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The rumen and potassium homeostasis: a model[*]

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ABSTRACT

The ruminant has to be able to regulate potassium metabolism over an extremely wide range of intake. When related to bodyweight, dietary potassium intake of ruminants frequently exceeds that of humans by a factor of over ten, and experimental situations suggest that the toxic threshold for ingestion of potassium is considerably higher. While a number of studies have addressed the outstanding ability of the ruminant kidney to extrude potassium, the role of the rumen in maintaining potassium homeostasis in sheep and cows by serving as a reservoir for the redistribution of potassium has received considerably less attention. Based on current and past studies at the level of the cell, the tissue and the animal, we present a model for the regulation of ruminal transport that may help in the understanding of the variable responses of the rumen epithelium to dietary potassium intake, and the role of magnesium in mediating these responses.

KEY WORDS: rumen, potassium, magnesium, cation channel, sheep, patch-clamp

INTRODUCTION

The ability of ruminants to produce milk and gain weight while ingesting grass and hay relies on their ability to deal with large amounts of potassium. When related to body weight, the upper limit of daily voluntary human potassium intake is 1.4 mmol/kg·d (Giebisch, 1998). Conversely, ruminants have to be able to tolerate variations in K^+ uptake from 3.8 mmol/kg·d (Scott, 1969) to almost 20 mmol/kg·d (Rabinowitz et al., 1984); with direct toxic effects only seen after intraruminal infusion of savagely high concentrations of 40 mmol/kg·d (Bohman et al., 1969). Considering that the intake of potassium continues to increase worldwide due to modern fertilization techniques, surprisingly little information is available on the mechanism of potassium homeostasis in the ruminant.

As in humans, intestinal absorption of potassium in the ruminant species is rapid and almost total (90 %)(Khorasani et al., 1997). Thus, the contribution of

the rumen to total digestibility of K^+ has to be seen as small. Depending on dietary intake, an accumulation of the element in the ruminal cavity can be observed (Sellers and Dobson, 1960), with concentrations reaching 100 mmol/l so that as much as 31% of total body potassium can be found in the rumen (Belyea et al., 1978).

Thus, the rumen apparently functions as a redistribution space for potassium in addition to the classical intracellular compartment (Giebisch, 1998), and efflux of K^+ across the ruminal epithelium must be limited. Indeed, studies of K^+ secretion under low K^+ conditions suggest a very low permeability of the apical membrane to K^+ (Leonhard-Marek and Martens, 1996). However, an elevation of ruminal K^+ concentration strongly changes the potential difference over the tissue not only *in vitro* (Ferreira et al., 1966), but also *in vivo* (Martens and Blume, 1986; Scott, 1966; Sellers and Dobson, 1960), suggesting the opposite. It should be noted that the *in vivo* potential cannot represent a Nernst-equilibrium but has to be maintained by a steady flow of ions.

In order to resolve these seemingly contradictory observations, detailed knowledge is needed about the underlying conductances at the level of the cell. We demonstrate that the conductance of ruminal K^+ channels increases with the potassium concentration, so that high K^+ should depolarize the apical membrane as suggested (Leonhard-Marek and Martens, 1996). In addition, we demonstrate that cytosolic magnesium regulates sodium influx into ruminal cells not only in the absence of extracellular calcium as demonstrated previously (Stumpff et al., 2004; Leonhard-Marek et al., 2005), but also under physiological conditions. Based on these findings and a literature study, it is possible to establish a model for the regulation of potassium efflux from the rumen that may explain the ability of this organ to function as a redistribution space for potassium homeostasis.


MATERIALS AND METHODS

Cells from the rumen of freshly slaughtered sheep were harvested, cultured and measured essentially as reported previously (Abdoun et al., 2005; Leonhard-Marek et al., 2005). Patch-clamp measurements were performed using an EPC 9 patch-clamp amplifier (HEKA elektronic, Lambrecht, Germany) and TIDA for Windows Software (HEKA elektronic, Lambrecht, Germany). Current responses were recorded continuously at 100 Hz using a protocol that generated steps of 200 ms duration to voltages between –120 mV and 100 mV in 20 mV steps, returning to a holding potential of –40mV for 200 ms in between. In addition, conventional voltage pulse protocols were used that recorded data at a much higher sampling rate of 5 kHz and a stepsize of 10 mV within the same voltage

range.   Pipette solution contained (mmol·l^{-1}): 1 KH$_2$PO$_4$, 10 Hepes, 0.8 CaCl$_2$, 5 EGTA, 123 K-gluconate and 10 NaCl, with or without 0.9 MgSO$_4$, as indicated. Extracellular solutions contained: 1 NaH$_2$PO$_4$, 5 KCl, 10 Hepes, 1.7 CaCl$_2$, 0.9 MgCl$_2$ to which either 130 NaCl, 130 KCl or 130 choline chloride were added. All voltages were corrected for liquid junction potential.

RESULTS

Sodium conductance

As in a previous study (Leonhard-Marek et al., 2005; Stumpff et al., 2004), Na current could be stimulated by removing Ca^{2+} and Mg^{2+} from the bath, with current into the cell rising to 328 ± 82 %, n = 31, p = 0.009. Outward current rose less dramatically to 150 ± 19 %, (p = 0.01, n = 31). Cells depolarized from -27 ± 2 mV to -21 ± 3 mV (p = 0.0004). However, when external Na$^+$ was replaced by choline in a solution containing physiological concentrations of Ca^{2+} and Mg^{2+}, no significant effects could be observed, although a small reduction of inward and outward current could be detected (to 93 ± 5%, p = 0.09 and 92 ± 4 %, p = 0.07, n = 16). Reversal potential shifted slightly (p = 0.08) towards more negative values. Effects of removing Na$^+$ became significant after cytosolic Mg^{2+} was reduced by removing the ion from the pipette solution, with inward current dropping to 67 ± 5 %, n = 10, p = 0.006. Outward current remained unchanged (p = 0.6) Reversal potential dropped to -43 ± 4 mV (p = 0.0002). (Fig 1.)
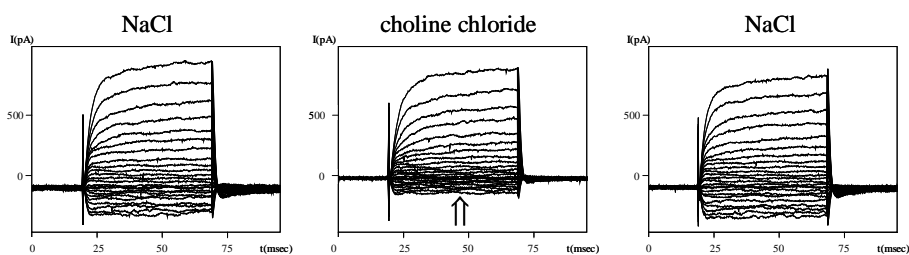
Fig 1. Whole cell currents of cell filled with a Mg^{2+}- free pipette solution. Inward current (⇑) was higher in NaCl bath solution than in choline chloride solution.

Potassium conductance

When external solution was changed from NaCl to KCl Ringer, inward current at -120 mV increased from 100% to 344 ± 52% (n = 20, p = 0.0002). In most cells, outward current could also be seen to increase to a mean value of 122 ± 10 % (p = 0.004). Reversal potential shifted from -29 ± 3 mV to -9 ± 3 mV. To

exclude an activation of chloride conductances by potassium, external chloride was removed from the solutions. In the presence of Na, outward current decreased to 70 ± 8 % (p = 0.02), and cells depolarized to -15 $\pm$ 3 mV (n = 13, p = 0.0002). Inward current did not change significantly (p = 0.4). Replacement of Na-gluconate by K-gluconate led to complete depolarization (-1$\pm$ 2, n = 18, p = 0.0001) with induction of inward current as before. Mean outward current in K-gluconate solution rose to 142 ± 22% (p = 0.07), suggesting that efflux of potassium was enhanced in the high potassium solution.


DISCUSSION

The results of this study show that the non-selective cation channel of the rumen is regulated by changes in cytosolic Mg^{2+} concentration not only in the absence of external Ca^{2+} (Stumpff et al., 2004; Leonhard-Marek et al., 2005) but also in physiological solutions. We were also able to show that the K^+ conductance of ruminal epithelial cells rises with an increase in K^+. In light of basolateral and apical expression of K^+ channels by the rumen in addition to the paracellular pathway (Leonhard-Marek and Martens, 1996), the question arises how the ruminal epithelium is able to maintain high gradients for K^+ from lumen to blood.

It is possible to estimate the amount of K^+ that would flow through the ruminal epithelium in the absence of any regulatory mechanisms. Under *in vivo* conditions, the potential across the ruminal epithelium changes from 30 mV to 60 mV (blood side positive) when ruminal K^+ is increased by 80 mmol/l (Scott, 1966). Assuming a conductance G_t of about 2.5 mS/cm^2 (Leonhard-Marek and Martens, 1996), and a ruminal absorptive surface area of 4 m^2 (Dobson, 1959), electrogenic flux should equal I = $G_t \cdot$ U or some 2.7 moles /day for sheep of 50 kg, or 54 mmol/kg·d. Since this (toxic) amount exceeds dietary intake of K^+ in this study and others (Martens and Blume, 1986; Sellers and Dobson, 1960), we suggest that the potential represents an increase in the active transport of Na^+.

But how is the conductance of the apical membrane for Na^+ adjusted? The well-known detrimental effects of K^+ on ruminal Mg^{2+} uptake may provide a clue (Martens and Schweigel, 2000). Reduction of cytosolic Mg^{2+} concentration should increase the openprobability of the apical non-selective cation channel, stimulate apical influx of Na^+ and basolateral efflux via the Na^+/K^+-ATPase. The corresponding rise in transepithelial potential should reduce the electrochemical gradient for the efflux of K^+. Elevation of ruminal K^+ concentration should follow and is observed, as is the corresponding absorption of appropriate amounts of Na^+ from the rumen to maintain ruminal osmolarity (Scott, 1966; Sellers and Dobson, 1960; Warner and Stacy, 1972). While most of this Na^+ is

eventually recirculated with saliva (Silanikove and Tadmor, 1989), urinary excretion of Na^+ increases almost immediately after ingestion of a high K^+ meal (1-3 hours) (Suttle and Field, 1967), long before the bulk of ruminal fluid reaches the small intestine. Onset of K^+ excretion in sheep switched to a high K^+ diet is equally rapid and not related to a rise in plasma K^+ (Rabinowitz et al., 1984), and may reflect effects of Na^+ on K^+ absorption in the distal nephron (Giebisch, 1998).

It appears that the negative effects of a reduction in the uptake of magnesium by high dietary intake of K^+ (Martens and Schweigel, 2000) are balanced by the positive effects of a redistribution of K^+ into the ruminal cavity. However, the mechanisms that allow ruminants to compensate for high intakes of K^+ may be exhausted by the increasing accumulation of K^+ in soils and forage, related to K^+ directly or to the associated organic anions (Goff, 2004). Finally, it should be noted that maintenance of a high rumen-blood K^+ gradient requires energy. Seen from this perspective, reducing the use of high potassium fertilizers in farming may become an increasingly attractive economic alternative.
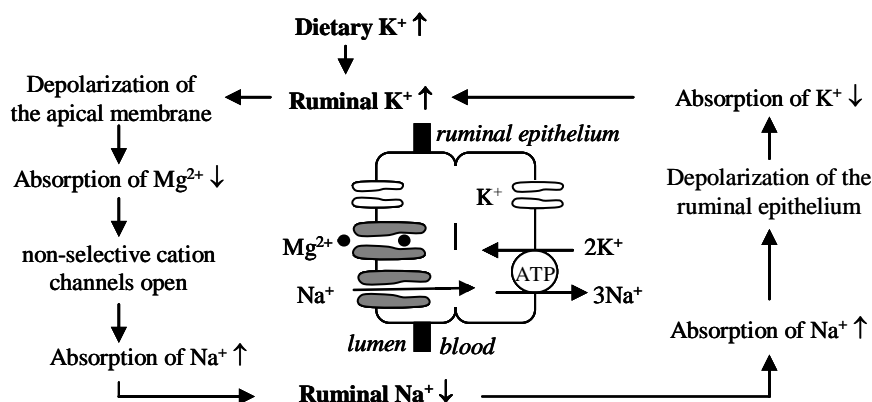
Fig 2: Model of ruminal transport. Efflux of K^+ through the ruminal epithelium is controlled by the transepithelial potential. The accumulation of K in the rumen allows this organ to function as an additional reserviour for the redistribution of potassium.

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THE RUMEN AND POTASSIUM HOMEOSTASIS

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