Journal of Animal and Feed Sciences, 16, Suppl. 2, 2007, 543–547

Effects of anions on Ca absorption across the rumen

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ABSTRACT

Feeding anions has a positive effect on Ca homeostasis in cows. Since some studies suggest a non-acid-base effect of anions, we studied their effects on the microclimate of the rumen surface and on ruminal Ca absorption. Ruminal surface pH was increased by sulphate, as previously shown for chloride. However, while chloride and short-chain fatty acids stimulate ruminal Ca absorption, sulphate had no consistent effect on Ca flux rates. These observations underline that anions also contribute to Ca homeostasis by different gastrointestinal effects.

KEY WORDS: calcium absorption, anions, rumen, hypocalcaemia, milk fever

INTRODUCTION

Feeding chlorides, sulphates or providing short-chain fatty acids (SCFA) through a high grain diet has a positive effect on Ca homeostasis in cows (Mellau et al., 2004), and can help to avoid hypocalcaemia after parturition. These effects are partly explained by the induction of a metabolic acidosis which changes the responsiveness of the hormonal pathways involved in Ca homeostasis (Goff and Horst, 2003). In addition, Cl and SCFA are also able to stimulate Ca absorption across the rumen wall (Leonhard-Marek et al., 2007). In that study sulphate had no effect on ruminal Ca absorption. Roche et al. (2002) state, however, that the positive influence of sulphate on Ca homeostasis in cows can not solely be explained on the basis of changes in acid-base-balance; and in another study preduodenal Ca absorption was correlated to preduodenal sulphate absorption (Khorasani et al., 1997).

Sulphur is absorbed from the rumen (Kandylis and Bray, 1987), and different anion exchangers have been shown at the mRNA level in rumen epithelium (Bilk

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et al., 2005). In other tissues, these exchangers transport sulphate in exchange for bicarbonate or OH^- (Mount and Romero, 2004), and they might likewise contribute to ruminal sulphate absorption. We have shown recently, that an increased absorption rate of Cl (in exchange for bicarbonate or OH^-) alkalizes the pH of the ruminal surface (Leonhard-Marek et al., 2006), and it is known that different Ca channels increase their conductance with alkaline pH (Pietrobon et al., 1989; Vennekens et al., 2001). We therefore wanted to study the effects of ruminal sulphate in the microclimate of the rumen surface and the significance of the different anions for Ca absorption in more detail.

MATERIAL AND METHODS

Tissues and incubation

Pieces of the ventral rumen wall were taken from slaughtered sheep within 5 min after bleeding and immediately immersed in a buffer solution, where the mucosa was stripped from the underlying muscle layers and the serosa. Mucosal tissues were mounted between the two halves of conventional Ussing incubation chambers and connected to buffer reservoirs on each side, that were continuously stirred by the use of a gas-lift system. The chambers were connected to a computer controlled voltage clamp device, and the experiments were performed under short-circuit conditions in standard buffer solutions as described previously (Leonhard-Marek et al., 2006).

Ca flux rates

Unidirectional flux rates of Ca were measured using ^{45}Ca as a tracer, which was added as $^{45}CaCl_2$ (specific activity >370 GBq/g) in each chamber to the mucosal or the serosal side of the epithelia, respectively. After an equilibration period of 20 min, buffer samples of 500 µl were taken at 30 min intervals and replaced by aliquots of the same unlabelled solution. Radioactivity was measured in a conventional liquid scintillation counter. Flux rates were calculated from the rate of tracer appearance on the other side of the epithelia (Leonhard-Marek et al., 2007). The effects of different anions on Ca flux rates were tested with adjacent pieces of ruminal epithelia from the same sheep.

Measurements of surface pH

The surface pH (pH_s) of the epithelia was measured according to the method of Genz et al. (1999) with 5-N-hexadecanoyl-aminofluorescein (HAF) as a pH-sensitive

fluorescent dye which inserts in the outer leaflet of plasma membranes with the hexadecanoyl chain. For pH measurement a piece of stripped ruminal epithelium was mounted in a microperfusion chamber on the stage of a fluorescence microscope with the mucosal side directed to the objective. The fluorescence intensity of HAF (530 nm) was measured at two excitation wavelengths (436 and 485 nm) using an inverse microscope (Axiovert 35M, Zeiss, Oberkochen, Germany) equipped with a photomultiplier as described previously (Leonhard-Marek et al., 2006).

Statistical analysis

Results are given as means±SEM. *n* designates the numbers of tissues. Statistical significance was evaluated using analysis of variances or Student's t test, paired or unpaired as appropriate.

RESULTS

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. When the epithelia were bathed in a low chloride buffer (10 mmol/l Cl) on the mucosal side, pH_s values amounted to 7.47±0.03 (n=10). This surface pH was increased by 0.10±0.004 (n=6; $P<0.001$) when the sulphate concentration was increased from 0 to 29 mmol/l on the luminal side. In the absence of bicarbonate (HEPES-buffered solution) the surface pH only increased by 0.03±0.01 (n=4) due to an increase of 29 mmol/l in luminal sulphate concentration. However, this change was not significant.

The sulphate induced increase in ruminal surface pH indicates that a stimulation of Ca conductances in rumen epithelial cells might be possible. However, we had not seen an increased Ca absorption in a sulphate buffer (Leonhard-Marek et al., 2007). In the same study we had shown that the presence of 30 mmol/l sulphate in the solutions reduced the concentration of free Ca ions by about 70%, which raises the possibility of a strong negative effect of sulphate on ruminal Ca absorption. To investigate this hypothesis we maximally stimulated Ca absorption across one group of epithelia by the combined presence of SCFA (60 mmol/l) and Cl (68 mmol/l) and compared the Ca absorption rates with those of adjacent epithelia that were incubated in the presence of SCFA (60 mmol/l) and 29 mmol/l sulphate (at the expense of 58 mmol/l Cl). In two different groups of animals this comparison showed no consistent effect of sulphate on Ca absorption. Ca flux rates from the mucosal to the serosal side amounted to 20.3±1.4 and 19.5±2.1 nmol $cm^{-2}\,h^{-1}$ in the presence of Cl and SCFA (n=12 and 14), while the respective sulphate groups showed Ca flux rates of 17.6±0.9 and 21.5±4.6 nmol $cm^{-2}\,h^{-1}$ (n=12 and 4).

DISCUSSION

Both sulphate and gluconate reduce the concentration of free Ca ions in solutions (Leonhard-Marek et al., 2007). However, while the addition of gluconate did reduce the absorptive flux of Ca in the presence of Cl and SCFA in this former study, the addition of sulphate had no effect on SCFA-stimulated Ca flux rates in the present study. This suggests that sulphate besides reducing ionized Ca has also a stimulatory effect on Ca absorption across the rumen wall, resulting in no change of Ca absorption under *in vitro* conditions. A possible mechanism for this stimulatory action is revealed by the pH measurements. An increase in surface pH in the presence of sulphate will open a non-selective cation conductance in the apical membrane of rumen epithelial cells (Leonhard-Marek et al., 2006) and should also increase the driving force for a $Ca^{2+}/2H^{+}$ exchanger, that has been suggested to contribute additionally to Ca absorption (Schröder et al., 1997; Wadhwa and Care, 2000). Both mechanisms could allow for an increased Ca uptake into the epithelium. This is underlined by *in vivo* studies (Wagner, 1998), which have shown, that an increase in luminal pH increases Ca absorption from the temporarily isolated washed rumen. While the pH at the epithelial surface of the rumen is positively correlated with Ca absorption, the pH in the ruminal contents of fed animals might determine, where Ca is absorbed.

Increasing the pH in the luminal contents under normal feeding conditions has been shown to reduce the concentration of ultrafiltrable Ca and increase the proportion of Ca bound to suspended material of dietary food (Storry, 1961; Johnson and Aubrey Jones, 1989). This will retain Ca in the rumen. Decreasing luminal pH and thereby increasing the ultrafiltrable fraction of Ca will allow a quicker passage of Ca to abomasal or intestinal regions.

An acute increase in Ca concentration on the luminal side allows for a dramatic increase of absorptive Ca flux across bovine rumen epithelium *in vitro* (Ricken, 2005), pointing to the great absorptive capacitiy of the rumen for Ca absorption that can be utilized when adding Ca salts to the diet.

CONCLUSIONS

We have shown that chloride and sulphate increases the pH of the ruminal surface and might thereby have a positive effect on ruminal Ca absorption. Short-chain fatty acids that are partly absorbed *via* $SCFA^{-}/HCO_3^{-}$ exchange might have similar effects on surface pH. While Cl and SCFA anions are able to stimulate Ca absorption across the rumen, sulphate had no effect, since potential positive effects at the epithelial surface are antagonized by a reduction of free Ca ions. These observations underline that the anions fed to prevent or treat a hypocalcaemic condition can also have important gastrointestinal effects on Ca homeostasis.

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