Effect of chloride on pH microclimate and electrogenic Na\(^+\) absorption across the rumen epithelium of goat and sheep

S. Leonhard-Marek, G. Breves, and R. Busche

Department of Physiology and Department of Biochemistry, School of Veterinary Medicine, Hannover, Germany

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Abstract

Leonhard-Marek, S., G. Breves, and R. Busche. Effect of chloride on pH microclimate and electrogenic Na\(^+\) absorption across the rumen epithelium of goat and sheep. Am J Physiol Gastrointest Liver Physiol 291: G246–G252, 2006. First published February 16, 2006; doi:10.1152/ajpgi.00419.2005—Active Na\(^+\) absorption across rumen epithelium comprises Na\(^+\)/H\(^+\) exchange and a nonselective cation conductance (NSCC). Luminal chloride is able to stimulate Na\(^+\) absorption, which has been attributed to an interaction between Cl\(^-\)/HCO\(_3\)\(^-\) and Na\(^+\)/H\(^+\) exchangers. However, isolated rumen epithelial cells also express a Cl\(^-\) conductance. We investigated whether Cl\(^-\) has an additional effect on electrogenic Na\(^+\) absorption via NSCC. NSCC was estimated from short-circuit current (\(I_{sc}\)) across epithelia of goat and sheep rumen in Ussing chambers. Epithelial surface pH (pH\(_s\)) was measured with 5-N-hexadecanoyl-aminomethoxycarbonyl-N-glutamate. Membrane potentials were measured with microelectrodes. Luminal, but not serosal, Cl\(^-\) stimulated the Ca\(^{2+}\) and Mg\(^{2+}\) sensitive \(I_{sc}\). This effect was independent of the replacing anion (gluconate or acetate) and of the presence of bicarbonate. The mean pH\(_s\) of rumen epithelium amounted to 7.47 ± 0.03 in a low-Cl\(^-\) solution. It was increased by 0.21 pH units when luminal Cl\(^-\) was increased from 10 to 68 mM. Increasing mucosal pH from 7.5 to 8.0 also increased the Ca\(^{2+}\) and Mg\(^{2+}\) sensitive \(I_{sc}\) and transepithelial conductance and reduced the fractional resistance of the apical membrane. Luminal Cl\(^-\) depolarized the apical membrane of rumen epithelium. 5-Nitro-2-(3-phenylpropylamino)-benzoate reduced the divalent cation sensitive \(I_{sc}\), but only in low-Cl\(^-\) solutions. The results show that luminal Cl\(^-\) can increase the microclimate pH via apical Cl\(^-\)/HCO\(_3\)\(^-\) or Cl\(^-\)/OH\(^-\) exchangers. Electrogenic Na\(^+\) absorption via NSCC increases with pH, explaining part of the Cl\(^-\) effects on Na\(^+\) absorption. The data further show that the Cl\(^-\) conductance of rumen epithelium must be located at the basolateral membrane.

Electrolyte transport; sodium absorption; forestomach; ruminants; microclimate

The rumen, the biggest forestomach of ruminants, has a high significance for the absorption of sodium. Sodium intake with food is not very high (between 15 and 40 g/day for a cow, 2 g/day for a sheep), but during eating and especially during ruminating, substantial amounts of sodium enter the forestomachs with the saliva. This Na\(^+\) secretion by the salivary glands may reach more than 500 g/day in cows and 35 g/day in sheep (2, 14). Up to 50% of endogenous Na\(^+\) secretion is absorbed across the epithelium of the rumen (10), where two pathways contribute to active Na\(^+\) absorption: an electrogenic Na\(^+\)/H\(^+\) exchange and an electrogenic Na\(^+\) conductance (25). Electrogenic Na\(^+\) uptake is especially important under conditions of sodium deficiency, when Na\(^+\) intake and Na\(^+\) concentration in the saliva are low (23). Ruminal electrogenic Na\(^+\) uptake occurs via a nonselective cation conductance (NSCC) which is regulated by extracellular Ca\(^{2+}\) and Mg\(^{2+}\) and by intracellular Mg\(^{2+}\) ions (19, 22).

Previous studies have shown that luminal chloride is able to stimulate Na\(^+\) absorption across the rumen wall in cattle and sheep (8, 24). This has been attributed to an interaction between Cl\(^-\)/HCO\(_3\)\(^-\) and Na\(^+\)/H\(^+\) exchange via the intracellular pH. Linear regression on Na\(^+\) and Cl\(^-\) fluxes showed a high correlation coefficient between both transport rates and a coupling ratio of about 0.7 Cl\(^-\)/1 Na\(^+\) in cattle (9) and 0.6 Cl\(^-\)/1 Na\(^+\) in sheep (24). This coupling ratio suggests an indirect rather than a direct coupling mechanism. Alternatively, chloride absorption across the rumen, in addition to interacting with Na\(^+\)/H\(^+\) exchange, might have an additional effect on Na\(^+\) conductance.

We wanted to know whether luminal chloride might have an effect on the electrogenic part of Na\(^+\) absorption via NSCC. For this purpose we measured the effect of chloride gradients on the Ca\(^{2+}\)-sensitive Na\(^+\) current in Ussing chambers, on the apical membrane potential of rumen epithelial cells, and on the pH of the epithelial surface.

Our data suggest that the level of chloride on the luminal side can change the pH microclimate in the stratum corneum of the epithelial layer, which, in turn, increases the conductance for Na\(^+\) ions.

Methods

Tissues. The protocol of the animal treatment was approved and its conduct supervised by the respective animal protection officer of the institution. Adult male and female sheep and goats were killed by stunning with a commercial abattoir shooting apparatus and by bleeding from the carotids. Pieces of the ventral rumen wall were taken from slaughtered animals within 5 min after bleeding and immediately immersed in a buffer solution at 38°C, where the mucosa was stripped from the underlying muscle layers and the serosa.

Incubation and electrical measurements. Mucosal tissues were mounted between the two halves of incubation chambers with an exposed area of 1 or 3.14 cm\(^2\) (20). We minimized edge damage by placing rings of silicon rubber on both sides of the tissues. Ussing chambers were connected to a computer-controlled voltage clamp and microelectrode device (Biomedical Instruments Munich, Germany). Transepithelial potential differences (\(V_{TE}\)) were measured through buffer solution agar bridges.

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and calomel electrodes with reference to the mucosal solution. Trans-
etheliial conductances \((G_t)\) were determined from the changes in \(V_i\),
caused by bipolar current pulses of 100 \(\mu\)A/cm\(^2\) of 500-ms duration.

The currents were passed through buffer solution agar bridges con-
ected to Ag/AgCl electrodes in 3 M KCl (Ussing chambers) or
through rings of Ag/AgCl electrodes placed in each half of the
microelectrode chamber. In each setup, fluid resistances and junction
potentials were measured before mounting the mucosal tissues and
corrected for during the experiments. The experiments were per-
formed under short-circuit conditions.

**Microelectrodes.** Conventional microelectrodes were pulled from
borosilicate glass (outer diameter 1.2 or 1.5 mm) and filled with 0.5 M
KCl, yielding resistances of 15–30 M\(\Omega\). We impaled rumen epithelial
cells across the apical membrane using a motorized micromanipulator
with piezo element and measured the apical membrane potential \((V_a)\)
with reference to the mucosal solution. Impalements were accepted if
1) the change in \(V_a\) was abrupt while advancing into the tissue, 2) \(V_a\)
remained stable for at least 1 min, and 3) \(V_a\) returned to 0 ± 3 mV
after withdrawing the electrode.

**Measurements of surface \(pH\).** The surface \(pH\) (\(pH_s\)) of the epithelia
was measured according to the method of Genz et al. (12). 5-
Nitro-2-(3-phenylpropylamino)-benzoate (NPPB)

**Chemicals.** 5-Nitro-2-(3-phenylpropylamino)-benzoate (NPPB)
was dissolved in dimethyl sulfoxide (DMSO) and added in a volume
of 2 \(\mu\)l DMSO per 10 ml buffer solution. This DMSO volume
produced no electrophysiological effects in control tissues incubated
in parallel. The pH-sensitive fluorescent dye HAF was purchased from
Hexascan (Deisenhofen, Germany). All other chemicals were of analytical grade
and were obtained from Merck (Darmstadt, Germany).

**Statistics.** Results are means ± SE; \(n\) designates the numbers of
tissues or cells. Statistical significance was evaluated using analysis of
variance or Student’s \(t\)-test, paired or unpaired as appropriate.

**RESULTS**

**Effects of chloride on the \(Ca^{2+}\)- and \(Mg^{2+}\)-sensitive \(Na^+\) current.** Isolated rumen epithelia from goat or sheep were
incubated in standard buffer solution under short-circuit con-
ditions. Changing to \(Ca^{2+}\)- and \(Mg^{2+}\)-free conditions on
the luminal side increased short-circuit current \((I_{sc})\), and addition
of \(Ca^{2+}\) and \(Mg^{2+}\) ions to the luminal side decreased \(I_{sc}\), both
as previously shown (19). Reducing the Cl\(^-\) concentration on
both sides of rumen epithelia had no effect on the baseline current but decreased the subsequent divalent cation-sensitive $I_{sc}$ (Fig. 2). This could be shown with epithelia from sheep and goat rumen.

We then changed repeatedly to Ca$^{2+}$/H$^{+}$- and Mg$^{2+}$/H$^{+}$-free conditions on the luminal side, while manipulating the Cl$^{-}$/H$^{+}$ concentration only on the luminal side, only on the serosal side, or on both sides of the epithelia. These experiments showed that luminal, but not serosal, Cl$^{-}$ accounted for the effects on the divalent cation-sensitive $I_{sc}$ in sheep and goat (Fig. 3).

For these first experiments we had exchanged chloride for gluconate in the bathing solutions. Thus the results might have been due to a decrease in Cl$^{-}$ or an increase in gluconate concentration. To discriminate between both possibilities, we tested whether the replacing anion might be responsible for the effect attributed to chloride. Exchanging chloride for gluconate or acetate had the same immediate effect on $I_{sc}$ (Fig. 4). This Cl$^{-}$ effect was also shown in the absence of bicarbonate (HEPES-buffered solution, Fig. 4).

A closer inspection of the time course of $I_{sc}$ measured at different Cl$^{-}$ concentrations showed two additional phenomena (Fig. 5). A drop in Cl$^{-}$ concentration was followed by an $I_{sc}$ decrease to the minimum value shown in Fig. 4. From this minimum value, $I_{sc}$ increased again when the epithelia were bathed in a low-Cl$^{-}$, high-acetate solution (from 2.73 ± 0.25 to 3.65 ± 0.50 μeq cm$^{-2}$ h$^{-1}$ within 10 min, $n = 6$, $P < 0.05$). In contrast, $I_{sc}$ remained on the minimum value when the epithelia were bathed in a low-Cl$^{-}$, high-gluconate solution (2.10 ± 0.36 vs. 2.17 ± 0.34 μeq cm$^{-2}$ h$^{-1}$ 10 min later, $n = 6$). The exchange to a high-Cl$^{-}$ solution was followed by an overshoot in $I_{sc}$ (Fig. 5), which declined to a plateau value corresponding to the $I_{sc}$ under high-Cl$^{-}$ conditions shown in Fig. 4.

**Effects of chloride on apical membrane potential.** Whole-cell patch-clamp experiments with isolated rumen epithelial cells have recently shown that these cells express a conductance for chloride (22). This Cl$^{-}$ conductance is not signifi-

![Fig. 2](http://ajpgi.physiology.org/)

**Fig. 2.** Influence of chloride on the short-circuit current ($I_{sc}$) across goat rumen epithelium in the presence (gray bars) and in the absence (open bars) of Ca$^{2+}$ and Mg$^{2+}$ on the mucosal side. The given Cl$^{-}$ concentrations were present on both sides of the epithelia. Different solutions were applied consecutively to the same epithelia. Values are means ± SE; $n = 5$, *$P < 0.05$ vs. 124 mM Cl$^{-}$.

![Fig. 3](http://ajpgi.physiology.org/)

**Fig. 3.** Mucosal Cl$^{-}$ stimulates the $I_{sc}$ across goat and sheep rumen epithelium in the absence of Ca$^{2+}$ and Mg$^{2+}$ on the mucosal side. Different solutions were applied consecutively to 3 neighboring epithelia from goat and 2 neighboring epithelia from sheep rumen. Values are means ± SE; $n = 5$ (goat) and $n = 4$ (sheep), *$P < 0.05$ vs. 124 mM Cl$^{-}$ (mucosal and serosal).

![Fig. 4](http://ajpgi.physiology.org/)

**Fig. 4.** Immediate effect of a reduced Cl$^{-}$ concentration on the $I_{sc}$ across goat rumen in the absence of Ca$^{2+}$ and Mg$^{2+}$ on the mucosal side. Cl$^{-}$ was replaced with acetate or gluconate and in the presence or absence of bicarbonate as indicated. The replacing anion did not influence this effect of Cl$^{-}$ on $I_{sc}$. Values are means ± SE; number of experiments is given in parentheses.
cantly altered by changes in external Ca\(^{2+}\) and Mg\(^{2+}\) ions (22) but might have an influence on the uptake of cations. To test whether Cl\(^{-}\) conductances are also present in the intact tissue and whether luminal chloride might be able to alter the electric driving force for the uptake of cations, we impaled rumen epithelia with microelectrodes and measured the apical membrane potential at different mucosal Cl\(^{-}\) concentrations. Serosal Cl\(^{-}\) was kept constant at 120 mM.

Increasing the mucosal Cl\(^{-}\) concentration from 0 to 120 mM induced a depolarization of the apical membrane in the presence and in the absence of divalent cations on the mucosal side (Fig. 6). In the presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions, mucosal Cl\(^{-}\)-depolarized the V\(_{a}\) from −55.9 ± 6.5 mV (0 mM Cl\(^{-}\) mucosal) to −48.8 ± 6.0 mV (120 mM Cl\(^{-}\) mucosal, n = 4, P < 0.01) without a significant effect on the fractional resistance of the apical membrane (fR\(_a\), 71 ± 2% and 69 ± 2%). In the absence of Ca\(^{2+}\) and Mg\(^{2+}\) ions, an increase in luminal Cl\(^{-}\) depolarized V\(_{a}\) from −44.8 ± 3.5 mV (0 mM Cl\(^{-}\)) to −34.4 ± 3.2 (120 mM Cl\(^{-}\), n = 7, P < 0.001) and tended to reduce the fR\(_a\) from 39 ± 5% to 34 ± 4% (P = 0.09). Membrane potentials were corrected for junction potentials according to Barry (3). Mucosal Ca\(^{2+}\) and Mg\(^{2+}\) ions increased fR\(_a\) (Fig. 6), as previously shown by Lang and Martens (18).

**Mucosal NPPB reduced I\(_{sc}\) in low-chloride buffer.** The arylinabenoatoate NPPB has been shown to block Cl\(^{-}\) channels and Cl\(^{-}\) transporters (6). When we applied 50 μM NPPB to the mucosal side of sheep rumen epithelium, in the absence of Ca\(^{2+}\) and Mg\(^{2+}\), NPPB tended to decrease I\(_{sc}\) from 4.38 ± 0.69 to 3.95 ± 0.86 μeq·cm\(^{-2}\)·h\(^{-1}\) (n = 4). In the standard buffer including 120 mM Cl\(^{-}\), this reduction, however, was not different from the time- and solvent-dependent control (ΔI\(_{sc}\) = −0.43 ± 0.18 due to NPPB in DMSO vs. ΔI\(_{sc}\) = −0.25 ± 0.06 μeq·cm\(^{-2}\)·h\(^{-1}\) due to DMSO alone). In contrast, at low Cl\(^{-}\) concentrations on the luminal side (10 mM Cl\(^{-}\)) NPPB reduced I\(_{sc}\) by 1.35 ± 0.37 μeq·cm\(^{-2}\)·h\(^{-1}\), which differed significantly from the time- and solvent-dependent decrease of 0.13 ± 0.15 (n = 4). Experiments with goat rumen showed similar results. In the standard (high Cl\(^{-}\)) buffer 50 μM NPPB reduced I\(_{sc}\) by 0.42 ± 0.09 μeq·cm\(^{-2}\)·h\(^{-1}\) (n = 5), whereas the time- and solvent (DMSO)-dependent decrease in I\(_{sc}\) amounted only to 0.14 ± 0.04 μeq·cm\(^{-2}\)·h\(^{-1}\) (n = 5). As in sheep, these experiments were performed under divalent cation-free conditions on the mucosal side. Because of the lower standard error, this NPPB effect was significantly different from the time-dependent decrease (P < 0.05). Again, the NPPB effect was significantly higher under Cl\(^{-}\)-free conditions on the luminal side (ΔI\(_{sc}\) = −1.73 ± 0.24 μeq·cm\(^{-2}\)·h\(^{-1}\), n = 3, P < 0.001 vs. NPPB effect under high-Cl\(^{-}\) conditions).

This NPPB action becoming obvious or enhanced at reduced Cl\(^{-}\) concentrations suggests competition with the Cl\(^{-}\) binding site of a transporter, rather than block of a channel.

**Effects of chloride on pH.** Chloride absorption and bicarbonate secretion are partly dependent on each other in vivo; therefore, Cl\(^{-}\)/HCO\(_{3}^{-}\) transporters have been suggested to be involved in ruminal Cl\(^{-}\) absorption. Recently, the expression of different anion exchangers within the rumen papillae of sheep has been shown at the mRNA level (4). For the guinea pig colon it was shown that an apically located Cl\(^{-}\)/HCO\(_{3}^{-}\) exchanger affects the pH (12). We therefore tested whether chloride might exert an effect on the ruminal pH microclimate via these exchangers.

Incubating sheep ruminal epithelia with the pH-sensitive dye HAF for 20 min anchored the fatty acid tail of this dye molecule in the outermost layer of the epithelium (Fig. 1). When the epithelia were bathed in a low-chloride buffer (10 mM Cl\(^{-}\)) on the mucosal side, the pH charged to 7.47 ± 0.03 (n = 10). This pH charged increased by 0.21 ± 0.01 (n = 10, P < 0.001) when the Cl\(^{-}\) concentration was increased to 68 mM on the luminal side (Fig. 7).

A similar observation could be made in the absence of bicarbonate. In a HEPES-buffered solution the pH charged amounted to 7.55 ± 0.03 under low-Cl\(^{-}\) conditions on the mucosal side (25 mM Cl\(^{-}\)), pH charged increased by 0.12 ± 0.01 (n = 5, P < 0.001) upon change to a high-Cl\(^{-}\) buffer on the luminal side (125 mM Cl\(^{-}\)).

**Effect of mucosal pH on I\(_{sc}\).** To determine whether a small step in pH charged might have an effect on the Na\(^{+}\) current via NSCC,
we varied mucosal pH and measured basal and divalent cation-sensitive $I_{sc}$ and $G_t$ across sheep rumen. This was done under SCFA- and bicarbonate-free conditions to minimize Na$^+$ transport via Na$^+$/H$^+$ exchange.

In the presence of Ca$^{2+}$ and Mg$^{2+}$ on the mucosal side the increase in mucosal pH from 7.5 to 8.0 had no effect on $I_{sc}$ ($\Delta I_{sc} = 0.09 \pm 0.09 \mu$eq cm$^{-2}$ h$^{-1}$) and $G_t$ ($\Delta G_t = 0.16 \pm 0.18$ mS/cm$^2$, $n = 9$). When the same epithelia were incubated in the absence of divalent cations on the mucosal side, the increase in mucosal pH from 7.5 to 8.0 raised $I_{sc}$ by $0.19 \pm 0.07 \mu$eq cm$^{-2}$ h$^{-1}$ ($P < 0.05$) and $G_t$ by $0.39 \pm 0.07$ mS/cm$^2$ ($P < 0.001$).

Effect of mucosal pH on apical membrane potential. If an increase in luminal pH is able to enhance the Na$^+$ current through the divalent cation-sensitive pathway as suggested by the increases in $I_{sc}$ and $G_t$, then this should decrease the $R_D$ and depolarize the $V_a$. We therefore performed additional microelectrode experiments with sheep rumen under divalent cation-free conditions on the mucosal side and tested for the effect of a luminal pH increase from 7.5 to 8.0 on the $V_a$ and the $R_D$.

An increase in luminal pH depolarized the mean $V_a$ from $-25.7$ mV (at pH 7.5) to $-24.5$ mV (at pH 8.0) with a $\Delta V_a$ of $1.2 \pm 0.3$ mV ($n = 5$, $P < 0.01$) and decreased the mean $R_D$ from 49% to 45% with a $\Delta R_D$ of 4% ($n = 5$, $P < 0.05$).

**DISCUSSION**

**Physiological role of electrogenic Na$^+$ transport in the rumen.** In rumen epithelium the electrogenic Na$^+$ transport mechanism works in parallel with a Na$^+$/H$^+$ exchange. At high concentrations Na$^+$ is mainly transported via the electroneutral pathway, whereas at low-Na$^+$ concentrations the electrogenic pathway predominates (23). High-energy diets have been shown to increase ruminal sodium absorption. This has been attributed to an increased transport via Na$^+$/H$^+$ exchange, since the stimulation could be blocked by a high dose of aniloidine (1 mM). Na$^+$/H$^+$ exchange was also stimulated by the luminal presence of short-chain fatty acids (SCFA) and by a slight acidification of luminal pH (11). High-protein diets, on the other hand, can alkalinize the rumen contents. Under these conditions electrogenic Na$^+$ absorption gains more importance, since a higher mucosal pH decreases the stimulatory effects of SCFA and CO$_2$ on ruminal Na$^+$/H$^+$ exchange (11).

A higher pH also reduces the concentration of free Ca$^{2+}$ and Mg$^{2+}$ ions in rumen fluid (13), which would at the same time relieve the blocking effect of these cations on the electrogenic Na$^+$ transport. Sodium transport might be enhanced further if pH had a direct effect on the Na$^+$ conductance. Such a direct stimulatory effect of mucosal pH on the Ca$^{2+}$- and Mg$^{2+}$-sensitive Na$^+$ current across rumen epithelium (see below).

**Chloride effects via Cl$^-$ channels.** In the current study chloride was able to stimulate the Ca$^{2+}$- and Mg$^{2+}$-sensitive Na$^+$ current across the rumen, and this stimulation could be attributed to the presence of Cl$^-$ on the luminal side. Recent patch-clamp experiments with isolated rumen epithelial cells (REC) have shown that REC express a Cl$^-$ conductance (22). If this conductance was present in the intact tissue, and if it were localized in the apical membrane of epithelial cells, then an increase in luminal Cl$^-$ should hyperpolarize the transporting cells and thereby increase the driving force for the uptake of cations. We tested for this hypothesis with microelectrode experiments where we impaled rumen epithelium via the apical membrane. In contrast to the assumptions, an increase in luminal Cl$^-$ concentration did not hyperpolarize, but depolarized the rumen epithelium (Fig. 6). This observation strongly argues against an apical localization of Cl$^-$ channels in ruminal epithelium.

The Cl$^-$ conductances should thus be localized in the basolateral membranes of rumen epithelial cells and might contribute to the transepithelial transport of Cl$^-$, as suggested for basolateral localized CIC-2 channels in the distal colon of guinea pigs (7). An increase in luminal Cl$^-$ concentration would then accumulate intracellular Cl$^-$ via electroneutral uptake across the apical membrane; this would be followed by electrogenic extrusion of Cl$^-$ across the basolateral membrane, thereby depolarizing the cell interior. A basolaterally localized Cl$^-$ conductance would thus be in line with the microelectrode experiments, whereas the Cl$^-$ effect on electronic Na$^+$ transport must have another reason.

**Chloride effects via anion exchangers.** The current concept of chloride absorption across rumen epithelium involves an electroneutral Cl$^-$ uptake across the apical membrane via Cl$^-$/HCO$_3^-$ exchange, since removal of HCO$_3^-$/CO$_2$ decreased the Cl$^-$ flux from the mucosal to the serosal side (8, 26). The hereby induced changes in HCO$_3^-$ secretion might have an effect on local pH values. Studies with isolated REC (4) have shown that these cells need extracellular Cl$^-$ to recover from an alkaline load, suggesting apical and/or basolateral localized anion exchangers. We used the pH-sensitive dye HAF to investigate whether Cl$^-$ ions were able to change the pH microenvironment at the ruminal surface. HAF incorporates in the apical membrane of the outermost epithelial cells and inside epithelial cells that belong to the stratum corneum, the exterior cell layer of rumen epithelium (Fig. 1). Our data show that an increase in luminal Cl$^-$ concentration increases the pH of the epithelial surface. This would be in line with an apically localized Cl$^-$/HCO$_3^-$ exchange.

Investigating the effects of different combinations of SCFA on the pH microclimate was beyond the scope of the present study, but the regulatory increase in $I_{sc}$ in an acetate buffer (Fig. 5) suggests that an exchange of acetate for bicarbonate might as well have an effect on the pH, as it was described for butyrate at the basolateral pH$_b$ in the cecum by Kirschberger et
al. (15) and therefore could also affect $I_{sc}$. This would be in line with the concept of SCFA being partly absorbed via SCFA/ HCO$_3^-$ exchange across rumen epithelium (16).

The present study shows additionally that an increase in luminal Cl$^-$ was also able to increase pH$_i$ in the absence of bicarbonate in the bathing solutions. This allows two explanations. First, cell metabolism might have provided sufficient HCO$_3^-$ to run the exchanger even in the absence of extracellular HCO$_3^-$/CO$_2$. We would, however, have expected a reduced exchange activity under these conditions since a switch from HCO$_3^-$/CO$_2$-free to HCO$_3^-$/CO$_2$-containing solutions is able to stimulate Cl$^-$ absorption (26). This argues against the first explanation. Second, bicarbonate might be replaced by OH$^-$ anions and allow for Cl$^-$ absorption via Cl$^-$/OH$^-$ exchange. Different anion exchangers have been shown at the mRNA level in rumen epithelium and cultured ruminal epithelial cells (4), including AE2, DRA, and PAT1. These exchangers generally have a high affinity for HCO$_3^-$ but can also transport OH$^-$ instead and function as a Cl$^-$/OH$^-$ exchange (27, 28). This latter explanation is supported by the observation that as for the pH effects, the presence of bicarbonate was also not necessary for the Cl$^-$ effects on $I_{sc}$.

**Effects of mucosal pH.** The fluorescence experiments had shown that a variation in luminal Cl$^-$ was able to change the epithelial pH$_i$ by about 0.2 units to alkaline values. This is a pH range where electrogenic Na$^+$ absorption gains importance for overall ruminal Na$^+$ absorption. We therefore had to test whether a small change in luminal pH would be able to increase the Ca$^{2+}$- and Mg$^{2+}$- sensitive $I_{sc}$. When divalent cations were present at the mucosal side, a variation in mucosal pH from 7.5 to 8.0 had no effect on $I_{sc}$ or $G_i$. This is in line with the observation that an increase in mucosal Cl$^-$ had no effect on $I_{sc}$ in the mucosal presence of divergent cations. In the mucosal absence of divalent cations the same pH step from 7.5 to 8.0 increased $I_{sc}$ and $G_i$. These experiments were done with Na$^+$ as the main cation in the buffer solution; so, the increase in $I_{sc}$ and $G_i$ should reflect an increased Na$^+$ absorption through the Ca$^{2+}$-sensitive pathway at pH 8.0. Note that a change from low to high Cl$^-$ on the luminal side induced a small overshoot in the pH$_i$ as well as in the $I_{sc}$ recordings.

Microelectrode experiments under divergent cation-free conditions on the mucosal side showed that an increase in mucosal pH from 7.5 to 8.0 decreased the $fR_a$ and induced a small depolarization of the cells. Both observations are in line with a pH-dependent increase of the Ca$^{2+}$-sensitive conductance and an enhanced Na$^+$ diffusion via this pathway. The pH-induced changes in membrane potential seem very small; they are, however, in line with the changes in transepithelial potential that could be calculated from the Ussing chamber experiments. Furthermore, the increased Cl$^-$ effects on membrane potential and $fR_a$ seen in the absence of divergent cations are in the same range as the pH-dependent effects discussed here. These observations are in line with a Cl$^-$-dependent change in pH$_i$ followed by an increased Na$^+$ current through the divalent cation-sensitive pathway.

In conclusion, luminal chloride is able to increase the electrogenic (Ca$^{2+}$- and Mg$^{2+}$-sensitive) Na$^+$ absorption across the rumen epithelium of sheep. Our data suggest that this increase is mediated via an increased activity of luminal Cl$^-$/HCO$_3^-$ or Cl$^-$/OH$^-$ exchange, an increased pH in the microclimate of the epithelial surface, and a pH effect on the nonselective cation conductance responsible for Na$^+$ absorption. The data further show that the Cl$^-$ conductance of rumen epithelium must be located at the basolateral membrane.

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