Na transport in sheep rumen is modulated by voltage-dependent cation conductance in apical membrane

INGO LANG1 AND HOLGER MARTENS2

1Department of Veterinary Physiology, Free University of Berlin, 14163 Berlin; and 2Department of Zoophysiology and Cell Biology, University of Potsdam, 14471 Potsdam, Germany

Lang, Ingo, and Holger Martens. Na transport in sheep rumen is modulated by voltage-dependent cation conductance in apical membrane. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G609–G618, 1999.—The effects of clamping the transepithelial potential difference (PD; mucosa reference) have been studied in sheep rumen epithelium. Pieces of ruminal epithelium were examined in Ussing chambers, in a part of the experiments combined with conventional intracellular recordings. After equilibration, the tissue conductance (Gt) was 2.50 ± 0.09 mS/cm², the potential difference of the apical membrane (PDa) was −47 ± 2 mV, and the fractional resistance of the apical membrane (Rfa) was 68 ± 2% under short-circuit conditions. Hyperpolarization of the tissue (bloodside positive) depolarized PDa, decreased Rfa, and increased Gt significantly. Clamping PDa at negative values caused converse effects on PDa and Rfa. All changes were completely reversible. The determination of individual conductances revealed that the conductance of the apical membrane increased almost linearly with depolarization of PDa. The PD-dependent changes were significantly reduced by total replacement of Na. These observations support the assumption of a PD-dependent conductance in the apical membrane that permits enhanced apical uptake of Na even at depolarized PDa. This mechanism appears to be important for the regulation of osmotic pressure in forestomach fluid.

sheep rumen; sodium transport; potential difference-dependent conductance

The rumen is an important site of Na absorption in the digestive system of sheep, and it has long been known that the absorption of Na from the rumen is mediated by an active transport mechanism (8). This conclusion has been supported by all the subsequent in vitro studies (7, 17, 28), which have further revealed that the flux in net Na (JNa) is considerably higher than the (Na-dependent) short-circuit current (Isc). The discrepancy between Isc and JNa has led to the assumption of two parallel transport mechanisms for Na, namely electrogenic and electroneutral (7, 28). These mechanisms enable the rumen epithelium to cope with the wide range of ruminal Na concentrations between 21 mmol/l (31) and 145 mmol/l (2). At low Na concentrations, Na is mainly transported via the electrogenic pathway, whereas, at high Na concentrations, the electroneutral Na/H exchange mechanism is predominant (26). However, this extended knowledge of ruminal Na transport does not explain a very old observation: an increase of K intake and, consequently, of ruminal K concentration enhances Na absorption from the rumen (38, 42); this causes a very close and reciprocal relationship between ruminal Na and K concentration, i.e., the concentration of Na is low at high K and vice versa. Consequently, the sum of the Na and K concentrations in ruminal fluid is kept almost constant (38). The physiological meaning of this mechanism can readily be appreciated, because the K-dependent Na absorption prevents an increase of osmotic pressure in the ruminal fluid and hence a flow of water into the forestomachs when diets with a high K content are consumed. The underlying mechanism of the K-dependent Na transport is unknown. Stacy and Warner (42) have suggested a stimulation of Na absorption by an increase of luminal osmotic pressure. In a previous study, we tested the hypothesis that the K-dependent Na transport is mediated by electroneutral Na-K-2Cl cotransport, but mucosal addition of furosemide or bumetanide (1 mM), which are potent inhibitors of Na-K-2Cl cotransport, does not change Na fluxes in isolated epithelia of sheep rumen (28). Alternatively, a link between the electrogenic Na transport and the ruminal K concentration appears to be contradictory and not in accord with the electrophysiological consequences of high ruminal K concentrations. An increase in ruminal K concentration depolarizes the apical membrane of rumen epithelium (25) and hence reduces the driving force for apical Na uptake. Furthermore, a positive correlation between the ruminal log of the K concentration and the transepithelial potential difference (PD; bloodside positive) has been demonstrated (11); this would enhance passive (paracellular) backflow of Na from the blood into the rumen. These effects would obviously reduce net Na absorption, in contrast to the well-known experimental observation of enhanced Na transport at high ruminal K.

A different explanation for these contradictions might be provided by our unpublished observation that the rumen epithelium exhibits a reversible increase in total tissue conductance (Gt) at increasing transmural PD (bloodside positive). If this change in Gt is primarily or solely located in the apical membrane, the following working hypothesis could explain K-dependent Na transport: an increase in ruminal K concentration depolarizes the apical membrane and increases or induces a PD-dependent cation conductance, which enhances Na uptake (despite a reduced electrical driving force) and finally increases transepithelial Na transport via the basolateral Na-K-ATPase. The aim of the present study was to test this hypothesis with the use of Ussing chambers and microelectrode techniques.
METHODS

Animals. The sheep used varied in breed, age, and sex. They had liberal access to drinking water and hay. The animals were intended for human consumption and killed at a local slaughterhouse.

Epithelia. Immediately after slaughter, pieces of the ventral rumen sac were excised. They were immersed in a transport buffer solution (maintained at 38°C and gassed with carbogen) and stripped of the attached muscle layers and the serosa.

Ussing chambers. Pieces of epithelia were mounted between the halves of an Ussing chamber with an exposed area of 0.95 cm². Edge damage was minimized by rings of silicon rubber between the chamber halves and the tissue.

Above each side of the tissue was a reservoir with 16 ml buffer solution, maintained at 38°C and continuously stirred by a gas lift system with oxygen.

For the experiments with microelectrodes, a modified Ussing chamber with a tissue area of 0.79 cm² was used. The chamber had a volume of 0.6 ml on the mucosal side and 0.5 ml on the serosal side. Both halves were perfused with oxygenated buffer solution at a flow rate of 14 ml/min; the solution was heated to 38°C immediately before entering the chamber.

Microelectrodes. Microelectrodes were pulled from filamented borosilicate glass and filled with 0.5 M KCl, yielding resistances of 15–25 MΩ. Rumen epithelial cells were impaled across the apical membrane with a motorized micromanipulator with a piezo element. The PD of the apical membrane (PDₐ) was measured with reference to the mucosal solution. PDₐ and PDₐ were observed on an oscilloscope. Impalements were accepted if 1) there was an abrupt fall in PDₐ during advancing of the microelectrode, 2) PDₐ remained stable for at least 1 min, and 3) PDₐ returned to 0 ± 3 mV on withdrawal of the electrode.

Electrical measurements. The preparations in the conventional Ussing chambers were connected to a computer-controlled voltage-clamp device (AC-microclamp, Aachen, Germany). The PDₐ was measured through KCl (3 M) agar bridges near the tissue and calomel electrodes. External current could be passed through the epithelium via another pair of agar bridges. The Iₛ and the technical current (Iₜ) needed to clamp PDₐ to defined voltages were recorded. The Gₛ was determined from the change in PDₐ caused by unidirectional current pulses superimposed on Iₛ. The pulse duration was 500 ms, with the start of the recording after 250 ms. In the microelectrode studies, transepithelial voltage pulses had an amplitude of 10 mV and a duration of 160 ms. The pulse frequency was 0.5 Hz. Pulses were generated and measurements were performed with a microelectrode amplifier and voltage-clamp device (Biomedical Instruments, Munich, Germany). The fractional resistance of the apical membrane (Rₛ) was calculated from the pulse-induced changes in PDₐ relative to the changes in PDₐ (Rₛ = ΔPDₐ/ΔPDₐ). PDₐ, Gₛ, Iₛ, PDₐ, Rₛ, and the electrode resistance were permanently displayed on a chart recorder and stored on a PC.

Calculations. The method for the calculation of membrane resistances described by Frömter and Gebler (12) was modified. The voltage divider ratio α = Rₛ/Rₛ (where Rₛ is resistance of the apical membrane and Rₛ is resistance of the basolateral membrane) was calculated as Rₛ/(1 – Rₛ). It was assumed that the use of Ca- and Mg-free solution on the mucosal side altered only the Rₛ membrane and did not influence the parallel shunt pathway. The parameters measured with no divalent cations in the mucosal solution are marked with an asterisk: Rₛ, Rₛ, and resistance of the paracellular pathway (Rₚ) were calculated as:

\[ R₁ = \frac{\alpha \cdot (\alpha - \alpha^*) \cdot R_s \cdot R_p^*}{(1 + \alpha) \cdot (1 + \alpha^*) \cdot (R_s - R_p^*)} \]

\[ R₂ = \frac{(\alpha - \alpha^*) \cdot R_s \cdot R_p^*}{(1 + \alpha) \cdot (1 + \alpha^*) \cdot (R_s - R_p^*)} \]

\[ Rₚ = \frac{(\alpha - \alpha^*) \cdot R_s \cdot R_p^*}{(1 + \alpha) \cdot R_p^* - (1 + \alpha^*) \cdot R_s} \]

These resistances are converted to conductances in Results.

Solutions. The buffer solution used for the transport of the epithelia from the slaughterhouse to the laboratory contained (in mmol/l) 1.2 CaCl₂, 1.2 MgCl₂, 2.4 Na₂HPO₄, 0.4 NaH₂PO₄, 50.0 NaHCO₃, 5.0 KCl, 115.0 NaCl, and 5.0 glucose. It was gassed with carbogen. The other solutions were gassed with oxygen and buffered to pH 7.4 with Tris. Osmolarity was adjusted to 300 mosmol/l with mannitol. The control solution contained (in mmol/l) 2.0 K₂HPO₄, 1.0 KH₂PO₄, 10.0 glucose, 8.0 MOPS, 120.0 NaCl, 1.0 CaCl₂, and 1.0 MgCl₂. In the Na-free solution, Na₂ was replaced by N-methyl-D-glucamine (NMDG). The nominally Ca- and Mg-free solutions contained 1.0 mmol/l EDTA and no added CaCl₂ or MgCl₂.

Statistics. Results are given as means ± SE or as single values; n is the number of tissues in the Ussing chamber studies or the number of impalements in the microelectrode studies. Statistical comparisons were made by a paired Student’s t-test; P values of < 0.05 were considered significant.

RESULTS

Capacitive currents. During the passage of short pulses across a tissue as complex as the rumen epithelium, capacitance effects occur. To check the duration of the capacitive currents and to choose a pulse duration long enough to avoid artifacts in the calculation of Gₛ, we damped the epithelium to +40 mV with a rectangular voltage pulse and observed Iₛ at a high time resolution. Figure 1 shows an example of such a recording. In Fig. 1B it is evident that after 160 ms, the earliest time we used for the calculation of Gₛ, capacitive currents have disappeared.

Voltage dependence of Gₛ. The PD of the rumen epithelium was linearly correlated with increasing log of the K concentration in the ruminal fluid with a range of 20–60 mV (bloodside positive; Ref. 11). The applied PD included this physiological range and was extended to negative values, from −80 mV to +80 mV. The PD-Gₛ relationship is shown in Fig. 2A. Only small alterations of Gₛ were seen when PDₛ was changed from −80 to 0 mV. In contrast, Gₛ curvilinearly increased from 2.5 ± 0.09 μS/cm² under short-circuit conditions (0 mV) to 3.68 ± 0.13 μS/cm² at 80 mV (P < 0.05). It should be noted that alterations of Gₛ were pronounced at physiological (in vivo) PDₛ (20–60 mV). The PD-induced variations of Gₛ were completely reversible and independent of the sequence of the applied PDₛ (stepwise from minus to plus or alternating polarity pulses). The PDₛ-Iₛ relationship from the same tissues is given in Fig. 2B. The Iₛ intercept at 0 PDₛ represents the Iₛ (12.4 ± 1.27 μA/cm²). Despite the noticeably enhanced
Gt at positive PDt, the PDt-Gt relationship deviated only slightly from linearity. To analyze this discrepancy, we calculated a current (Icalc), with the clamped PDt and the measured Gt (Fig. 2B). The difference between Icalc and It when PDt = 0 mV represented the Isc. Moreover, at any other PDt, this difference represented the current caused by active rheogenic ion transport (Iact; Fig. 2B). Because Icalc was always higher than the measured It, active electrogentic ion movement must have contributed to the PDt; this consequently reduced the measured It at a given PDt.

Na and PD-dependent changes of Gt and It, Iact significantly increased with positive PDt. When PDt = 80 mV, Iact was 76.2 ± 3.1 µA/cm². At negative PDt up to −60 mV, there was no significant difference between Isc and Iact. A possible explanation for the difference between Iact and Isc could be an electrogentic Na transport in the mucosal-serosal direction that is stimulated at positive PDt. This would be in agreement with the well-known in vivo observations and our working hypothesis. To examine whether Na represents or significantly contributes to this current, Na was replaced by NMDG on both sides of the tissues, and the responses of Gt and It were measured upon the alteration of PDt. The PDt-Gt relationship is given in Fig. 3A. Na replacement significantly reduced Gt, from 2.49 ± 0.14 to 1.98 ± 0.09 mS/cm², under short-circuit conditions. A small PD-dependent increase of Gt was still present under Na-free conditions; at 80 mV, a Gt of 2.43 ± 0.10 mS/cm² was obtained. This is an increase of 24 ± 1.7%, which

Fig. 1. A: typical example of effect of a rectangular voltage pulse hyperpolarizing rumen epithelium to +40 mV on technical current (It). Pulse duration was 10 s, and sampling frequency was 100 Hz. After capacitive currents at onset of pulse, there was no further change in It, B: part of A at a higher time resolution. In our experiments, determination of tissue conductance (Gt) with short pulses was performed 250 or 160 ms after onset of pulses. Figure clearly shows that, after this time, capacitive currents have disappeared.

Fig. 2. A: transepithelial potential difference (PDt)-Gt relationship. Hyperpolarization of tissue strongly enhances Gt; n = 30. B: PDt-It relationships of same tissues as in A are represented. With positive PDt, relationship deviates slightly from linearity but less than expected from PDt-dependent increase in Gt (A). We calculated a current from PDt and Gt, namely Icalc. Difference between these two currents represents active rheogenic ion transport (Iact). Under short-circuit conditions, this current is by definition identical to short-circuit current (Isc). At positive PDt, it significantly increases. This may be explained by increased rheogenic Na transport.
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EFFECTS OF PDt ON ELECTROGENIC NA TRANSPORT

Under Na-free conditions, IµA/cm². The most important information deduced from this experiment was the calculation of Iact at positive PDt. 

Because the concurrent changes of PDa and PDt were not known, these PD were measured under voltage clamp conditions of PDt from -80 to +80 mV by mucosal impalement with microelectrodes. The relationship between PDt and PDa is almost linear: PDt = 0.66 PDa - 47.7 mV (r² = 0.92), n = 15.

Voltage dependence of fRa. The working hypothesis in this study is the assumption that the depolarization of PDa by high ruminal K concentrations increases or induces a PD-dependent (cation) conductance in the apical membrane, this may explain the increase in Gt upon hyperpolarization of PDt (bloodside positive). If this assumption is correct, the measurement of the fractional resistance, fRa = Ra/(Ra + Rb), should be sensitive upon manipulation of PDa. The determination of these parameters has been possible by microelectrodes for many years (12). Figure 4 shows a representative mucosal impalement of rumen epithelium. PDt, Gt, PDa, and fRa were recorded simultaneously. Alterations of PDt caused the known effects on Gt. A hyperpolarization was accompanied by a decrease in PDa and fRa. All PD-induced alterations were completely reversible. Figure 5 summarizes the relationship between PDa, Gt, and fRa and clearly shows the PD-dependent reciprocal relationship of Gt and fRa. An increase in Gt is accompanied by a decline in fRa and vice versa. It is worthwhile mentioning that fRa linearly decreased within the physiological range of PDt (20–60 mV). A very close and linear correlation was obtained between PDa and fRa (Fig. 6).

Calculation of the membrane conductances. The preceding experiments demonstrated that the rumen epithelium exhibited PD-dependent alterations of Gt and fRa, supporting the assumption of changes in the conductance(s) in the apical membrane. Because fRa only represents a quotient, information regarding the absolute changes is still lacking. An analysis of epithelial resistances is possible when only one resistance can be altered reversibly. In tissues with classic electrogenic Na transport, this manipulation can easily be performed by mucosal addition of amiloride, which is a potent and reversible inhibitor of the Na channel (12). The electrogenic Na transport of the rumen epithelium is amiloride insensitive (30), but removal of the divalent cations Ca and Mg from the mucosal side significantly enhances Gt (and ISc) of sheep rumen epithelium (24). This reversible change of Gt was therefore used for the determination of resistances. Hence, we repeated the microelectrode studies with or without divalent cations in the mucosal solution at PDt of 0, 20, 40, 60, and 80 mV (negative PDt were omitted because they caused a large and irreversible increase of Gt upon removal of Ca and Mg), and we recorded Gt, PDa, and fRa from the same impalement. The data thus obtained

Fig. 3. A: PDt-Gt relationship. After control experiment, same tissue samples were incubated with Na-free solution. Under each voltage clamp condition, Gt was calculated under both control and Na-free conditions. Under Na-free conditions in which PDt = 0 mV, Iact = ISc and is almost zero. Under control conditions, active rheogenic ion transport is significantly more stimulated by a hyperpolarization of the epithelium than under Na-free conditions.

B: PDt-Iact relationship. Replacement of Na abolished the ISc almost completely, from 13.5 ± 2.32 µA/cm² (control) to 1.1 ± 1.00 µA/cm². The most important information deduced from this experiment was the calculation of Iact (Fig. 3B). Under Na-free conditions, Iact increased to 31.0 ± 2.3 µA/cm² at PDt = 80 mV. This is 29.9 ± 2.6 µA/cm² larger than ISc. In controls from the same animals, this difference is 75.7 ± 4.8 µA/cm², which is significantly higher. The difference between treatment and control may indicate that Na contributes to the enhanced Iact at positive PDt.

PDt and PDa. Because the concurrent changes of PDa and/or the PD of the basolateral membrane (PDb) when altering PDt were not known, these PD were measured under voltage clamp conditions of PDt from -80 to +80 mV by mucosal impalement with microelectrodes. The relationship between PDt and PDa is almost linear: PDa = 0.66 PDt - 47.7 mV (r² = 0.92), n = 15.

Figures 4-6 show the relationship between PDt, PDa, Gt, fRa, and Iact. These figures indicate that PDt-induced changes in PDa, Gt, fRa, and Iact are also less distinct; Iact increased to 31.0 ± 2.3 µA/cm² at PDt = 80 mV. This is 29.9 ± 2.6 µA/cm² larger than ISc. In controls from the same animals, this difference is 75.7 ± 4.8 µA/cm², which is significantly higher. The difference between treatment and control may indicate that Na contributes to the enhanced Iact at positive PDt.
are summarized in Table 1. Removal of Ca and Mg from the mucosal solution caused a significant increase in $G_t$ and $I_{sc}$, depolarized $PD_a$, and reduced $fR_a$ significantly under short-circuit conditions. Furthermore, the known PD-dependent alterations of $G_t$ and $fR_a$ were also observed in the absence of mucosal Ca and Mg. The calculation of the individual resistances clearly showed that conductance of the apical membrane ($G_a$) was significantly enlarged from $1.34 \pm 0.14 \, \text{mS/cm}^2$ under short-circuit conditions to $2.60 \pm 0.22 \, \text{mS/cm}^2$ at $80 \, \text{mV PD}_t$ (Fig. 7A). Because conductance of the basolateral membrane ($G_b$) and conductance of the paracellular pathway ($G_p$) (despite some scatter of the data at $80 \, \text{mV}$) remained unchanged (Fig. 7, B and C), the PD-dependent changes of $G_t$ must have been located in the apical membrane.

**DISCUSSION**

Previous studies have shown that an increase of apical K concentrations elevates $PD_t$ (9, 11), depolarizes $PD_a$ (25), and enhances Na absorption from the rumen of sheep (8, 38). Because K-dependent electroneutral Na transport has not been demonstrated in sheep rumen (28), it has been hypothesised that the depolarization of $PD_a$ activates a PD-dependent cation conductance in the apical membrane; this mediates enhanced apical Na uptake and finally Na transport across the rumen epithelium. The results of the present study support this hypothesis.

$PD_t$ and $G_t$. The rumen epithelium clearly exhibits PD-dependent changes of $G_t$; these are completely reversible and independent of the sequence of applied

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**Fig. 4.** Original tracing of an impalement under various voltage-clamped conditions. With a hyperpolarization of the epithelium ($PD_t$ positive), $G_t$ increases, potential difference of apical membrane ($PD_a$) depolarizes, and fractional resistance of the apical membrane ($fR_a$) decreases. For technical reasons, no calculation of $G_t$ or $fR_a$ could be performed with first voltage pulse after changing $PD_t$. This is indicated by dotted lines in traces of $G_t$ and $fR_a$.

**Fig. 5.** $G_t$ and $fR_a$ were recorded simultaneously under various $PD_t$ ($n = 15$). Every increase in $G_t$ is accompanied by a fall in $fR_a$ and vice versa.

**Fig. 6.** $fR_a$ becomes significantly smaller when apical membrane is depolarized. Each symbol stands for an individual recording ($n = 15$). Linear regressions have been fitted to the plots (solid lines). Means ± SE of the regression equations are: $fR_a(\%) = (-0.2 \pm 0.0) \cdot PD_a(\text{mV}) + (57 \pm 2); r^2 = 0.93 \pm 0.01$. 

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PDₜ (stepwise from minus to plus or alternating polarity pulses). However, PDₜ-dependent changes of Gₜ are complicated by possible capacitance effects when short pulses are used. Sehested et al. (40) have determined, in studies with bovine rumen epithelium, time-dependent changes of PDₜ in response to a current. They have found a monoexponential buildup of PDₜ and a new steady state after 10 ms. This is in agreement with our own measurements (Fig. 1); Iₜ reaches a new steady state after some 50 ms. Because we use a time delay of 160 ms (microelectrode) or 250 ms (Ussing chamber) for the calculation of Gₜ, possible capacitive effects are highly unlikely.

A further problem of the applied method could be the keratinized multilayered structure of the rumen epithelium. The determination of cellular electrophysiological parameters relies on the assumption that the cells of the various layers are coupled and represent one intracellular compartment. This assumption is probably true. Henrikson (20) has described “complex intercellular channels” within the rumen epithelium; these permit the diffusion of Na through the different layers. Furthermore, we have not observed a change of PDₐ after successful mucosal impalement when the microelectrode is moved stepwise into deeper layers of the epithelium.

Gₜ is the sum of cellular conductances (Gₛ) and of the paracellular or shunt conductance (Gₛ; Ref. 35), and consequently any change in Gₜ could be caused by alterations of Gₛ and/or Gₛ. The usual Ussing chamber method does not discriminate between these alternatives. Impalements of the epithelial cells with microelectrodes help to localize alterations of Gₛ to the apical or the basolateral membrane and, if the resistance of one membrane can reversibly be changed, aid the calculation of apical, basolateral, and paracellular resistance. The FRₛ decreases significantly with increasing PDₜ and the simultaneous depolarization of PDₐ. The decrease in FRₛ is in keeping with the determination of Gₛ, Gₛ, and Gₛ by the use of the reversible Ca-sensitive alteration of Gₛ (and Iₛ). The response on removal of mucosal Ca and Mg is restricted to Gₛ, which is enhanced from 1.40 mS/cm² when PD₝ = 20 mV to 2.53 mS/cm² at 80 mV; this compensates the diminution of electrochemical PD for Na (ΔµNa) within this range of PDₜ and permits enhanced Na uptake across the apical membrane and transepithelial Na transport. Removal of divalent cations from the mucosal solution causes increases in Iₛ and Gₛ in sheep rumen epithelium and Jₙₑᵦ (ΔIₛ = ΔJₙₑᵦ; Ref. 25) and does not change mannitol fluxes, indicating that the permeability of the shunt pathway is not influenced. We have used this experimental design (± divalent cations) because the electrogenic Na transport in rumen epithelium is not amiloride sensitive (30).

PDₜ and Gₜ in other epithelia. PDₜ-dependent changes of Gₜ have been observed in many epithelia (15, 18, 34, 43). In tissues with the classic amiloride-sensitive Na transport, hyperpolarization (bloodside positive) causes a decrease of apical conductance and cellular current (33, 34) and an increase of transmural resistance (13). Consequently, Na transport is diminished with increasing hyperpolarization of the tissue (5). Since single-channel recordings of amiloride-sensitive Na channels of A6 cells exhibit near linearity in their current-voltage (I-V) relationship over a range of ±80 mV (16), the decrease of Na transport may be explained by the reduced driving force. This generally accepted I-V relationship of amiloride-sensitive Na transport is in contrast to the observation in the present study and suggests that the electrogenic Na transport in sheep rumen, which is amiloride-insensitive (30) and modulated by apical divalent cations (24), is a distinct Na pathway. However, it should be mentioned that a voltage-sensitive (the open probability increases with voltage) amiloride-blockable Na channel has been described in cultured human sweat duct cells (21) and in A6 cells (16). Recently, Marunaka et al. (32) have reported an amiloride-sensitive Na-permeable nonselective cation channel in the apical membrane of fetal alveolar epithelium with an increased open probability when the apical membrane is depolarized.

Depolarization of the apical membrane potential results in a marked decrease of FRₛ in gallbladder epithelium (14, 15, 43). Indeed, depolarization of PDₚ has disclosed the presence of a voltage-dependent apical K conductance in these studies. Because the rumen epithelium exhibits a K conductance in the apical membrane (25), a possible contribution of this conductance to the PD-dependent change of FRₛ cannot be excluded. An increased open probability of the putative K channel on depolarization would enhance K exit from the cell and transepithelial K transport in the serosal-

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Table 1. Effects of Ca- and Mg-free conditions at positive PDₜ

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
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<tr>
<td>Gₛ, mS/cm²</td>
<td>2.32±0.14</td>
<td>2.44±0.14</td>
<td>2.57±0.17</td>
<td>2.70±0.18</td>
<td>2.88±0.18</td>
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<tr>
<td>Mg- and Ca-free Gₛ, mS/cm²</td>
<td>3.21±0.12</td>
<td>3.19±0.11</td>
<td>3.28±0.11</td>
<td>3.44±0.11</td>
<td>3.65±0.12</td>
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<tr>
<td>PDₚ, mV</td>
<td>−54±2</td>
<td>−38±2</td>
<td>−24±2</td>
<td>−10±2</td>
<td>+4±3</td>
</tr>
<tr>
<td>Mg- and Ca-free PDₚ, mV</td>
<td>−34±4</td>
<td>−23±4</td>
<td>−14±3</td>
<td>−4±3</td>
<td>+6±3</td>
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<tr>
<td>FRₛ, %</td>
<td>74±1</td>
<td>72±1</td>
<td>69±1</td>
<td>66±1</td>
<td>62±1</td>
</tr>
<tr>
<td>Mg- and Ca-free FRₛ, %</td>
<td>49±3</td>
<td>49±3</td>
<td>47±3</td>
<td>44±3</td>
<td>42±3</td>
</tr>
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</table>

Values are means ± SE; n = 12. Removal of divalent cations has significant effects on all parameters displayed. Tissue conductance (Gₛ) is increased, apical membrane is depolarized, and fractional apical resistance (FRₛ) is decreased. Potential difference (PD) dependence of Gₛ and FRₛ is less distinct under Ca- and Mg-free conditions. PDₚ, potential difference of the apical membrane; PDₜ, transepithelial PD.
mucosal direction and would consequently decrease or abolish \( I_{\text{act}} \), in contrast to the obtained data. As mentioned above, the depolarization of \( PD_a \) increases cation absorption and not secretion. However, uncertainties still remain. The K current of sheep rumen epithelium is very small (25) and might be outnumbered or probably overwhelmed by electrogenic Na transport. García-Díaz et al. (14), Stoddard and Reuss (43), and Gunter-Smith (15) have been able to abolish the PD-dependent changes of \( R_f \) in gallbladder epithelium by mucosal addition of the K channel blocker tetraethylammonium (TEA) or Ba. These inhibitors do not block the apical K conductance of sheep rumen epithelium (TEA; Martens, unpublished observations) or do so only to a small extent (Ba; Ref. 25). Present knowledge of ion transport in rumen epithelium supports the assumption of a K (25) and a Na (7) conductance in the apical membrane. The PD-dependent alteration of \( R_f \) could hence include the Na and/or the K conductance, or, alternatively, it could be a conductance that is silent at normal \( PD_a \) and activated by depolarization. These alternatives can only be distinguished by single-channel studies.

Ruminal K, \( PD_t \), and \( PD_a \). Because PD of the rumen epithelium exhibits in vivo variations from 20 to 60 mV (bloodside positive), the discussion here is restricted to this range of \( PD_t \) (11, 39). It is well known that the \( PD_t \) of sheep rumen is positively correlated with the rumen log of the K concentration (11, 39, 41). This correlation has been studied in more detail as it has become evident that the absorption of Mg from the rumen, which is essential for maintaining Mg homeostasis in ruminants (45), is disturbed with increasing \( PD_t \) (6, 27). Leonhard-Marek and Martens (25) have found that \( PD_a \) is depolarized and \( PD_t \) hyperpolarized with increasing apical K concentration. In their study, \( \Delta PD_a \) accounts for 0.6 \( \Delta PD_t \), which is in excellent agreement with the present results and shows that the K-dependent alterations of \( PD_t \), \( PD_a \), and \( G_t \) can easily be simulated with a voltage clamp. The depolarization of \( PD_a \) reduces the driving force for cation uptake and might be the predominant reason for decreased Mg absorption upon high ruminal K (25). In contrast, Na transport is not disturbed, despite the reduced \( \Delta H_{Na}\) and can even be enhanced under these conditions, because the reduced \( \Delta \mu_{Na} \) is compensated by an increase of conductance of the Na pathway.

Another observation in keeping with a PD-dependent electrogenic Na absorption is the difference between the current calculated from \( PD_t \) and \( G_t \), \( I_{\text{calc}} \), and the current used by the voltage clamp at a given \( PD_t \). At positive \( PD_t \), \( I_{\text{calc}} \) is consistently higher than \( I_t \). This difference between measured \( I_t \) and calculated current, \( I_{\text{act}} \), increases with increasing hyperpolarization of the tissue. This may be caused by an increased electrogenic Na absorption. Because replacement of Na significantly reduces the increase in \( I_{\text{act}} \) in agreement with the

**Fig. 7.** A: conductance of the apical membrane (\( G_a \)) was calculated from data with Ca- and Mg-free solution on mucosal side of epithelium and with control conditions \( n = 12 \) according to the method described by Frömter and Gebler (12). There is a significant potential dependence of \( G_a \); high transepithelial PD leads to an increase in \( G_a \). Data from one impalement are represented by the same symbol in A, B, and C. B: no significant change in conductance of the basolateral membrane (\( G_b \)) resulting from \( PD_t \) changes can be detected. C: conductance of the paracellular pathway (\( G_p \)) is unaffected by changes in \( PD_t \). At higher \( PD_t \), data are not normally distributed. Therefore, a Wilcoxon signed-rank test was performed. No significant changes could be detected.
underlying working hypothesis, electrogenic Na transport might represent the additional ion transport. Of course, we cannot exclude possible contributions from other ions, as indicated by the observation that even the total replacement of Na does not abolish the increase in $I_{sc}$.

Ruminal Na transport. It is well established that Na is absorbed from the rumen by active transport (8). All in vitro studies have confirmed this conclusion and have shown that $I_{sc}$ is always less than $J_{Na}^{net}$ (7, 10, 28); this has led to the assumption of two parallel Na transport mechanisms: an electrogenic and an electroneutral Na transport (7). The electroneutral transport is probably mediated by apical Na/H exchange, since high mucosal concentrations of amiloride (1 mM) inhibit $J_{Na}^{net}$ significantly (28) and other inhibitors of electroneutral Na transport (chlorothiazide, furosemide, or bumetanide) do not influence $J_{Na}^{net}$ (28). The lack of effect of the loop diuretics furosemide and bumetanide make it very unlikely that the K-dependent Na transport is represented by an electroneutral Na transport.

The assumption of electrogenic Na transport in addition to electroneutral transport relies on three observations. $I_{sc}$ is abolished by Na replacement, $I_{sc}$ is also abolished by the serosal addition of ouabain (9), and $I_{sc}$ equals $J_{Na}^{net}$ when Cl− and HCO$_3$− are replaced by SO$_4$− and tricine (7). These observations closely resemble results from studies with Na-transporting tissues, such as amphibian skin (19) or rabbit distal colon (46). However, significant differences are observed. Low mucosal concentrations of amiloride, which effectively block electrogenic Na transport in the tissues mentioned above, are without an effect on $I_{sc}$ in rumen epithelium (30). Mucosal removal of divalent cations enhances $I_{sc}$ and $J_{Na}^{net}$ considerably (24), indicating that the electrogenic Na transport in forestomach epithelia exhibits properties distinct from the classic Na-absorbing tissues, which is further substantiated by the data of the present study.

The proposed model of electrogenic Na transport, including a PD-dependent conductance in the apical membrane, is complicated by the observation that an inflow of Na should depolarize PD$_a$, with a consequent increase in the PD-dependent conductance that would further enhance Na inflow and hence the depolarization of PD$_a$. This positive feedback (and vicious circle) of Na transport does not take place, as increasing Na concentrations lead to the saturation of $I_{sc}$ [Michaels-Menten constant ($K_m$) = 31.9 mM, maximal transport capacity = 1.18 µeq·cm$^{-2}$·h$^{-1}$; Ref. 26]. It has been shown in several tissues that PD$_a$ varies only within narrow limits when the Na transport is altered (see review in Ref. 36). This regulation of PD$_a$ seems to result from a parallel change of Na transport rate and basolateral K conductance (36). We have unsuccessfully attempted to alter PD$_a$ of rumen epithelium by a rapid change of mucosal Na concentrations during mucosal impalement with microelectrodes. The changes in PD$_a$ are very small or even absent, a finding that may be explained by Ohm's law: the Na current of tens of µA/cm$^2$ passes through a R$_a$ of several hundred Ω·cm$^2$, causing a voltage deflection of some millivolts. Moreover, the apical K conductance could explain this observation: a possible depolarization of PD$_a$ should enhance K efflux and hence repolarize PD$_a$. The apical K conductance appears to accomplish two important functions that are essential for the understanding of ruminal Na transport and that are closely associated with PD$_a$: at low ruminal K concentrations, PD$_a$ is stabilized, thus maintaining normal Na transport, whereas high ruminal K concentrations depolarize PD$_a$ and activate the PD-dependent conductance, which permits enhanced Na absorption.

The findings of the present study are in contrast to the early observations of Stacy and Warner (42), who have suggested a stimulation of Na transport by an increase of apical osmotic pressure, because intraruminal application of KCl or a mixture of mannitol and urea raises the osmotic pressure of ruminal fluid to ~400 mosmol/l and enhances Na absorption from the rumen. The effect of KCl is much more plausibly explained by the results of the present study in which we assume a PD-dependent cation conductance. It is well known that urea is very rapidly hydrolyzed by microbial urease to NH$_4$+, which causes K-like alteration of PD$_a$ and $I_{sc}$ (4). Bodeker et al. (4) suggested that NH$_4$+ passes through the K conductance in the apical membrane, which might have similar effects on PD$_a$ and PD-dependent cation conductance. These conclusions are (indirectly) substantiated by recent studies in our laboratory showing that an increase of apical osmotic pressure by mannitol decreases Na transport (23).

Physiological implication of K-dependent Na transport. One of the characteristics of digestive physiology in ruminants is the high secretion rate of isotonic saliva: 10–20 l/day in sheep (22) and 98–190 l/day in cows (1). The Na concentration in mixed saliva is up to 168 mM and determines the Na concentration in the ruminal fluid, which is some 18 mM lower than in mixed saliva (2). Consequently, Na as the major cation in saliva and rumen content predominantly influences the osmotic pressure of ruminal fluid, which varies around isotonicity, being hypotonic (243–272 mosmol/l) before and hypertonic (~400 mosmol/l) after a meal (48). K is the most abundant mineral in plants and is rapidly dissolved in the forestomach fluid (38). Indeed, a close linear correlation has been observed between K intake and ruminal K concentrations (38). Because the ruminal K exceeds 100 mosmol/l at high K intake, the sum of Na and K should add up to 200–250 mosmol/l, resulting in high luminal osmotic pressure and inflow of water into the rumen (49). This water movement may impair the homeostasis of extracellular fluid and plasma volume, because the secretion of saliva per se causes the withdrawal of large amounts of electrolytes and water from the extracellular fluid volume, which is reduced after a meal (44) and which is accompanied by a reduction of the plasma volume (3). These possible complications are effectively prevented by the K-dependent Na absorption from the rumen that keeps
the sum of Na and K relatively low (some 140 mosmol/l) and almost constant (38). Surprisingly, this important interaction between K concentration and Na transport has rarely been considered in Na transport studies. The results of the present study offer, for the first time, a tentative model for this K-dependent Na absorption.

I-V relationship in epithelia. The epithelial and cellular effects of clamping the PD, Na transport have been investigated for many years (37), and it has been established that epithelia exhibit electrical rectification and rarely behave as Ohm resistors (13, 18). Since the perturbation of PD, induces ion movement and, consequently, changes of the driving forces for ions, two extreme experimental conditions are usually used for the study of the I-V relationship: “instantaneous” and “steady state.” (For a careful discussion of these problems, see Ref. 37.) High ruminal K concentrations cause the sustained (steady state) alteration of cellular and transepithelial potential differences. We have simulated this condition by choosing a pulse duration of 30 s in the Ussing chamber studies. The obtained ratio of \(\Delta P_D / \Delta P_D_{\text{t}}\) (0.6) is in excellent agreement with data from Leonhard-Marek and Martens (25), who have induced electrophysiological alteration by increasing apical K. Furthermore, the K-induced depolarization of PD, and hyperpolarization of PD, with the well-known effect on ruminal Mg transport can be simulated by simple electrical manipulation of the tissue (29). These observations support the conclusion that the experimental conditions in the present study represent the K-dependent electrophysiological alteration of ruminal epithelium.

The analysis of our data is based on the calculation of \(G_\text{t}\) and \(fR_\text{a}\) from the pulse-induced changes in PD, and PD,\(\text{t}\). As we have shown, \(G_\text{t}\) and \(G_\text{a}\) are potential dependent, a finding that implies a methodical problem: each pulse that is generated by the voltage-clamp device changes \(G_\text{t}\) and transepithelial potential differences. We have simulated this condition by choosing a pulse duration of 30 s and hence all data are subjected to an inevitable systematic error. However, these deviations are much smaller than the observed effects caused by depolarization or hyperpolarization. The same problems are present with the calculation of \(fR_\text{a}\) as discussed by van Driesche and DeWolff (47).

In conclusion, it has been known for decades that an increase in ruminal K concentration is accompanied by an enhanced Na absorption from the rumen; this causes a decrease in Na concentration and maintains the sum of Na and K concentrations in the ruminal fluid almost constant (38). The data obtained in the present study support the assumption of a tentative model of K-dependent Na transport in the rumen epithelium. Depolarization of PD, by high ruminal K concentration increases a PD-dependent conductance, which permits an enhanced apical Na uptake, despite the reduced driving force across this membrane. Because the reverse observations have been made in studies with classic amiloride-sensitive Na transport in frog skin (5, 33), the electrogenic Ca-sensitive amiloride-insensitive Na transport in sheep rumen appears to be a distinct and novel mechanism of epithelial Na movement, which, to our knowledge, has not been described in other epithelial tissues.

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