Journal of Animal and Feed Sciences, 13, Suppl. 1, 2004, 281–284

Protein kinase C is involved in the regulation of Na^+ transport across rumen epithelium

S. Leonhard-Marek[1] and G. Breves

Department of Physiology, School of Veterinary Medicine Hannover
Bischofsholer Damm 15/102, D-30173 Hannover, Germany

ABSTRACT

Electrogenic Na^+-transport across rumen epithelium occurs *via* a non-selective cation channel, blocked by luminal Ca^{2+} and Mg^{2+} and by cytosolic Mg^{2+}. The role of protein kinase C (PKC) in the regulation of this pathway was studied with isolated epithelia from goat rumen incubated in Ussing chambers. Omission of Ca^{2+} and Mg^{2+} from the luminal side induced an increase in short circuit current followed by a decline. This down-regulation of Na^+-current was delayed after activation of PKC with 6-[N-decylamino]-4-hydroxymethylinole. Na^+-current declined again after inhibition of PKC with chelerythrine, showing that PKC contributes to the regulation of ruminal electrogenic Na^+-transport.

KEY WORDS: non-selective cation channel, protein kinase C, sodium transport, forestomach

INTRODUCTION

Rumen epithelium exhibits a non-selective cation conductance, which permits the passage of Na^+ and other monovalent cations in the absence of divalent cations. This conductance is blocked by Ca^{2+} and Mg^{2+} ions from the luminal side and by Mg^{2+} ions from the cytosolic side (Leonhard-Marek, 2002; Stumpff et al., 2004). In other epithelia, protein kinase C (PKC) is involved in the regulation of Na^+ transport *via* the epithelial Na^+ channel ENaC. PKC stimulates Na^+ transport across frog skin (Civan et al., 1991), whereas it decreases Na^+ channel activity in epithelia from the urinary tract (e.g., Ling and Eaton, 1989). We wanted to know whether protein kinase C is involved in the regulation of electrogenic Na^+ transport across the rumen.

[1] Corresponding author: e-mail: sabine.leonhard-marek@tiho-hannover.de

MATERIAL AND METHODS

Isolated epithelia from the rumen of slaughtered goats were incubated in Ussing chambers under short circuit conditions. Epithelia were bathed in a standard bicarbonate buffer, which contained indomethacine (10^{-5} mol l^{-1}) in order to avoid a prestimulation of the tissues *via* prostaglandines. Divalent free solutions contained 0.5 mmol l^{-1} EGTA. We used 6-[N-decylamino]-4-hydroxymethyl-inole (DHI) as an activator of PKC and chelerythrine chloride (CC) as a specific inhibitor of PKC. DHI and CC were added to both sides of the epithelia in a concentration of 2 µmol l^{-1}.

RESULTS

The omission of Ca^{2+} and Mg^{2+} ions from the luminal side opened the non-selective cation conductance which could be measured as an increase in short circuit current (Isc) and transepithelial conductance. Isc remained on a plateau value for about 15 min and then declined slowly (Figure 1).

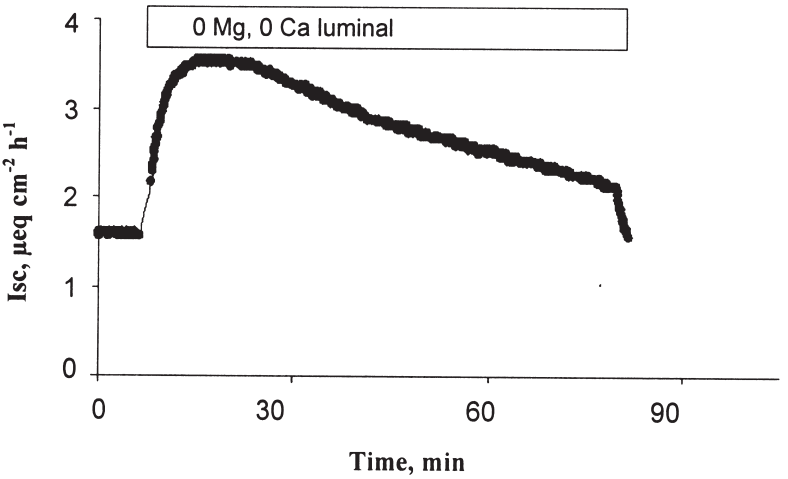

Figure 1. Omission of divalent cations from the luminal side increased the short circuit current (Isc) across rumen epithelium (example from goat rumen). The Isc plateau was followed by a down-regulation. Re-addition of Ca and Mg at the end of the experiment induced an immediate decline in Isc towards baseline levels

This time-dependent decrease in Isc measured over 30 min without any additions was -0.52±0.05 µeq cm^{-2} h^{-1} (n=12). When stimulating PKC with DHI shortly after the Isc plateau, as shown in figure 2, Isc decreased much less (-0.34±0.05 µeq cm^{-2} h^{-1} in 30 min, n=9; $P<0.05$).

When we then added CC in the presence of DHI, thereby blocking the protein kinase C again, the Isc also started to decrease again (Figure 2, Δ Isc 0-15 min

after CC addition: -0.20±0.02 μeq cm^{-2} h^{-1}, n=8). This was significantly different (P<0.05) from the time-dependent decrease in the only presence of DHI as measured simultaneously in other pieces of rumen epithelia (-0.06±0.01 μeq cm^{-2} h^{-1}, from 30 to 45 min after DHI addition, n=3).

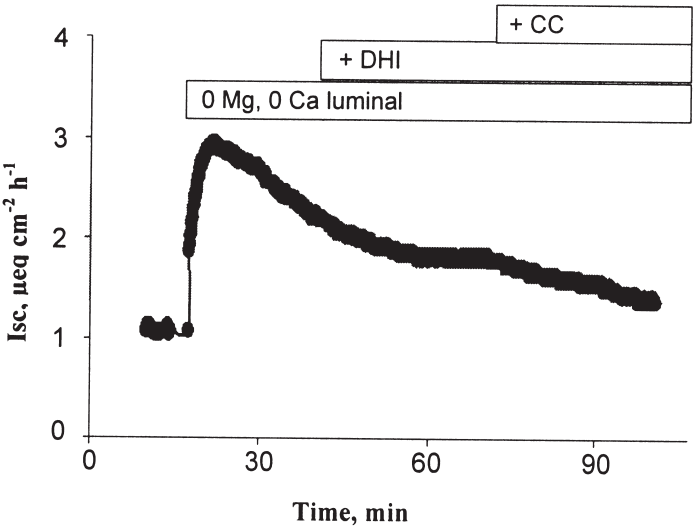

Figure 2. DHI inhibited and chelerythrine (CC) increased the down-regulation of the divalent cation sensitive current (example from goat rumen)

The addition of CC shortly after the Isc plateau resulted in an Isc decrease (Δ Isc) of -0.60±0.05 μeq cm^{-2} h^{-1} (n=9) measured over the next 30 min which was not significantly different from the mere time-dependent decrease of -0.52±0.05 μeq cm^{-2} h^{-1} (n=12) shown above.

DHI and CC given in the presence of divalent cations on the luminal side had no effect on the Isc (n=10).

DISCUSSION

The increase in Isc after luminal omission of Ca^{2+} and Mg^{2+} ions reflects an increase in Na^+ transport as shown earlier (Rübbelke, 1998). We opened this pathway for a longer time and observed that the increase in Na^+ current was followed by a decline (Figure 1). The decline in Isc from the peak value indicates that autoregulatory mechanisms exist to regulate Na^+ uptake to the activity of the Na^+/K^+-ATPase. This is in agreement with the classical Na^+ transport *via* ENaC (Turnheim, 1994) and with observations in the omasum of sheep (Schultheiss and Martens, 1999).

One of the factors that regulate Na^+ entry has been shown to be the ubiquitous protein kinase C. However, observations in this field were contradictory. While

PKC increases transepithelial Na^+ transport across frog and toad skin, PKC inhibited Na^+ transport across epithelia from the urinary tract. This means that regulation of epithelial Na^+ permeability by protein kinase C is tissue specific (Chalfant et al., 1996).

The contribution of protein kinase C to Na^+ transport across the rumen resembles its action on frog and toad skin, albeit the pathways for Na^+ uptake are different between skin and rumen.

CONCLUSIONS

We have shown that protein kinase C can be activated in rumen epithelia, and that it is involved in the regulation of electrogenic Na^+ transport processes across the rumen wall.

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