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Effect of ammonia on Na^+ transport across isolated rumen epithelium of sheep is diet dependent

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The cellular uptake of ammonia affects the intracellular pH (pH_i) of polar and non-polar cells. A predominant uptake of NH_3 and its intracellular protonation tend to alkalinise the cytoplasm, whereas a predominant uptake of NH_4^+ acidifies the cytoplasm by reversing this reaction. Hence, the well-known absorption of ammonia across the rumen epithelium probably causes a change in the pH_i. The magnitude and direction of this change in pH_i (acid or alkaline) depends on the relative transport rates of NH_3 and NH_4^+. Consequently, the intracellular availability of protons will influence the activity of the Na^+–H^+ exchanger, which could affect transepithelial Na^+ transport. The aim of the present study has been to test this possible interaction between ruminal ammonia concentrations and Na^+ transport. The term ammonia is used to designate the sum of the protonated (NH_4^+) and unprotonated (NH_3) forms. Isolated ruminal epithelium of sheep was investigated by using the Ussing-chamber technique *in vitro*. The present results indicate that ammonia inhibits Na^+ transport across the rumen epithelium of hay-fed sheep, probably by binding intracellular protons and thus inhibiting Na^+–H^+ exchange. By contrast, ammonia stimulates Na^+ transport in concentrate-fed and urea-fed sheep, which develop an adaptation mechanism in the form of an increased metabolism of ammonia in the rumen mucosa and/or an increased permeability of rumen epithelium to the charged ammonium ion (NH_4^+). Intracellular dissociation of NH_4^+ increases the availability of protons, which stimulate Na^+–H^+ exchange. This positive effect of ruminal ammonia on Na^+ absorption may significantly contribute to the regulation of osmotic pressure of the ruminal fluid, because intraruminal ammonia concentrations up to 40 mmol/l have been reported.

Rumen: Sheep: Na^+ transport: Ammonia

Ammonia absorption across the rumen epithelia occurs predominantly by simple diffusion of the non-ionised form of ammonia (NH_3) because of its lipophility and lack of charge (Bödeker *et al.* 1990; Remond *et al.* 1993). Recently, however, it has been concluded that NH_4^+ takes part in total ammonia transport most probably via a quinidine-sensitive K^+-channel in the apical membrane of sheep rumen epithelium (Bödeker & Kemkowski, 1996).

Na^+–H^+ exchange (NHE) is the predominant, electrically silent Na^+ transport mechanism in sheep rumen epithelium (Martens *et al.* 1991) and internal H^+, independent of its role as a substrate for exchange with external Na^+, has an important modifier role as an allosteric activator of the Na^+–H^+ exchanger (Aronson *et al.* 1982). The intracellular pH (pH_i) of the rumen epithelial cell has been reported to have an effect on Na^+ uptake (Gäbel *et al.* 1996; Schweigel *et al.* 2000) and NHE contributes to the regulation of pH_i in rumen epithelial cells (Müller *et al.* 2000).

It is well known that the cellular uptake of ammonia affects the pH_i of polar (Kikeri *et al.* 1992; Heitzmann *et al.* 2000; Müller *et al.* 2000) and non-polar (Burckhardt & Frömter, 1992; Nagaraja & Brookes, 1998) cells. A predominant uptake of NH_4^+ acidifies the cytoplasm by release of protons, whereas a predominant NH_3 uptake and its intracellular protonation tend to alkalinise the cytoplasm. Consequently, electroneutral Na^+ transport via NHE could be affected, because the availability of H^+ varies according to the predominant uptake of NH_4^+ or NH_3. Because the concentration of ammonia and the pH of the ruminal fluid exhibit a wide variation, the relative concentrations of NH_3 and NH_4^+ change according to the Henderson–Hasselbalch equation. At a pH of 7·4 some 1 % or 0·15 mmol/l of the total ammonia is NH_3 at a concentration of 15 mmol/l. At a typical pH of 6·4 only 0·1 % is NH_3.

Recent studies from our laboratory have shown that ammonia inhibits electroneutral Na^+ transport across the rumen epithelium of hay-fed sheep in a dose-dependent manner (Abdoun & Martens, 1999).

Abbreviations: I_{sc}, short-circuit current; J_{ms}, mucosal-to-serosal flux; J_{net}, net flux; ME, metabolisable energy; NHE, Na^+–H^+exchange; PD, potential difference; pH_i, intracellular pH.

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The rumen epithelium metabolises ammonia and much of the ammonia taken up by the sheep rumen epithelium from the mucosal solution is not accounted for in the serosal solution (Bödeker *et al.* 1992). Such a mechanism might aid adaptation to a wide variation of ruminal ammonia concentrations attributable to variable intakes of N.

These observations have led to us to investigate the diet-dependence of interactions between ruminal ammonia concentrations and Na^+ transport across the rumen epithelium.

Materials and methods

Isolated epithelial tissues were used from five groups of sheep fed different diets for at least 3 weeks. Group (A) was fed hay. Because three batches of hay were used, the composition of the hay exhibited some variation (g/kg): crude protein, 129–163; fat, 23–27; crude fibre, 228–268; ash, 81–92; K, 26–34; Na, 1·8–3·1. Group (B) was fed hay + 800 g concentrate. The composition was (g/kg): crude protein, 160; crude fibre, 130; fat, 30; ash, 95; metabolisable energy (ME), 5·9 MJ/kg. Group (C) was fed hay + 400 g maize starch. The composition was (g/kg): crude protein, 9; fat, 2; ash, 1; ME, 13·45 MJ/kg. Group (D) was fed hay + 200 g milled and pelleted wheat (12·38 MJ ME/kg). The diet for group (E) consisted of hay + 200 g milled wheat mixed with 50 g urea, which was then pelleted. In all studies, hay was supplied *ad libitum* and the concentrate, maize starch and wheat were offered in equal portions at 07.00 hours and 15.00 hours. The sheep were 1–2 years old and had a body weight of 50–60 kg. The sheep had access to a lick-stone and water.

The preparation and incubation of rumen epithelium has been described in detail by Martens *et al.* (1987). Hay was offered always overnight and the sheep were killed in a local slaughter house at 07.30 hours without the morning portion of concentrate, maize starch, wheat or wheat + urea. At 2 to 3 min after death and exsanguination, the reticulo-rumen was removed from the abdominal cavity. A 150 cm^2 piece of rumen wall was taken from the ventral sac.

The pieces were first carefully cleaned by immersion in a buffer solution. The epithelium was then stripped from the muscle layer and the isolated epithelium was rapidly taken (within 10 min) to the laboratory in a buffer solution kept at 38°C, cut into squares (3 × 3 cm) and mounted between the two halves of an Ussing chamber to give an exposed serosal area of 3·14 cm^2. Edge damage was minimised by rings of silicon rubber on both sides of the tissue. During preparation and transport, the buffer solution was gassed with O_2–CO_2 (95:5, v/v). The mounted tissues were bathed on each side with 18 ml buffer solution by using a gas-lift system and were gassed with O_2–CO_2 (95:5, v/v) at 38°C. The standard electrolyte solution contained (mmol/l): Na^+, 90; K^+, 5; Ca^{2+}, 1; Mg^{2+}, 2; HCO_3^-, 25; Cl^-, 59; $H_2PO_4^-$, 1; HPO_4^{2-}, 2; acetate, 25; propionate, 10; butyrate, 5; glucose, 10; D(-)-N-methyl-D-glucamine-hydrochloric acid, 30. In the ammonia-containing buffer solutions (5, 15 and 30 mmol/l), D(-)-N-methyl-D-glucamine-hydrochloric acid was replaced by equimolar NH_4Cl. Radioisotopes ($^{22}Na^+$) were obtained from Amersham, Braunschweig, Germany. All reagents were of analytical grade.

Incubation procedure

The time from killing the sheep to mounting the epithelium was 20–30 min and a further period of 20 min was allowed for equilibration of the epithelium with the standard buffer solution in the Ussing chamber. At the end of the equilibration period the standard buffer solution of the mucosal side was replaced by the ammonia-containing buffer. Unidirectional fluxes of Na^+ were measured by using $^{22}Na^+$. The isotopes were added to one side of the epithelium and the tissues were incubated for 30 min to allow equilibration of the isotope.

Fluxes were calculated from the rate of the appearance of tracer on the other side of the epithelium within 60 min. Paired determinations of Na^+ fluxes were accepted only if the conductances differed by less than 25 %.

Unidirectional mucosal-to-serosal flux (J_{ms}) and serosal-to-mucosal flux and the net fluxes (J_{net}) of Na^+ were calculated from samples taken at the beginning and the end of each flux period. J_{net} was calculated as the difference between oppositely directed unidirectional fluxes.

Ammonia flux rate across the rumen epithelium was determined by measuring the rate of total ammonia (NH_3+NH_4^+) disappearance from the mucosal side and its appearance in the buffer solution at the serosal side, by using an ion analyser (EA 940; Orion; Boston, MA, USA).

Electrical measurements were continuously obtained with the aid of a computer-controlled voltage-clamp device (AC Micro-Clamp, Aachen, Germany). KCl–agar bridges were positioned near each surface of the tissue and connected to calomel electrodes for the measurement of the transepithelial potential difference (PD). Polyethylene bridges filled with bathing solution and agar for the application of current were inserted into the chambers approximately 3 cm from the surface of the tissue so that a uniform density of current flow could be assumed. The junction potential and the fluid resistance of the buffer between the tips of the PD-sensing bridges was determined before the tissue was mounted and subsequently corrected by the computer-controlled voltage clamp. The tissues were incubated under short-circuit conditions, as determined by the experimental protocol. The tissue was alternatively pulsed with a positive or negative 100 μA pulse of 1 s duration. The displacement in PD caused by the pulse was measured and, from the change in PD and pulse amplitude, the tissue conductance was calculated and printed out, together with the short-circuit current (I_{sc}) and the transepithelial PD every min.

Radioactivity

$^{22}Na^+$ was assayed by using a well-type crystal counter (LKB Wallace-Perkin Elmer, Überlingen, Germany).

Statistical analysis

Statistical evaluations were carried out by means of SPSS program version 10.0 for Windows. Results are given as

mean values with their standard errors. ANOVA was carried out in the form of a repeated measurement model. In the case of a significant difference between groups (ammonia concentration), Dunnett's test was performed (control *v*. ammonia). Significant effects of the treatment were reported at $P<0{\cdot}05$.

Results

Electrophysiological parameters

Short-circuit current and tissue conductance. Luminal ammonia caused a concentration-dependent increase of I_{sc}, which was significant at 15 and 30 mmol/l (see Tables 1–4), in all epithelia. This change in I_{sc} represented the flow of NH_4^+ through K^+ channels in the luminal membrane (Bödeker & Kemkowski, 1996) and exhibited significant differences between the feeding regimens. An almost linear correlation was found between luminal ammonia concentration and increase of I_{sc} (I_{sc} after mucosal addition of ammonia minus I_{sc} before mucosal addition of ammonia) in concentrate-fed ($y=0{\cdot}13+0{\cdot}021x$; r 0·94) and urea-fed sheep ($y=0{\cdot}05+0{\cdot}028x$; r 0·98) (Fig. 1). In hay-fed and maize-starch-fed sheep, the change of I_{sc} appeared to be saturated at an ammonia concentration greater than 15 mmol/l (Fig. 1). Corresponding alterations of tissue conductance were observed in all groups (significant at 30 mmol/l in concentrate-fed animals and in the urea group; Tables 2 and 4).

Na^+ transport rates

Hay-fed sheep. Increasing mucosal ammonia concentrations (5, 15 and 30 mmol/l) significantly ($P<0{\cdot}05$) decreased the J_{ms} and J_{net} of Na^+ across the rumen epithelium of hay-fed sheep (Table 1). This inhibitory effect of ammonia on Na^+ transport followed Michaelis–Menten kinetics (Fig. 2) and allowed (Lineweaver and Burk plot) the determination of the Michaelis–Menten constant (8·33 mmol NH_4Cl/l) and the maximal inhibitory rate in the J_{net} of Na^+ (2·08 μeq/cm^2 per h).

Concentrate-fed sheep. Increasing luminal ammonia concentrations significantly ($P<0{\cdot}05$) stimulated J_{ms} (30 mmol/l) and J_{net} (15 and 30 mmol/l) (Table 2). This stimulation of Na^+ transport by luminal ammonia in concentrate-fed sheep suggests a process of adaptation in the rumen epithelium.

Maize-fed sheep. Sheep fed maize starch daily showed slightly higher (though not significant) J_{net} of Na^+ (3·07 (SE 0·53) μeq/cm^2 per h) compared with hay-fed sheep

Table 1. Effect of increasing mucosal ammonia concentration on Na^+ transport and the electrophysiology of isolated rumen epithelium of hay-fed sheep†

(Mean values with their standard errors)

	Na^+ fluxes (μeq/cm per h)						I_{sc} (μeq/cm per h)		G_t (mS/cm^2)			
	J_{ms}		J_{sm}		J_{net}							
NH_4Cl (mmol/l)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*N*‡	*n*§
0	3·90	0·43	1·14	0·12	2·76	0·39	0·80	0·07	2·81	0·29	6	9
5	3·09	0·22	1·12	0·17	1·97*	0·19	0·94	0·05	3·03	0·38	6	9
15	2·34*	0·27	0·86	0·09	1·48*	0·25	1·22*	0·12	2·94	0·31	6	9
30	1·91*	0·12	0·86	0·05	1·05*	0·12	1·26*	0·04	3·08	0·20	6	7

J_{ms}, mucosal-to-serosal flux of Na^+; J_{sm}, serosal-to-mucosal flux of Na^+; J_{net}, net flux of Na^+; I_{sc}, short-circuit current; G_t, conductance of the epithelium.
* Mean value was significantly different from that of the control group (0 mmol NH_4Cl/l) ($P<0{\cdot}05$).
† For details of diet and procedures, see p. 752.
‡ Number of experimental animals.
§ Number of epithelial tissue samples per treatment group.

Table 2. Effect of increasing mucosal ammonia concentration on Na^+ transport and the electrophysiology of isolated rumen epithelium of concentrate-fed sheep†

(Mean values with their standard errors)

	Na^+ fluxes (μeq/cm per h)						I_{sc} (μeq/cm per h)		G_t (mS/cm^2)			
	J_{ms}		J_{sm}		J_{net}							
NH_4Cl (mmol/l)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*N*‡	*n*§
0	4·20	0·50	1·12	0·15	3·08	0·37	0·76	0·06	2·46	0·20	6	8
5	4·69	0·58	1·03	0·09	3·66	0·49	0·93	0·08	2·63	0·20	6	9
15	5·16	0·68	1·01	0·19	4·15*	0·42	1·31*	0·09	3·06	0·30	6	10
30	5·60*	0·74	0·97	0·09	4·63*	0·68	1·47*	0·09	3·51*	0·28	6	9

J_{ms}, mucosal-to-serosal flux of Na^+; J_{sm}, serosal-to-mucosal flux of Na^+; J_{net}, net flux of Na^+; I_{sc}, short-circuit current; G_t, conductance of the epithelium.
* Mean value was significantly different from that of the control group (0 mmol NH_4Cl/l) ($P<0{\cdot}05$).
† For details of diet and procedures, see p. 752.
‡ Number of experimental animals.
§ Number of epithelial tissue samples per treatment group.

Table 3. Effect of increasing mucosal ammonia concentration on Na^+ transport and the electrophysiology of isolated rumen epithelium of maize-fed sheep†

(Mean values with their standard errors)

	Na^+ fluxes (μeq/cm per h)						I_{sc} (μeq/cm per h)		G_t (mS/cm^2)			
	J_{ms}		J_{sm}		J_{net}							
NH_4Cl (mmol/l)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*N*‡	*n*§
0	4·09	0·49	1·02	0·07	3·07	0·53	0·69	0·11	2·40	0·28	4	6
5	3·95	0·27	0·91	0·08	3·04	0·24	1·03	0·05	2·63	0·35	4	5
15	3·13	0·24	0·83*	0·06	2·30	0·21	1·17*	0·07	2·75	0·33	4	5
30	3·08	0·31	0·85*	0·05	2·23	0·68	1·41*	0·05	2·89	0·26	4	6

J_{ms}, mucosal-to-serosal flux of Na^+; J_{sm}, serosal-to-mucosal flux of Na^+; J_{net}, net flux of Na^+; I_{sc}, short-circuit current; G_t, conductance of the epithelium.
* Mean value was significantly different from that of the control group (0 mmol NH_4Cl/l) ($P<0·05$).
† For details of diet and procedures, see p. 752.
‡ Number of experimental animals.
§ Number of epithelial tissue samples per treatment group.

Table 4. Effect of increasing mucosal ammonia concentration on Na^+ flux rates across the rumen epithelium of wheat- and urea-fed sheep‡

(Mean values with their standard errors)

	Na^+ fluxes (μeq/cm per h)						I_{sc} (μeq/cm per h)		G_t (mS/cm^2)			
	J_{ms}		J_{sm}		J_{net}							
NH_4Cl (mmol/l)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*N*§	*n*‖
Wheat group												
0	4·11	0·40	1·16	0·13	2·95	0·31	0·79	0·08	2·98	0·13	4	6
15	3·11	0·65	0·92	0·14	2·19	0·62	1·20†	0·09	3·04	0·18	4	6
30	2·06†	0·20	0·76	0·04	1·30†	0·16	1·28†	0·09	3·32	0·17	4	6
Urea group												
0	3·47	0·45	1·23	0·14	2·24	0·34	0·89	0·10	2·91	0·18	3	5
15	4·13	0·59	1·08	0·13	3·05	0·48	1·44†	0·05	3·45	0·33	3	4
30	4·48*	0·62	1·07	0·14	3·41†	0·49	1·72†	0·08	4·03†	0·40	3	4

J_{ms}, mucosal-to-serosal flux of Na^+; J_{sm}, serosal-to-mucosal flux of Na^+; J_{net}, net flux of Na^+; I_{sc}, short-circuit current; G_t, conductance of the epithelium.
* Mean value was marginally significantly different from that of the control for the urea group (0 mmol NH_4Cl/l) ($P=0·051$).
† Within a group, mean value was significantly different from that of the control group (0 mmol NH_4Cl/l) ($P<0·05$).
‡ For details of diet and procedures, see p. 752.
§ Number of experimental animals.
‖ Number of epithelial tissue samples per treatment group.

(2·76 (SE 0·39) μeq/cm^2 per h). Increasing luminal ammonia concentrations reduced J_{ms} and J_{net} by some 25–28 % at 30 mmol ammonia/l (Table 3), but this decrease was not significant.

Urea-fed sheep. Increasing luminal ammonia concentrations significantly stimulated both J_{ms} and J_{net} of Na^+ across the rumen epithelium of urea-fed sheep, whereas Na^+ flux rates across the rumen epithelium of wheat-fed sheep were significantly ($P<0·05$) inhibited (Table 4), indicating that the increased N intake induced adaptation.

Total ammonia flux rates. The mucosal disappearance rate of total ammonia (30 mmol/l) is higher than the serosal appearance rate in both hay-fed and concentrate-fed sheep indicating intra-epithelial metabolism of ammonia. The mucosal disappearance rate is, however, significantly ($P<0·05$) higher whereas the serosal appearance rate is significantly ($P<0·05$) lower in concentrate-fed sheep (Table 5).

Discussion

The results of the present study indicate that diet alters the function of rumen epithelium, modulating the effect of ammonia on Na^+ transport. Surprisingly, ammonia significantly inhibited Na^+ transport at pH 7·4 in a dose-dependent manner at physiological concentrations in hay-fed sheep. NHE represents the predominant Na^+ transport mechanism in sheep rumen epithelium (Martens *et al.* 1991) and NHE appeared to be nearly abolished at 30 mmol ammonia/l, because J_{net} and I_{sc} (corrected for the NH_4^+-dependent current), which accounts for electrogenic Na^+ transport, had almost the same magnitude (see Table 1). The abolition of electroneutral Na^+ transport via NHE can further be deduced from the maximal inhibitory rate in the J_{net} of Na^+. The I_{sc} represents electrogenic Na^+ transport and was 0·80 μeq/cm^2 per h under control conditions (see Table 1). The difference between I_{sc} (0·80 μeq/cm^2 per h) and the J_{net} of Na^+ (2·76 μeq/cm^2 per h; Table 1) mirrored electroneutral Na^+ transport with 1·96 μeq/cm^2 per h. This was almost identical to the maximal inhibitory rate in the J_{net} of Na^+ of 2·08 μeq/cm^2 per h. The inhibition of Na^+ absorption by ammonia may have physiological consequences, because the absorptive capacity of the rumen partly counterbalances the high net secretion into the forestomachs via saliva. Martens *et al.* (2001) have recently discussed the possible absorptive capacity of the

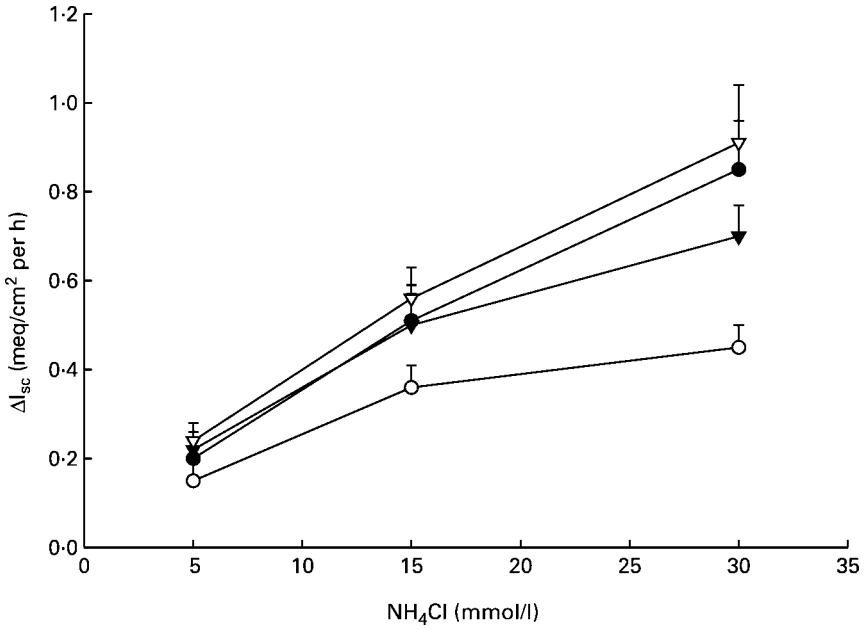

Fig. 1. Increase of short-circuit current (ΔI_{sc}) after mucosal addition of ammonia. ΔI_{sc} of concentrate-fed sheep (-●-; *n* 6) and urea-fed sheep (-Δ-; *n* 3) was significantly different from hay-fed animals (-○-; *n* 6) at 30 mmol ammonia/l ($P<0{\cdot}05$). Values are means, with their standard errors represented by vertical bars. (-▼-), Maize-fed sheep (*n* 4). For details, see Tables 1–4.

rumen of sheep for Na^+, which approaches almost 600 mmol Na/d.

The suggested increase of pH_i may lead to the augmented availability of HCO_3^- and hence enhance Cl^- transport via $Cl^- - HCO_3^-$ exchange, which is coupled to NHE by pH_i (Martens *et al.* 1991). Indeed, K Abdoun, K Wolf and H Martens (unpublished results) have observed an increase of Cl^- transport across sheep rumen epithelium at 30 mmol ammonia/l. The obtained data with epithelia from hay-fed sheep are consistent with the predictable effects of transepithelial NH_3 movement on Na^+ transport via NHE.

A diet of 800 g concentrate (128 g crude protein (20·5 g N); 4·72 MJ ME) fed in equal portions twice daily in addition to hay *ad libitum* caused totally different effects of ammonia on Na^+ transport *in vitro*. J_{ms} and J_{net} were significantly enhanced by some 33 % at 30 mmol ammonia/l. It should be noted that Na^+ transport under control conditions in concentrate-fed sheep was not different from that in hay-fed sheep (Tables 1 and 2); this does not agree

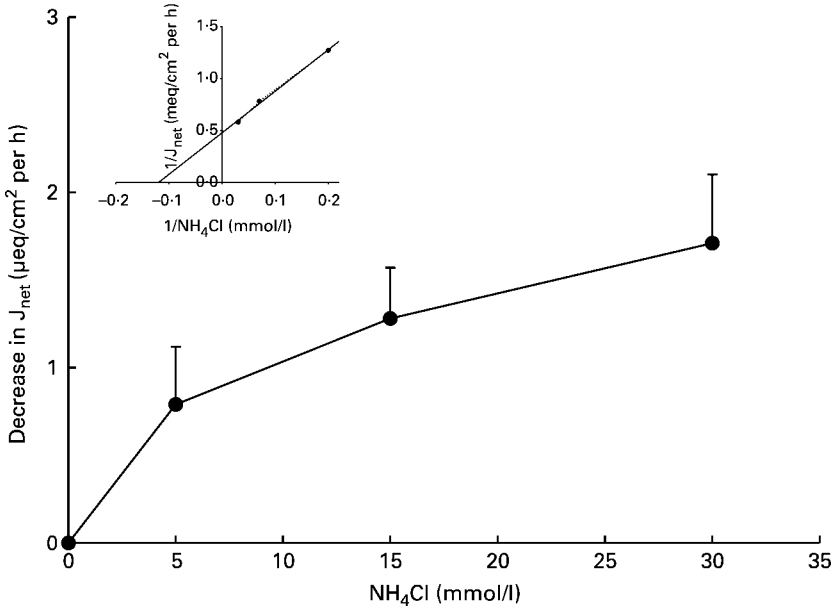

Fig. 2. The inhibitory effect of ammonia on net Na^+ flux (J_{net}) rate across the rumen epithelium of hay-fed sheep (four experimental animals; six epithelial tissue samples per treatment group). Inset, double reciprocal plot that reveals a Michaelis–Menten constant for the inhibitory effect of ammonia on J_{net} (ammonia concentrations *v.* the inhibition in J_{net}) of 8·33 mmol/l and maximal inhibitory rate in J_{net} of 2·08 μeq/cm per h.

Table 5. Mucosal disappearance and serosal appearance rate of total ammonia across the rumen epithelium of hay-fed and concentrate-fed sheep at a luminal ammonia concentration of 30 mmol/l†

(Mean values with their standard errors)

Diet	*n*‡	Mucosal disappearance rate (μmol/cm^2 per h)		Serosal appearance rate (μmol/cm^2 per h)	
Hay	7	4·66	0·28	3·96	0·22
Concentrate	9	6·26*	0·48	2·02*	0·34

* Mean value was significantly different to that for hay-fed animals ($P<0·05$).
† For details of diet and procedures, see p. 752.
‡ Number of epithelial tissue samples per treatment group.

with previous findings (Gäbel *et al.* 1987; Zanming *et al.* 2002). However, the concentrate intake was lower in the present study and hay was offered *ad libitum*.

Feeding concentrate changes both energy and N intake. Hence, an attempt was made to separate the effect of energy from possible alterations induced by ammonia. A daily supplement of 400 g maize starch provided the sheep with 5·38 MJ ME and a negligible 4 g crude protein. Again, it is worth noting that Na^+ transport under control conditions has the same magnitude as in hay-fed and concentrate-fed sheep (see Tables 1, 2 and 3). A concentration of 5 mmol ammonia/l did not change Na^+ transport rates and 15 or 30 mmol ammonia/l reduced J_{ms} or J_{net} (but not significantly) by some 20–30 %, which suggests minor effects of energy intake on adaptation and hints at an effect of ammonia. Indeed, 50 g urea or 1·66 M-ammonia induced an increase of J_{ms} ($P=0·051$) and of J_{net} ($P<0·05$) at 30 mmol luminal ammonia/l. By contrast, Na^+ transport rates, J_{ms} and J_{net}, were significantly inhibited at 30 mmol/l in the control group (200 g wheat).

Thus adaptation had occurred. However, the underlying mechanisms of adaptation are not well defined. It is well known that, *in vivo*, short-chain fatty acids (primarily butyrate) trigger the growth of rumen papillae (Sakata & Tamate, 1978). Recent *in vitro* studies support the conclusion that insulin, epidermal growth factor and insulin-like growth factor-1 are involved in stimulating cell growth of isolated ruminal cells (Baldwin, 1999). Similarly, Zanming *et al.* (2002) have observed higher insulin-like growth factor-1 concentrations in plasma, an increase in the size of papillae and surface of the rumen epithelium (atrium, ventral rumen and ventral blind sac) and an enhanced J_{net} of Na^+ across the isolated rumen epithelium (ventral rumen) in goats fed 1·1 kg concentrate/d and hay *ad libitum*. The data of Baldwin (1999) and of Zanming *et al.* (2002) support the suggestion that insulin-like growth factor-1 plays an important role in the adaptation of the rumen epithelium to energy-rich diets. Nevertheless, the cascade from enhanced insulin-like growth factor-1 to stimulation of Na^+ transport at high ruminal ammonia concentration is still obscure and unlikely in the urea-fed group. Probably local factors are also involved. Musch *et al.* (2001) have exposed the human colonic cell line C2/bbe to acetate, propionate and butyrate and found an increased NHE activity and protein expression in the apical membrane. The effect of urea feeding supports the assumption of a direct effect of ammonia on the epithelium.

The timescale of adaptation is not well defined. The size and number of rumen papillae in cows increase within 4–6 weeks after a change of diet from hay to hay plus concentrate (Dirksen *et al.* 1984). Adaptation to elevated ruminal ammonia concentration is much faster. An acute rise in ruminal ammonia concentration decreases Mg^{2+} absorption from the rumen (Head & Rook, 1955; Martens & Rayssiguier, 1980; Care *et al.* 1984). This effect disappears 3 d after a sudden increase in ruminal ammonia concentrations to some 40 mmol/l (Gäbel & Martens, 1986), which indicates a rapid adaptation and explains the transient effect of ruminal ammonia on Mg^{2+} absorption (Martens & Schweigel, 2000).

Adaptation obviously includes alterations induced by N intake or ruminal ammonia concentrations. Some suggestions can be made regarding the effect of N intake on Na^+ transport. Nocek *et al.* (1980) have shown that an increase of ruminal degradable protein (60 %) in the diet causes enhanced activity of glutamate dehydrogenase in the rumen epithelium of calves. This enzyme detoxifies ammonia (McLaren *et al.* 1961; Hoshino *et al.* 1966) and the findings of Nocek *et al.* (1980) are in agreement with our observations that, in concentrate-fed animals, the serosal appearance of ammonia is much lower compared with that in hay-fed animals despite higher luminal uptake. The synthesis of glutamate eliminates ammonia from the cytosol and reduces effects of ammonia on pH_i and electroneutral Na^+ transport via NHE and may contribute to decrease the risk of ammonia toxicity. Morris & Payne (1970) have shown that the tolerance of sheep to orally administered urea was positively related to dietary N intake.

The metabolism of ammonia might explain the abolition of inhibited Na^+ transport, but not the stimulation of NHE, which requires increased availability of H^+. Recent findings of Bödeker & Kemkowski (1996) support the assumption of NH_4^+ uptake through a K^+ channel in the apical membrane. Intracellular dissociation of NH_4^+ and release of H^+ would decrease pH_i and increase Na^+ transport mediated by NHE. Our data support the assumption of apical uptake of NH_4^+, because luminal ammonia induced an increase of I_{sc} in all cases (see Tables 1–4). The ΔI_{sc} (I_{sc} treatment minus I_{sc} control) is significantly higher in concentrate-fed (0·71 (SE 0·07) μeq/cm^2 per h) and urea-fed (0·83 (SE 0·10) μeq/cm^2 per h) sheep than in hay-fed animals (0·46 (SE 0·07) μeq/cm^2 per h), but not in maize-starch-fed sheep. This observation is in agreement with the assumption of the increased availability of H^+ attributable to NH_4^+ uptake. However, Na^+ transport is enhanced only in the concentrate-fed and the urea-fed group, but not in the maize-starch-fed sheep despite an almost identical ΔI_{sc}. Obviously, other factors contribute to the stimulating effect of ammonia on Na^+ transport. Metabolism of ammonia appears to be an unproven explanation.

In vitro studies always raise the question of how representative the results are for the normal *in vivo* situation. A compilation was made a few years ago of *in vivo* and *in vitro* data about the effect of K and the transmural PD of the rumen epithelium on Mg^{2+} absorption. The relative changes from all these studies agreed very

well (Leonhard-Marek *et al.* 1998). To the knowledge of the authors all *in vitro* studies about transport mechanisms of the rumen epithelium and possible effects on these mechanisms have been confirmed *in vivo* and vice versa.

In conclusion, ammonia decreases Na^+ transport via NHE across isolated rumen epithelia from hay-fed sheep and increases Na^+ transport in preparations from concentrate-fed and urea-fed sheep. The major reason for this alteration of Na^+ transport is probably the increase in N intake and the ruminal ammonia concentration. Because ruminal ammonia concentrations up to 40 mmol/l have been observed *in vivo*, an ammonia-dependent enhanced Na^+ absorption would prevent or reduce an increase of osmotic pressure in the ruminal fluid after a meal. Since hypertonic ruminal fluid increases water influx into the rumen (Dobson *et al.* 1976), decreases salivary flow (Warner & Stacy, 1977), food intake (Carter & Grovum, 1990) and short-chain fatty acids absorption (Bennink *et al.* 1978), ammonia-stimulated Na^+ absorption may contribute to normalise osmotic pressure and to diminish the possible negative side effects. Thus the positive interaction between ammonia and Na^+ absorption may be of practical importance for all feeding conditions with a rapid breakdown of protein.

Modulation of electroneutral Na^+ transport via NHE by CO_2 and HCO_3^- or short-chain fatty acids is well established. It appears from the results of the present study that the qualitative and quantitative effects of ammonia on Na^+ transport are as important as the influence of the classical modulators CO_2 and HCO_3^- or short-chain fatty acids.

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