J Comp Physiol B (2002) 172: 635–641
DOI 10.1007/s00360-002-0292-x

ORIGINAL PAPER

S. Leonhard-Marek

Divalent cations reduce the electrogenic transport of monovalent cations across rumen epithelium

Accepted: 18 July 2002 / Published online: 20 August 2002

Abstract The rumen epithelium of sheep and goats showed an increase in short circuit current (*I*sc) and transepithelial conductance (gt) upon mucosal removal of divalent cations. A divalent-sensitive *I*sc and gt were present in Na^+, K^+ or Rb^+ buffer, but nearly abolished in mucosal $NMDG^+$ (N-methyl-D-glucamine) buffer. High K buffer, addition of $BaCl_2$ or of ouabain on the serosal side also reduced or abolished the divalent-sensitive *I*sc. Mucosal Ca^{2+} was more potent in blocking *I*sc, but had the same potency as Mg^{2+} in blocking gt. A prolonged mucosal deprivation of Mg^{2+} ions increased gt, potential difference and basal as well as the Ca^{2+}-sensitive *I*sc. Mucosal addition of Mg^{2+} had a smaller effect on gt after serosal preincubation with Ba. The data suggest that rumen epithelial cells exhibit an apical non-selective cation conductance, which permits the passage of monovalents in the mucosal absence of divalents. The development of a divalent-sensitive *I*sc in Na buffer requires Na^+/K^+ pumps and K^+ recycling through Ba^{2+}-sensitive K^+ conductances on the basolateral side. This *I*sc is blocked by extracellular Ca^{2+} and both extracellular and intracellular Mg^{2+} ions. A prolonged deprivation of mucosal Mg^{2+} alone seems to affect intracellular Mg^{2+} in this Mg^{2+}-absorbing tissue.

Keywords Electrolyte transport · Sodium absorption · Calcium · Magnesium · Forestomach

Abbreviations *gt* transepithelial conductance · Δgt divalent-sensitive transepithelial conductance · *Isc* short circuit current · ΔIsc divalent-cation-sensitive short circuit current · $[Mg^{2+}]_i$ intracellular concentration of free Mg^{2+} ions · *NMDG* N-methyl-D-glucamine · *NSCC* non-selective cation conductance · *SCFA* short-chain fatty acids · *Vt* transepithelial potential difference

Communicated by G. Heldmaier

S. Leonhard-Marek
Department of Physiology,
School of Veterinary Medicine,
Bischofsholer Damm 15/102,
D-30173 Hannover, Germany
E-mail: sabine.leonhard-marek@tiho-hannover.de
Tel.: +49-511-8567634
Fax: +49-511-8567687

Introduction

Despite their multilayered cornified epithelium, the forestomachs of ruminants have a great absorptive capacity for short-chain fatty acids (SCFA) and electrolytes such as sodium, magnesium and calcium. The energy requirements of these animals are mainly met by the ruminal absorption of SCFA, and up to 50% of the sodium entering the forestomachs is absorbed across the rumen wall (Dobson 1959; Bergman 1990). Furthermore it is well established that the forestomachs are the main site of Mg^{2+} absorption in ruminants (Tomas and Potter 1976).

Ruminal electrogenic Na^+ transport differs from the classic Na^+-absorbing epithelia, since it is not blocked by low doses of amiloride (Martens and Gäbel 1988), but varies with the luminal concentration of divalent cations. Both net Na^+ absorption and short circuit current (*I*sc) as a measure of total electrogenic ion transport are markedly increased when the mucosal concentrations of free divalent cations such as Ca^{2+} and Mg^{2+} are decreased (Leonhard et al. 1990; Rübbelke 1998).

A Ca^{2+}-sensitive *I*sc has previously been shown in gastrointestinal epithelia from amphibia (Van Driessche et al. 1988; Krattenmacher et al. 1991), sheep (Schultheiss and Martens 1999), chicken (Heinz et al. 1991) and rabbit (Sellin and Dubinsky 1994) and probably represents a non-selective cation conductance (NSCC) in those tissues. The postulated functions for this conductance are Na^+, K^+ or Ca^{2+} transport or cell volume regulation (Schultheiss and Martens 1999).

Given the great significance of the rumen for the absorption of electrolytes, it was the aim of the present

study to characterize the divalent-cation-sensitive *I*sc (ΔIsc) of rumen tissue. The data obtained suggest that rumen epithelial cells likewise exhibit an apical NSCC, which permits the passage of monovalent cations in the mucosal absence of divalent cations.

Materials and methods

Tissues

Pieces of the ventral rumen wall were taken from slaughtered adult sheep or goats within 5 min after bleeding and immediately immersed in SCFA-containing buffer solution at 38 °C, where the mucosa was stripped from the underlying muscle layers and the serosa. Unless otherwise stated experiments were conducted with epithelia from sheep.

Electrical measurements

Mucosal tissues were mounted between the two halves of incubation chambers with an exposed area of 1 cm^2 or 3.14 cm^2. Edge damage was minimized by placing rings of silicon rubber on both sides of the tissues. Incubation chambers were connected to reservoirs containing 15 ml buffer solution on each side. The solutions were kept at 38 °C and were continuously stirred by the use of a gas lift system that supplied either 95% O_2/5% CO_2 or 100% O_2. The chambers were connected to a computer-controlled voltage clamp device (AC Microclamp, Aachen, Germany). Transepithelial potential differences (V_t) were measured through buffer solution agar bridges and calomel electrodes with reference to the mucosal solution. Tissue conductances (gt) were determined from the changes in V_t caused by bipolar current pulses of 100 μA cm^{-2} of 500 ms duration. The currents were passed through buffer solution agar bridges connected to Ag/AgCl electrodes in 3 M KCl. In each set-up fluid resistances and junction potentials were measured before mounting the mucosal tissues and corrected for during the experiments. The experiments were performed under short circuit conditions.

Solutions and chemicals

The standard solution contained (mmol l^{-1}): 140 Na^+, 5.4 K^+, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 124 Cl^-, 21 HCO_3^-, 2.4 HPO_4^{2-}, 0.6 $H_2PO_4^-$ and 10 glucose. In the SCFA buffer used to prepare and transport the tissues, 60 mmol l^{-1} Cl^- was replaced by 36 acetate, 15 propionate and 9 butyrate. To study the effect of other monovalent cations, 119 mmol l^{-1} Na^+ was replaced by K^+, Rb^+, or N-methyl-D-glucamine ($NMDG^+$). Ca^{2+}-free solutions contained 0.5 mmol l^{-1} EGTA. The pH of the solutions was 7.4, when gassed with 95% O_2/5% CO_2. Osmolarity was adjusted to 300 mosmol l^{-1} with mannitol. NMDG, EGTA and ionomycin were from Sigma (Deisenhofen, Germany). All other chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany).

Statistics

Results are given as means ± SEM. *n* designates the numbers of tissues, *N* the number of animals. Statistical significance was evaluated using analysis of variance (ANOVA) or Student's *t*-test, paired or unpaired as appropriate.

Results

Mucosal replacement of Ca^{2+} and Mg^{2+}

Changing the incubation conditions to Ca^{2+}-free and Mg^{2+}-free solutions on the mucosal side of sheep or goat rumen epithelia caused an increase in *I*sc and gt which reached a plateau value after about 20 min. Mucosal re-addition of Ca^{2+} and Mg^{2+} at the *I*sc plateau immediately decreased *I*sc and gt to control levels. Changing repeatedly between the presence and the absence of Ca^{2+} and Mg^{2+} ions on the mucosal side caused reproducible alterations in *I*sc and gt (Fig. 1A). A Ca^{2+}-sensitive *I*sc could still be shown after 7 h incubation. In some epithelia, the first *I*sc increase was somewhat smaller than the second and third increases. In most epithelia, basal *I*sc and the Ca^{2+}-sensitive *I*sc decreased with time (Fig. 1B).

Different monovalent cations

ΔIsc and divalent-sensitive gt (Δgt) could be shown when rumen epithelia were bathed in Na^+, K^+ or Rb^+ buffer on the mucosal side with no consistent differences between these three cations (Fig. 2). ΔIsc and Δgt were nearly abolished with $NMDG^+$ buffer on the mucosal side. A high K^+ buffer on the serosal side also abolished ΔIsc in the presence of Na^+ as the dominant mucosal cation, but had no effect on Δgt.

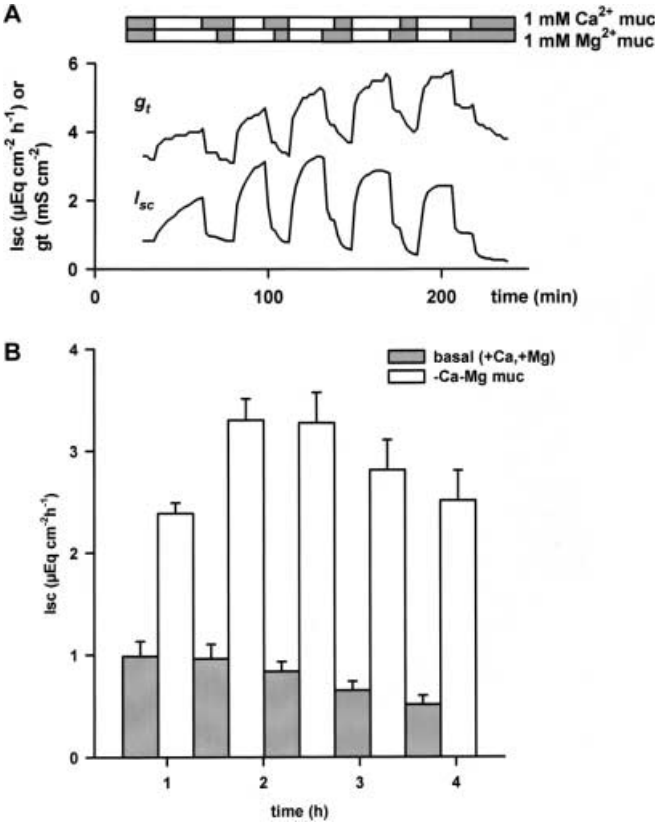

Fig. 1. **A** Effect of the mucosal presence (*grey boxes*) or absence (*white boxes*) of Ca^{2+} and Mg^{2+} ions on the short circuit current (*Isc*, *bottom line*) and the transepithelial conductance (g_t, *top line*) of rumen epithelium. **B** Basal and divalent sensitive *I*sc over time. Means ± SEM, $n/N = 8$ epithelia from four sheep

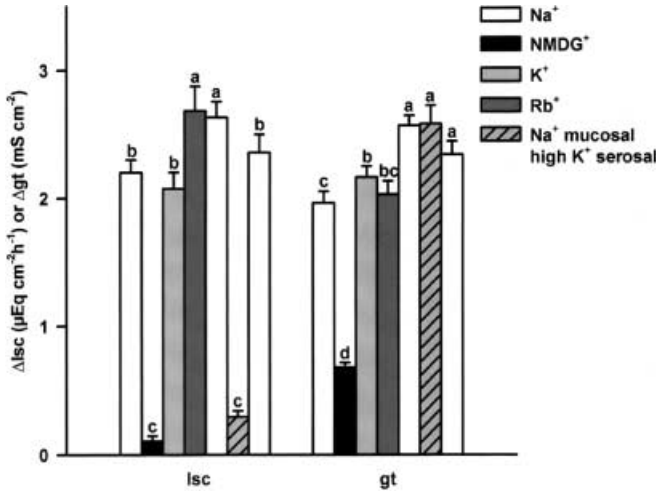

Fig. 2. Divalent sensitive *I*sc and gt in the presence of different monovalent cations on the mucosal side (Na^+, $NMDG^+$, K^+ or Rb^+) or on the serosal side (Na^+ or K^+). Means ± SEM, $n/N = 24$ epithelia from 11 sheep. *Columns with different letters* differ in $P < 0.05$

Effect of serosal $BaCl_2$

Reducing basolateral K^+ conductances by the addition of 1 mmol l^{-1} $BaCl_2$ to the serosal side reduced *I*sc by 1.12 ± 0.05 in the mucosal absence of divalent cations and by 0.20 ± 0.03 μEq cm^{-2} h^{-1} in their presence ($n = 6$, $P < 0.001$). Transepithelial conductance was reduced by 0.42 ± 0.06 mS cm^{-2} and 0.32 ± 0.05 mS cm^{-2} in these experiments.

Effect of ouabain

Blocking the basolateral Na^+/K^+-ATPase by serosal addition of ouabain under divalent cation free conditions on the mucosal side reduced the *I*sc by 70.9 ± 2.9% (1 μmol l^{-1} ouabain, $n = 3$), 71.3 ± 3.8% (10 μmol l^{-1}, $n = 4$) and 87.3 ± 6.5% (100 μmol l^{-1}, $n = 3$). There was a tendency for a higher ouabain effect at 100 μmol l^{-1} ($P = 0.06$). The addition of ouabain entailed a complete disappearance of the Δ*I*sc. The *I*sc increase due to mucosal elimination of Ca^{2+} and Mg^{2+} amounted to 0.93 ± 0.09 μEq cm^{-2} h^{-1} ($n = 10$) before and to 0.03 ± 0.03 μEq cm^{-2} h^{-1} after ouabain addition ($P < 0.05$ before vs. after ouabain).

Effects of Ca^{2+} versus Mg^{2+} ions on the mucosal side

On an equimolar basis (each 1 mmol l^{-1}), Ca^{2+} was more effective than Mg^{2+} in blocking the Δ*I*sc (Fig. 3). The addition of Ca^{2+} blocked 90.6 ± 0.9%, while the addition of Mg^{2+} blocked only 58.1 ± 2.4% of the Ca^{2+} and Mg^{2+} sensitive *I*sc. There was, however, no difference in their blocking potency on the Δgt. Ca^{2+} blocked 68.6 ± 2.0%, while Mg^{2+} blocked 62.4 ± 2.9% of the Ca^{2+} and Mg^{2+} sensitive gt.

Prolonged absence of mucosal Mg^{2+} ions

When only changing between the mucosal presence and absence of Ca^{2+} in the continued absence of mucosal Mg^{2+} ions (Mg was present on the serosal side), the appearance of the Ca^{2+}-sensitive *I*sc and gt across goat rumen epithelium changed depending on the duration of the mucosal Mg^{2+} absence (Fig. 4). A prolonged Mg^{2+} deprivation from the mucosal side allowed for a higher maximal increase of the Ca^{2+}-sensitive *I*sc and gt. An overshoot in *I*sc and gt as shown in Fig. 4 was observed in 11 epithelia from 6 goats, while another 10 epithelia from 10 goats showed an increase in the plateau values of the Ca^{2+}-sensitive *I*sc and gt. Re-addition of Mg^{2+} ions reduced the subsequent Ca^{2+}-sensitive and Mg^{2+}-sensitive *I*sc, as well as the basal values for *I*sc, gt and transepithelial potential difference (Fig. 5).

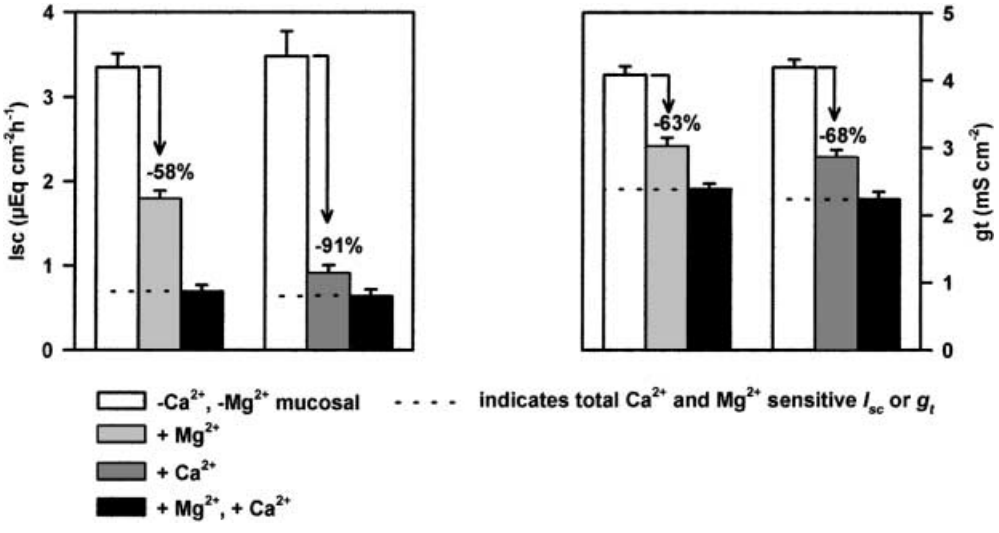

Fig. 3. Relative effects of mucosal Mg^{2+} or Ca^{2+} ions on *I*sc and gt. Means ± SEM, $n/N = 17$ and 12 epithelia from 9 sheep

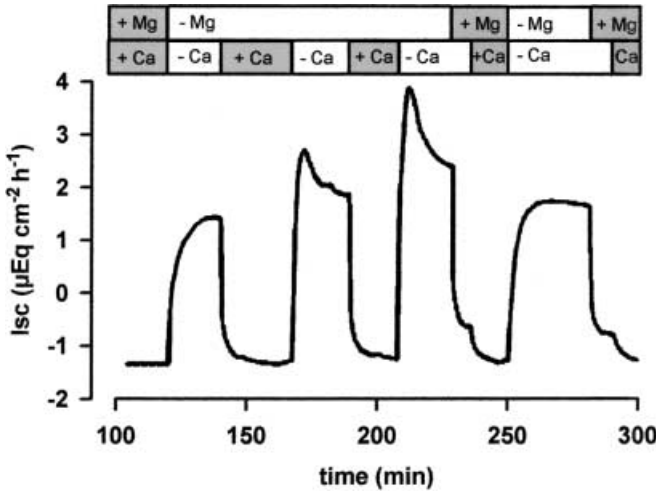

Fig. 4. Effect of a prolonged mucosal absence of Mg^{2+} ions on the Ca^{2+}-sensitive *I*sc across goat rumen. *Grey boxes*: mucosal presence of Mg^{2+} (*upper boxes*) or Ca^{2+} ions (*lower boxes*). *White boxes*: mucosal absence of Mg^{2+} or Ca^{2+} ions. Representative trace of 11 preparations from 6 goats

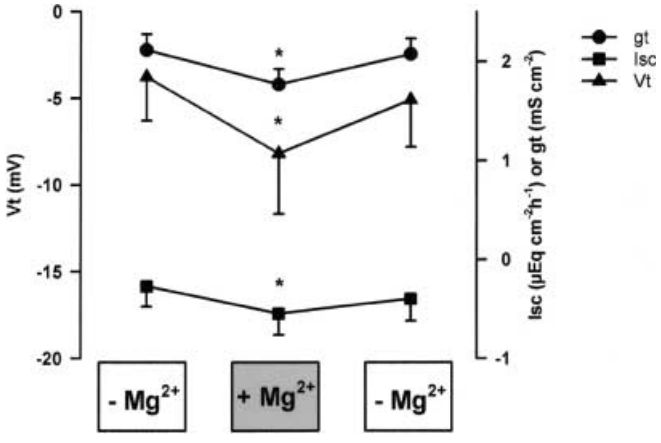

Fig. 5. Effect of mucosal Mg^{2+} ions on basal electrophysiological parameters. Means ± SEM, $n/N = 8$ epithelia from 8 goats; $^*P < 0.05$ versus $-Mg^{2+}$

In contrast to this, a continued mucosal absence of Ca^{2+} ions had no effect on the Mg^{2+}-sensitive *I*sc.

The negative *I*sc under basal conditions shown in Fig. 4 was not a general feature of goat rumen epithelia. Mean values for basal *I*sc across goat rumen amounted to -0.53 ± 0.11 μEq cm^{-2} h^{-1}, -0.09 ± 0.10 μEq cm^{-2} h^{-1}, $+0.89 \pm 0.06$ μEq cm^{-2} h^{-1} and $+1.30 \pm 0.05$ μEq cm^{-2} h^{-1} in four groups of animals (based on 24, 32, 36 and 54 epithelia from 9, 8, 10 and 9 goats, respectively).

Effect of mucosal Mg^{2+} in the presence of serosal barium

To investigate whether basolateral K^+ conductances might be involved in the effect of Mg^{2+} ions on gt, Mg^{2+} was added in the absence or presence of serosal barium. The mucosal addition of 1 mmol l^{-1} Mg^{2+} reduced gt by 1.4 ± 0.2 mS cm^{-2} in the absence of barium and by 1.2 ± 0.2 mS cm^{-2} in the presence of 1 mmol l^{-1} $BaCl_2$ on the serosal side ($n = 3$, $P < 0.05$, paired t-test). Serosal barium did not change the effect of a subsequent addition of 1 mmol l^{-1} Ca^{2+} ions on gt. Ca^{2+} reduced gt by 0.5 ± 0.1 mS cm^{-2} in the absence of barium and by 0.6 ± 0.2 mS cm^{-2} in the presence of barium.

Effect of serosal Mg^{2+}

The huge effect of mucosal Mg^{2+} deprivation alone was unexpected. To investigate the relative effects of mucosal versus serosal addition of Mg^{2+} ions, both conditions were tested in the mucosal absence of Ca^{2+} (Fig. 6). Serosal absence of Mg^{2+} increased *I*sc by 0.21 ± 0.03 μEq cm^{-2} h^{-1} and 0.40 ± 0.05 μEq cm^{-2} h^{-1} in the presence and absence of mucosal Mg^{2+}, respectively, while mucosal absence of Mg^{2+} increased *I*sc by 1.41 ± 0.22 μEq cm^{-2} h^{-1} and 1.60 ± 0.23 μEq cm^{-2} h^{-1} in the presence and absence of serosal Mg^{2+} ($n = 7$), respectively. Both effects were significant, but the influence of mucosal Mg^{2+} ions was much more pronounced. There was a statistically significant interaction ($P < 0.01$) between the effects of mucosal and serosal Mg^{2+} ions. The effects on gt were comparable, but did not depend on the Mg^{2+} concentration on the other side of the tissue (Fig. 6).

Effect of serosal ionomycin

In an attempt to separately increase intracellular Ca^{2+}, the mucosal solution was changed to divalent-free

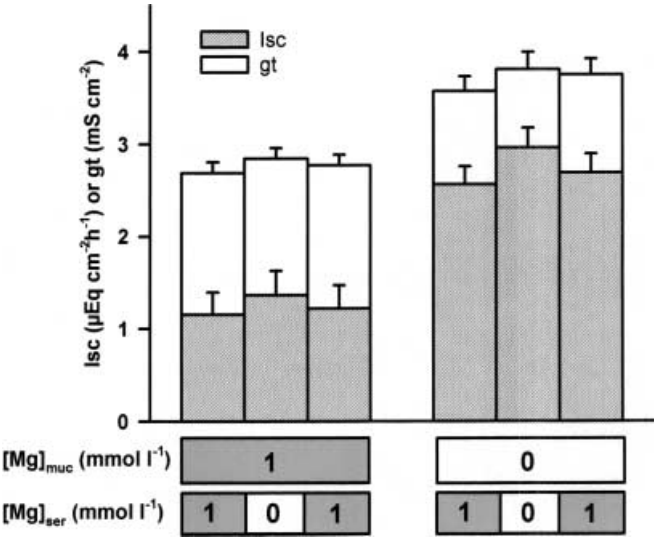

Fig. 6. Effect of serosal versus mucosal Mg^{2+} on *I*sc and gt in the absence of mucosal Ca^{2+}. Means ± SEM, $n/N = 7$ epithelia from 7 sheep, $P < 0.001$ for mucosal Mg, serosal Mg, and mucosal Mg×serosal Mg

conditions and then ionomycin (1 μmol l^{-1}) was added to the serosal side. Ionomycin addition reduced *I*sc from 1.98 ± 0.46 μEq cm^{-2} h^{-1} to 1.75 ± 0.43 μEq cm^{-2} h^{-1} within 20 min ($P < 0.01$, $n = 6$).

Effect of mucosal $BaCl_2$

In the mucosal absence of Ca^{2+} and Mg^{2+} ions, the addition of 1 mmol l^{-1} $BaCl_2$ to the mucosal side reduced *I*sc by 1.53 ± 0.13 μEq cm^{-2} h^{-1} and gt by 1.03 ± 0.11 mS cm^{-2}. In the mucosal presence of Ca^{2+} and Mg^{2+} ions, the same addition of $BaCl_2$ had significantly smaller effects. It increased *I*sc by 0.10 ± 0.00 μEq cm^{-2} h^{-1} and reduced gt by 0.42 ± 0.08 mS cm^{-2} ($n = 6$, $P < 0.01$ for both parameters vs. Ca^{2+}-free and Mg^{2+}-free).

Discussion

Divalent-cation-sensitive *I*sc

Removal of Ca^{2+} and Mg^{2+} ions from the mucosal side of the rumen epithelium opens an electrogenic conductance in the apical membrane. This is shown by the parallel increase in *I*sc and gt (Fig. 1A) and by the decrease of the fractional apical resistance together with a depolarization of the apical membrane potential of rumen epithelial cells (Lang and Martens 1999). The increase in *I*sc required the mucosal presence of small monovalent cations. Na^+, Rb^+ or K^+ as the main monovalent cations on the mucosal side allowed for comparable increases in *I*sc and gt, while *I*sc was nearly abolished and gt significantly reduced with $NMDG^+$ as the main mucosal cation (Fig. 2). In addition to this, Rübbelke (1998) had shown a comparable Ca^{2+}-sensitive increase in *I*sc when Na^+ was replaced by Cs^+ ions. In a Na-buffer the divalent-sensitive increase in *I*sc was accompanied by an increase in Na^+ flux from the mucosal to the serosal side in the same order of magnitude (Rübbelke 1998). The development of a Na^+ current across the tissue depends on the activity of the Na^+/K^+-ATPase and on the basolateral recycling of K^+ ions through Ba^{2+}-sensitive K^+ channels. This is shown by the decrease in Δ*I*sc after reducing the K^+ gradient between the cellular and serosal compartments (Fig. 2) or after addition of $BaCl_2$ to the serosal side. A prolonged exposure to Ca^{2+}-free solutions might disrupt epithelial tight junctions, which could have increased the passive and paracellular transport rates over the epithelium. Generally, the increase in gt upon removal of Ca^{2+} ions was associated with an increase in current over the tissues (Fig. 1), whereas an increased paracellular conductance should entail an increase in gt together with a decrease in *I*sc. In a few epithelia only, a second slower increase in gt started 30 min after exposure to a Ca^{2+}-free solution and was not associated with an increase in *I*sc. It might therefore represent the beginning of a decrease in paracellular resistance. However, the Na^+ flux from the serosal to the mucosal side as well as mannitol fluxes are not altered by the mucosal elimination of divalent cations (Rübbelke 1998), which speaks against a short time effect of mucosal Ca^{2+} ions on the paracellular pathway.

The effect of Ba^{2+} ions added to the mucosal side should be discussed in two ways. On the one hand, barium, as a divalent cation, might directly block this NSCC. This is supported by the observation that the effect of mucosal Ba^{2+} on the transepithelial conductance was more than doubled in the absence of Ca^{2+} and Mg^{2+} ions. On the other hand, the small increase in current after Ba^{2+} addition in the presence of Ca^{2+} and Mg^{2+} could be the equivalent of a reduced K secretion. This points to a possible effect of Ba^{2+} ions on luminal K^+ conductances in the present epithelia, which could have reduced the electric driving force for Na^+ uptake across the apical membranes. In older studies, however, K fluxes from the serosal to the mucosal side were barely reduced by mucosal Ba^{2+} ions (Leonhard 1990; Leonhard-Marek and Martens 1996).

Sides of Mg^{2+} block of Δ*I*sc

Controlled patch-clamp experiments to localize the divalent cation block to the extracellular or intracellular side of non-selective cation channels have been conducted in a few studies. An apical cation channel in toad urinary bladder was blocked by extracellular but not by intracellular Ca^{2+} (Das and Palmer 1989). In contrast to this a cation channel in cultured human keratinocytes showed a decrease in single-channel current when Ca^{2+} and/or Mg^{2+} were elevated on either side of the patch (Galietta et al. 1991). A high permeability to monovalent cations upon extracellular removal of divalent cations is also a feature of store-operated Ca^{2+} channels (Kerschbaum and Cahalan 1998), voltage-gated Ca^{2+} channels (Corry et al. 2001) and arachidonate-regulated Ca^{2+} channels (Mignen and Shuttleworth 2001). Currents through store-operated Ca^{2+} channels are also blocked by intracellular Mg^{2+}.

In the present study a prolonged Mg^{2+} deprivation from the mucosal side only allowed a higher maximal increase of the Ca^{2+}-sensitive *I*sc across goat rumen (Fig. 4). This finding was rather unexpected and suggests that not only extracellular but also intracellular Mg^{2+} ions may have an influence on the transport of monovalent cations across the rumen tissue. While extracellular Mg^{2+} ions are supposed to be removed from the mucosal side at every solution change, the concentration of free Mg^{2+} ions on the intracellular side ($[Mg^{2+}]_i$) seems to depend on the luminal availability over time. Experiments with isolated cells from rumen and other epithelia have shown that $[Mg^{2+}]_i$ is decreased when the cells are incubated in Mg^{2+}-free media over a prolonged period of time (Dai and Quamme 1991; Schweigel et al. 1999). Comparing the effects of mucosal

versus serosal Mg^{2+} on *I*sc and gt (Fig. 6) across the epithelium shows that the effects of mucosal Mg^{2+} were up to seven times higher than those of serosal Mg^{2+}. This observation together with the electrophysiological effects of a prolonged mucosal deprivation of Mg^{2+} ions (Fig. 4) suggests that in the rumen, as a Mg^{2+} absorbing epithelium, the luminal Mg^{2+} availability alone can change $[Mg^{2+}]_i$. This would interfere with the recently postulated function of $[Mg^{2+}]_i$ as a second messenger (Takaya et al. 2000).

The blocking effect of $[Mg^{2+}]_i$ on *I*sc might be either a direct one on the cation channel as in keratinocytes or an indirect one. As shown above, the magnitude of Na^+ current across the epithelium not only depends on the apical Na^+ conductance, but also on the basolateral K^+ conductance to allow for a high turnover of the Na^+/K^+-ATPase in the course of Na^+ absorption. Ruminal K^+ fluxes from serosal to mucosal can be more than doubled after serosal application of barium, which reveals a high K^+ recycling over the basolateral membrane (Leonhard-Marek and Martens 1996). Intracellular Mg^{2+} is responsible for the inward rectification of K^+ channels in a variety of tissues, i.e., for the block of outward currents (Nichols and Lopatin 1997). In the current study, the depressing effect of mucosal Mg^{2+} on gt was reduced after pre-incubation with serosal barium. This observation could point to an effect of Mg^{2+} on basolateral K^+conductances. It could also mean that Ba^{2+} had limited the amount of *I*sc to be inhibited with mucosal Mg^{2+}. However, the fact that barium did not change the effect of Ca^{2+} on gt contradicts this latter assumption.

If Mg reduces K^+ diffusion over the basolateral membrane, this should also have an impact on basal electrophysiological parameters. It should reduce the conductance and the potential difference of the basolateral membrane and thereby decrease the transepithelial conductance and potential difference. Both effects could be shown (Fig. 5). Thus, ruminal $[Mg^{2+}]_i$ might partly reduce the divalent cation sensitive *I*sc by decreasing basolateral K^+ recycling.

The side of the Ca^{2+} block of ruminal ΔIsc

Ionomycin was used in an attempt to increase intracellular Ca^{2+} in the absence of mucosal Ca^{2+}. The small decrease in *I*sc seen in these experiments was, however, in the same order of magnitude as mere time-dependent decreases in *I*sc in other epithelia. From the current study it seems, therefore, that Ca^{2+} ions block from the extracellular side.

Conclusions

The data suggest that rumen epithelial cells exhibit an apical NSCC, which permits the passage of monovalent cations in the mucosal absence of divalent cations. Whether this conductance is used in vivo as a Na, Ca or Mg transport pathway or whether it is involved in cell volume regulation, as postulated for other NSCC, remains to be investigated. The development of a divalent-cation-sensitive Na^+ current across the tissue requires Na^+/K^+ pumps and K^+ recycling through Ba^{2+}-sensitive K^+ conductances on the basolateral side.

Ca^{2+} and Mg^{2+} ions block the transepithelial current of monovalent cations from the extracellular side. Mg^{2+} ions seem to have an additional intracellular effect. The data further suggest that a prolonged deprivation of mucosal Mg^{2+} alone might decrease the concentration of intracellular Mg^{2+} in this Mg-absorbing tissue.

Acknowledgements This project was supported by the Deutsche Forschungsgemeinschaft, Le 824/2–1. The technical assistance of M. Burmester is greatly appreciated. The experiments comply with current German laws.

References

Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 70:567–590

Corry B, Allen TW, Kuyucak S, Chung S-H (2001) Mechanisms of permeation and selectivity in calcium channels. Biophys J 80:195–214

Dai L-J, Quamme GA (1991) Intracellular Mg^{2+} and magnesium depletion in isolated renal thick ascending limb cells. J Clin Invest 88:1255–1264

Das S, Palmer LG (1989) Extracellular Ca2+ controls outward rectification by apical cation channels in toad urinary bladder: patch–clamp and whole–bladder studies. J Membr Biol 107:157–168

Dobson A (1959) Active transport through the epithelium of the reticulo-rumen sac. J Physiol 146:235–251

Galietta LJ, Barone V, De Luca M, Romeo G (1991) Characterization of chloride and cation channels in cultured human keratinocytes. Pflügers Archiv 418:18–25

Heinz M, Krattenmacher R, Hoffmann B, Clauss W (1991) Different modes of electrogenic Na^+ absorption in the coprodeum of the chicken embryo: role of extracellular Ca^{2+}. J Comp Physiol A 161:363–370

Kerschbaum HH, Cahalan MD (1998) Monovalent permeability, rectification, and ionic block of store-operated calcium channels in Jurkat T lymphocytes. J Gen Physiol 111:521–537

Krattenmacher R, Voigt R, Heinz M, Clauss W (1991) Electrolyte transport through a cation-selective ion channel in large intestinal enterocytes of *Xenopus laevis*. J Exp Biol 155:275–290

Lang I, Martens H (1999) Na transport in sheep rumen is modulated by voltage-dependent cation conductance in apical membrane. Am J Physiol 277:G609–G618

Leonhard S (1990) In vitro studies on Mg transport through the rumen epithelium of sheep. Proposal of a model. Free University of Berlin, Inaugural-Dissertation

Leonhard S, Smith E, Martens H, Gäbel G, Ganzoni E (1990) Transport of magnesium across an isolated preparation of sheep rumen: a comparison of $MgCl_2$, Mg aspartate, Mg pidolate and Mg-EDTA. Magnesium Trace Elements 9:265–271

Leonhard-Marek S, Martens H (1996) Effects of potassium on magnesium transport across rumen epithelium. Am J Physiol 271:G1034–G1038

Martens H, Gäbel G (1988) Transport of Na and Cl across the epithelium of ruminant forestomachs: rumen and omasum. Comp Biochem Physiol A 90:569–575

Mignen O, Shuttleworth TJ (2001) Permeation of monovalent cations through the non-capacitative arachidonate-regulated Ca^{2+} channels in HEK293 cells. Comparison with endogenous store-operated channels. J Biol Chem 276:21365–21374

Nichols CG, Lopatin AN (1997) Inward rectifier potassium channels. Ann Rev Physiol 59:171–191

Rübbelke MK (1998) In vitro Untersuchungen des Pansenepithels von Schafen zur Charakterisierung eines elektrogenen, Calcium-sensitiven Natriumtransportes. Free University of Berlin, Inaugural-Dissertation

Schultheiss G, Martens H (1999) Ca-sensitive Na transport in sheep omasum. Am J Physiol 276:G1331–G1344

Schweigel M, Lang I, Martens H (1999) Mg^{2+} transport in sheep rumen epithelium: evidence for an electrodiffusive uptake mechanism. Am J Physiol 277:G976–G982

Sellin JH, Dubinsky WP (1994) Apical nonspecific cation conductances in rabbit cecum. Am J Physiol 266:G475–G484

Takaya J, Higashino H, Koobayashi Y (2000) Can magnesium act as a second messenger? Current data on translocation induced by various biologically active substances. Magnesium Res 13:139–146

Tomas FM, Potter BJ (1976) The site of magnesium absorption from the ruminant stomach. Br J Nutr 36:37–45

Van Driessche W, Simaels J, Aelvoet I, Erlij D (1988) Cation-selective channels in amphibian epithelia: electrophysiological properties and activation. Comp Biochem Physiol A 90:693–699