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# Interaction between ammonia, sodium and chloride transport across the rumen epithelium in sheep

Khalid Abdoun [a,*], Katarina Wolf [b], Holger Martens [b]

[a] *Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum, 13314 Shambat, Sudan*
[b] *Institute of Veterinary Physiology, Faculty of Veterinary Medicine, Free University of Berlin, Berlin, Germany*

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**Abstract**

The effect of increasing mucosal ammonia concentration [0 (control), 5, 15 and 30 mmol/l $NH_4Cl$] on the electrophysiology of isolated rumen epithelium and the transport of $Na^+$ and $Cl^-$ across it were studied, in vitro. Seventy-three isolated rumen epithelium samples from 14 sheep of different breeds, age and sex and solely fed on hay were used in this study. The presence of ammonia in the incubation solution on the mucosal side evoked a positive short-circuit current ($I_{sc}$) and increased the transepithelial conductance ($G_t$). The unidirectional flux rates of $Na^+$ were significantly ($p < 0.05$) reduced, while the mucosal to serosal and net flux rates for $Cl^-$ were significantly ($p < 0.05$) stimulated. It was determined that an 8 mmol/l intra-ruminal ammonia concentration could cause 50% inhibition of the maximal inhibitory effect of ammonia on Na absorption. It is proposed that ammonia interacts with the electroneutral $Na^+/H^+$ and $Cl^-/HCO_3^-$ exchangers of the rumen epithelium, probably by binding intracellular protons, thus elevating intracellular pH ($pH_i$) and consequently inhibiting $Na^+$ transport and stimulating $Cl^-$ transport across the isolated rumen epithelium of sheep. Inhibition of Na absorption could increase the osmolality of the rumen fluid, which has been reported to be a predisposing factor of diet-induced left displacement in the abomasum. Further, the increased osmolality of the rumen fluid could adversely affect the microbial growth and fermentation in the rumen.


*Keywords:* Sheep; Rumen; Ammonia; Sodium; Chloride

## 1. Introduction

From previous studies it has been shown that ammonia influences the intracellular pH ($pH_i$). The diffusion of $NH_3$ into the cell leads to an increase in intracellular pH due to its protonation ($NH_3 + H^+ \rightarrow NH_4^+$) (Brookes and Turner, 1993). This change in intracellular pH could have consequences on cellular functions, presumably the pH-dependent transport processes. Care et al. (1984) reported an inhibitory effect of intraruminal ammonia (30 mmol/l) on the $Mg^{2+}$ net absorption, associated with a reduction in $Na^+$ efflux.

Both $Na^+$ and $Cl^-$ are transported by active mechanisms from the mucosal to serosal area through the

* Corresponding author. Tel.: +249 133 290 53; fax: +249 133 126 38.
*E-mail address:* abdounn@yahoo.com (K. Abdoun).

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rumen epithelium (Stevens, 1964; Chien and Stevens, 1972; Ferreira et al., 1972; Harrison et al., 1975; Scharrer et al., 1983). There is compelling evidence regarding a $Na^+/H^+$ exchange in the rumen epithelium (Scharrer et al., 1983; Strozyk, 1987; Martens and Gäbel, 1988; Henseleit, 1991). Martens et al. (1991) proposed that the $Na^+/H^+$ exchange is the predominant electrically silent $Na^+$ transport mechanism in sheep rumen epithelium. Internal $H^+$, independent of its role as a substrate for exchange with external $Na^+$, has also had an important modifier role as an allosteric activator of the $Na^+/H^+$ exchanger (Aronson et al., 1982).

Martens et al. (1991), in ion replacement studies, convincingly demonstrated that $Cl^-$ transport is $HCO_3^-$ dependent, and as the replacement of $Cl^-$ or $HCO_3^-$ did not change the $I_{sc}$, it was reported that $Cl^-$ is also likely to be transported by an electrically silent mechanism. In many epithelia, a $Na^+/H^+$ exchange is combined with $Cl^-/HCO_3^-$ exchange system (Hopfer and Liedtke, 1987; Reuss and Stoddard, 1987), which leads to an indirect coupling between $Na^+$ and $Cl^-$ transport. This parallel exchange system of $Na^+/H^+$ and $Cl^-/HCO_3^-$ is related to the intracellular pH (Knickelbein et al., 1985).

In the rumen, large quantities of ammonia, mainly derived from microbial degradation of dietary protein and recycled urea, are produced. Thirty to eighty percentage of the dietary nitrogen is absorbed from the rumen as ammonia (McDonald, 1948), and transported via the portal vein to the liver, where it is detoxified by conversion to urea (Huntigton, 1986). Ammonia ($NH_3$) absorption across the rumen epithelium occurs predominantly by simple diffusion of the non-ionised state, as it is lipophilic and has a neutral electrical charge (Hogan, 1961; Chalmers et al., 1971; Bödeker et al., 1990; Remond et al., 1993). Recently however, it was shown that ammonium ($NH_4^+$) takes part in total ammonia transport, most probably via a quinidine sensitive $K^+$-channel (Bödeker and Kemkowski, 1996).

It was hypothesized that the absorption of the fermentation product ammonia from the rumen could elevate the intracellular pH, whereby $pH_i$-dependent electroneutral Na and Cl transport across the rumen epithelium could be affected. It was the intention of the present study to test this hypothesis by studying the effect of increased mucosal ammonia concentration on Na and Cl absorption from the rumen of sheep.

## 2. Materials and methods

Methods for the isolation, preparation and incubation of rumen epithelium were followed as described by Martens et al. (1987). Briefly, sheep of different ages, sex and breeds were slaughtered at a local slaughterhouse. Each animal served as an experimental unit, from which the rumen epithelial tissue was sampled and divided into the different experimental groups (treatments). This protocol is repeated several times on animals of different ages, sex and breeds. Two to three minutes after death and exsanguination, the rumen, along with reticulum was removed from the abdominal cavity. A 150 $cm^2$ sample of the rumen wall was dissected from the ventral sac. The samples were first carefully cleaned by immersion in a buffer solution. Then the epithelium was manually stripped from the muscle layer and the isolated epithelium was immediately taken to the laboratory in a buffer solution at 38 °C. The transport buffer and the bathing solution contained: 145.2 mmol/l $Na^+$, 5 mmol/l $K^+$, 1.2 mmol/l $Ca^{2+}$, 1.2 mmol/l $Mg^{2+}$, 124.8 mmol/l $Cl^-$, 25 mmol/l $HCO_3^-$, 2.4 mmol/l $HPO_4^{2-}$, 0.4 mmol/l $H_2PO_4^-$ and 5 mmol/l glucose. In the laboratory the epithelium was sliced into sections (3 cm × 3 cm) and mounted between the two halves of an Ussing chamber with the exposed serosal area of 3.14 $cm^2$. Rings of silicon rubber on both sides of the tissue minimized edge damage. During preparation and transport the buffer solution was gassed with 95% $O_2$ and 5% $CO_2$. The mounted tissues were bathed on each side with 18ml buffer solution using a gas lift system and gassed with 95% $O_2$ and 5% $CO_2$ or 100% $O_2$ ($HCO_3^-$-free buffer solutions) at 38 °C. The standard electrolyte solution contained: 90 mmol/l $Na^+$, 5 mmol/l $K^+$, 1mmol/l $Ca^{2+}$, 2 mmol/l $Mg^{2+}$, 25 mmol/l $HCO_3^-$, 59 mmol/l $Cl^-$, 1 mmol/l $H_2PO_4^-$, 2 mmol/l $HPO_4^{-2}$, 25 mmol/l acetate, 10 mmol/l propionate, 5 mmol/l butyrate, 10mmol/l glucose and 30 mmol/l D-(−)-*N*-methylglucamine-hydrochloric acid (NMDGCl). In ammonia containing buffer solutions (5, 15 and 30 mmol/l), equimolar $NH_4Cl$ replaced NMDGCl. In $HCO_3^-$-free buffer solutions, $NaHCO_3$ was replaced with Na gluconate. All chemicals used to formulate the different buffers were obtained from Sigma Chemical Co. (St. Louis, MO, USA) or from Merck (Darmstadt, Germany).