated Na transport (Table 4) (7, 14–15, 38), and the increase was inhibited by amiloride or S3226, further supporting the conclusion that electroneutral Na transport is mediated via NHE and via the isoform NHE3. A similar approach for identifying the isoform NHE3 was applied in a study of Na transport via Na+/H+ exchanger in rat nephron (41).

This observation is in contrast to the discussion of Graham et al. (16), who have demonstrated, by RT-PCR, the presence of NHE1–3 and NHE8 in bovine rumen and who conclude, from immunostaining, that NHE1 is “predominantly localized to the stratum granulosum with a progressive decrease toward stratum basale”. Consequently, the authors discuss this isoform with regard to the well-known interaction between the nonionic diffusional uptake of SCFA, the intracellular release of protons, the activation of NHE1, and Na influx.

The application of the specific inhibitor HOE642 in the present study does not support the conclusions of Graham et al. (16), and the demonstrated immunostaining of NHE3 predominately in the luminal layer of epithelium is in agreement with the effect of inhibitors. Special attention has been paid to the avoidance of unspecific immunostaining. No staining has been observed in the absence of the primary antibody and the detection of NHE3 in the kidney, and its absence in heart as negative controls supports the conclusion that NHE3 staining is specific.

<table>
<thead>
<tr>
<th>Table 2. Effects of mucosal addition of amiloride, HOE642, or S3226 on unidirectional Na flux rates of bovine rumen epithelium (without SCFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{\text{ms}}$ Na</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Amiloride, 1 mM</td>
</tr>
<tr>
<td>HOE642, 30 µM</td>
</tr>
<tr>
<td>S3226, 1 µM</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. Values in the same column bearing different superscripts are significantly different ($P < 0.05$).

<table>
<thead>
<tr>
<th>Table 3. Effects of mucosal addition of amiloride, HOE642, or S3226 on unidirectional Na flux rates of sheep rumen epithelium (without SCFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{\text{ms}}$ Na</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Amiloride, 1 mM</td>
</tr>
<tr>
<td>HOE642, 30 µM</td>
</tr>
<tr>
<td>S3226, 1 µM</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. Values in the same column bearing different superscripts are significantly different ($P < 0.05$) to the control.
The possible reasons for the discrepancy with the results of Graham et al. (16) are, however, not clear. It could be that NHE1 is present in the stratum granulosum but does not significantly contribute to transepithelial Na transport. NHE1 has been demonstrated in the stratum corneum of the epidermis (32) and is essential for the acidification of surface pH and the function of epidermal barrier. However, in contrast to the slightly acid pH (6.2–6.4) of the epidermis (3), the surface of the rumen epithelium exhibits an alkaline pH (7.4–7.5), which is modified by the luminal Cl concentration, supporting the assumption that the surface pH of this epithelium is mainly influenced by Cl\(^{-}\)/HCO\(_3\)\(^{-}\)-exchange (23).

A further observation supports the conclusion that transepithelial Na transport is mediated by NHE3 and not by NHE1. Schweigel et al. (36) have shown that an increase of the luminal osmotic pressure causes a significant inhibition of \(J_{\text{ms}}\) and \(J_{\text{net}}\) Na and, after a detailed discussion, conclude that this inhibition of \(J_{\text{ms}}\) and \(J_{\text{net}}\) Na is caused by an effect of osmotic pressure on NHE3 activity. By contrast, hyperosmolarity causes a rapid alkalinization in isolated rumen epithelial cells, indicating an increased NHE1 exchange activity (36). The assumption of NHE3-mediated electroneutral transport is further supported by the observation that cAMP inhibits Na transport across the rumen epithelium (12, 42). As is well

<table>
<thead>
<tr>
<th>Group</th>
<th>(I_{\text{ms}}) Na</th>
<th>(I_{\text{sm}}) Na</th>
<th>(I_{\text{net}}) Na</th>
<th>(I_{\text{sc}})</th>
<th>(G_{\text{t}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.71 ± 1.31(a)</td>
<td>1.77 ± 0.49</td>
<td>3.94 ± 1.42(a)</td>
<td>1.16 ± 0.42(a)</td>
<td>4.04 ± 0.45</td>
</tr>
<tr>
<td>Amiloride, 1 mM</td>
<td>2.39 ± 0.45(b)</td>
<td>1.45 ± 0.30</td>
<td>0.94 ± 0.54(b)</td>
<td>0.85 ± 0.18</td>
<td>4.69 ± 1.18</td>
</tr>
<tr>
<td>HOE642, 30 (\mu)M</td>
<td>5.35 ± 1.10</td>
<td>1.66 ± 0.31</td>
<td>3.69 ± 1.16</td>
<td>1.18 ± 0.22</td>
<td>5.02 ± 1.14</td>
</tr>
<tr>
<td>S3226, 1 (\mu)M</td>
<td>2.22 ± 0.67(b)</td>
<td>1.55 ± 0.30</td>
<td>0.67 ± 0.22(c)</td>
<td>0.59 ± 0.23(b)</td>
<td>4.62 ± 1.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Values in the same column bearing different superscripts are significantly different (\(P < 0.05\)) from the control.

Table 4. Effects of mucosal addition of amiloride, HOE642, or S3226, on unidirectional Na flux rates of sheep rumen epithelium (with mucosal SCFA)

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Fig. 2. Immunolocalization of NHE3. A: ovine rumen epithelium (blue): Immunostaining for NHE3 is most intense at the lumen facing membrane in cells of the stratum granulosum (sg) with a decrease in intensity across the stratum spinosum (ssp) and stratum basale (sb). Note the negative staining in the stratum corneum (sc). B: negative control with the respective preimmune serum (diluted 1:1,500) and counterstained with Mayer’s Hemalum solution. C: positive tissue control: rat kidney (4). D: negative tissue control: ovine heart.
established, cAMP inhibits NHE3 via the activation of PKA and not NHE1 (31).

On the other hand, NHE1 activity has been demonstrated in studies of the regulation of pH\textsubscript{t} in isolated REC (29, 36). In these studies, HOE642 and S3226 (36) or HOE694 (29) significantly reduces the recovery of pH\textsubscript{t} after an acid load by butyrate. Interestingly, the effects of HOE642 and S3226 are almost additive, indicating the activity of both isoforms in REC (36).

Graham et al. (16) have further demonstrated the presence of mRNA of NHE3 and NHE1 in bovine rumen; this is in agreement with our findings in sheep rumen (36). However, immunostaining by Graham et al. (16) with regard to NHE has only been performed with antibodies against NHE1 and NHE2, but not against NHE3. The reasons for the restriction to NHE1 and NHE2 are not known, and the authors do not further mention NHE3, despite detection of its mRNA.

Irrespective of the presence or absence of SCFA, amiloride, or S3226 (significantly) reduces I\textsubscript{sc} (Tables 3 and 4, Fig. 1), suggesting that electrogenic Na transport is also inhibited by blocking the PD-dependent cation conductance of the apical membrane (20, 22, 25). However, the change in I\textsubscript{sc} occurs with some delay after the mucosal addition of amiloride or S3226. The effect on I\textsubscript{sc} might thus indirectly be caused by the inhibition of NHE3, the decrease of intracellular pH possibly causing changes of ion conductance and hence of the flow and direction of ions. This conclusion of indirect effects is supported by the observation that amiloride in the micromolar range, which inhibits the classical epithelial Na channel, is without effect on NHE and I\textsubscript{sc} in sheep rumen (28). Notably, in the presence of mucosal amiloride or S3226, J\textsubscript{net} and I\textsubscript{sc} are not significantly different, but J\textsubscript{net} is numerically higher (Table 4). This small gap probably represents J\textsubscript{net} K flux in the serosal-mucosal direction (24).

Significance and Perspectives

The interaction between SCFA and NHE3 demonstrated here is of significant physiological importance under in vivo conditions because the apical uptake of SCFA during feeding causes intracellular release of protons. Acidification of the cytosol by proton release would be cytotoxic without the observed coordination of proton extrusion via NHE. Apical NHE3 activity is, therefore, the first line of defense against protons that are known to inhibit NHE1 and not NHE3. This supposition of Na uptake via NHE3 is in agreement with our previous finding that low concentrations of amiloride that are known to inhibit NHE1 do not change Na transport (28) and with the observation that cAMP inhibits Na transport (12, 42). NHE1 activity is present in ruminal epithelial cells and is involved in the regulation of pH\textsubscript{t} (29, 38) but probably does not contribute to transepithelial Na transport. Hence, classical “job sharing” in epithelia between NHE3 (transepithelial Na transport) and NHE1 (regulation of pH\textsubscript{t}) probably also occurs in the rumen epithelium. This is in line with the presence of NHE1 as shown by Graham et al. (16) but contradicts their conclusions.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


