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THE EFFECT OF ACETAZOLAMIDE ON ION TRANSPORT ACROSS ISOLATED SHEEP RUMEN EPITHELIUM

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SUMMARY

1. The net fluxes of sodium and chloride from the lumen to the blood side of isolated sheep rumen epithelium were reduced by treatment of both sides of the epithelium with acetazolamide.

2. The changes in the net fluxes of sodium and chloride were significantly correlated and showed recovery after removal of acetazolamide.

3. The net flux of potassium from blood to lumen side of the epithelium was not affected by treatment with acetazolamide.

4. It is suggested that acetazolamide blocks coupled sodium and chloride transport which may be mediated through 'low-activity' carbonic anhydrase enzymes.

INTRODUCTION

In their studies of active chloride transport across gastric mucosa both Hogben (1955) and Durbin & Heinz (1958) have shown that the sulphonamide-derivative drug, acetazolamide, inhibits the electrogenic flux of chloride across that tissue. Although the forestomachs of the ruminant animals, such as cattle and sheep, have a simple squamous stratified epithelium which is keratinized and structurally dissimilar to the glandular secretory epithelium of the acid-secreting stomach, evidence has accumulated which supports the original suggestion of Sperber & Hydén (1952) that active transport of chloride can occur from rumen to blood. Stevens (1964) showed that isolated sheets of rumen epithelium from cattle and goats actively transported sodium and chloride from rumen to

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blood and both Chien & Stevens (1972) and Ferreira, Harrison, Keynes & Zurich (1972) showed that part of the active sodium transport across cattle and sheep rumen epithelium was dependent on the presence of chloride ions.

In further investigations on the coupling mechanisms of ion transport across isolated sheep rumen epithelium, we have now studied the action of acetazolamide on the fluxes of sodium, potassium and chloride ions.

METHODS

Epithelium preparation, apparatus and Ringer solutions. Most of the details of the technique, apparatus and solutions used have been published previously (see Ferreira, Harrison & Keynes, 1966, and Harrison, Keynes, Rankin & Zurich, 1975). The small chambers described by Ferreira *et al.* (1972) were used in all of these experiments.

Acetazolamide (Diamox; Lederle Laboratory Division, Cyanamid of Great Britain Ltd, London: 500 mg) as the sodium salt (mol. wt. 244·2) was dissolved in 10 ml. sterile water and then diluted to 20 ml. with a 90 mM sucrose solution. This produced a stock solution of about 100 mM acetazolamide which was approximately iso-osmotic with the Ringer solutions used and had a sodium concentration of about 160 mM. Addition of 0·1 ml. or 1 ml. stock solution to the 10 ml. Ringer solution bathing each side of the epithelium gave a concentration of 1 or 10 mM acetazolamide. In the latter case the chloride and potassium concentrations in the bathing solutions were reduced by about 10 %. However, acetazolamide was always added to both sides of the epithelium and the appropriate measured concentrations of each ion were used in flux calculations. The alkaline pH of the stock solution reduced the hydrogen ion concentration of the Ringer solution (from pH 7·5 to 8·0). In the later experiments the pH was adjusted to 7·5 using a few drops of N-HCl and, in some cases, replacing Na_2HPO_4 in the standard Ringer solution by NaH_2PO_4. No differences were observed in the effects of Diamox when these adjustments were made to the Ringer solutions and the results of all observations have been combined in this paper.

Flux determinations. ^{42}KCl, $^{22}NaCl$, $^{24}NaCl$ and $Na^{36}Cl$ solutions were obtained from the Radiochemical Centre, Amersham. Various combinations of a short half-life isotope and a long half-life one were used in double-labelled experiments. The experimental handling and counting of the isotopes were as described previously (Harrison *et al.* 1975). Counts of ^{42}K and ^{24}Na were corrected for isotope decay; samples from double-labelled experiments with ^{42}K as one isotope were recounted for the second isotope after an interval of 7 days. With ^{24}Na, 10 days were allowed before recounting.

Analytical. Sodium, potassium and chloride concentrations and osmolality of Ringer solutions were determined as described previously (Harrison *et al.* 1975).

RESULTS

Sodium transport

In studying the effect on the unidirectional sodium fluxes, observations were made in eleven *paired* experiments treated with 10 mM Diamox and four *paired* experiments treated with 1 mM Diamox. Initially, the protocol of the experiments was to make control observations for four to

six 30 min periods after adding isotope to either blood or rumen sides of the preparations, and then to add Diamox to both sides of the epithelium during two further 30 min periods. Diamox caused a slight decline in sodium fluxes and short-circuit current which appeared no greater than that observed in some untreated preparations. For the last nine experiments using 10 mM Diamox and the last two using only 1 mM, the protocol was changed to allow four control periods of 30 min followed by two periods with Diamox added to the Ringer solutions, and finally three or four 30 min periods when normal Ringer again bathed the epithelium.

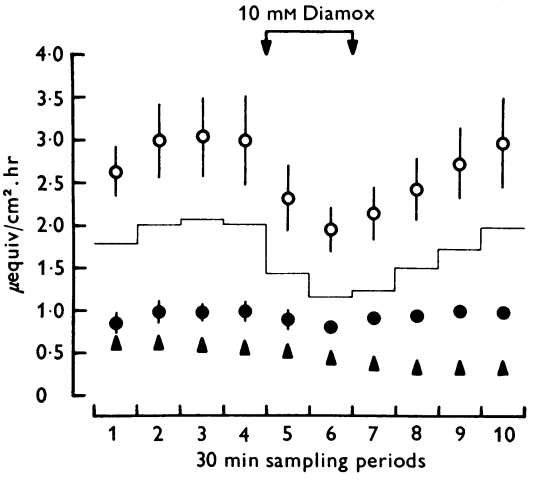

Fig. 1. The averaged results of nine *paired* experiments in which the sodium fluxes and short-circuit current were measured before, during and after the treatment of both sides of the epithelium with Diamox (10 mM). Isotope was added to the rumen or blood side of the preparation at the start of the first 30 min period after equilibration. ●, flux from rumen to blood; ○, flux from blood to rumen; ▲, short-circuit current; ——, net flux. The length of the line drawn through each flux value is $\pm 1 \times$ S.E. of mean; for the short-circuit current the S.E. of the mean was always less than $\pm 0{\cdot}06$ μequiv/cm².hr.

The averaged data obtained in the nine *paired* experiments when the epithelium was treated with 10 mM Diamox are illustrated in Fig. 1. To allow comparison with earlier work from our laboratory, the actual measurements made in (*a*) period three after addition of isotope, (*b*) period six which was the second Diamox treated period, and (*c*) period nine which was the third recovery period, are summarized in Table 1. There was a definite reduction in the unidirectional flux of sodium from rumen to blood and this caused a sharp fall in the net flux from rumen to blood. In subsequent periods the unidirectional and net fluxes returned to nearly the pre-treatment level by the fourth recovery period. The fluxes

TABLE 1. Sodium and current fluxes passing across isolated sheets of rumen epithelium during the third, sixth and ninth 30 min periods after the addition of isotope to the rumen side (*R–B*) or the blood side (*B–R*). *Paired* pieces of epithelium were taken from the same animal. Diamox (10 mM) was added to both sides of the epithelium for the fifth and sixth periods

	Sodium fluxes (μmole/cm^2.hr)						Short-circuit current fluxes (μequiv/cm^2.hr)		
	R–B Period			*B–R* Period			Period		
Expt. no.	3	6	9	3	6	9	3	6	9
281	—	—	—	0·71	0·66	0·86	0·45	0·33	0·23
282	2·65	2·09	1·92	—	—	—	0·34	0·24	0·12
306	—	—	—	0·82	0·69	(0·76)*	0·91	0·73	0·64
307	4·64	2·78	4·03	—	—	—	0·77	0·71	0·60
318	—	—	—	0·90	0·72	(0·70)*	0·75	0·26	(0·18)*
319	1·42	0·92	(1·19)*	—	—	—	0·67	0·29	(0·33)*
322	—	—	—	0·61	0·80	0·88	0·69	0·61	0·44
323	3·13	1·81	2·41	—	—	—	0·78	0·62	0·46
324	—	—	—	1·54	1·24	1·24	0·59	0·43	0·25
325	3·60	2·83	2·84	—	—	—	0·78	0·67	0·45
326	—	—	—	1·34	0·88	1·22	0·60	0·59	0·37
327	2·15	1·54	2·05	—	—	—	0·52	0·42	0·31
328	—	—	—	0·87	0·80	0·92	0·41	0·36	0·26
329	5·69	3·12	4·86	—	—	—	0·16	0·21	0·11
330	—	—	—	0·91	0·63	0·85	0·60	0·44	0·29
331	1·48	1·07	1·35	—	—	—	0·51	0·34	0·27
332	—	—	—	1·14	0·78	1·05	0·72	0·55	0·35
333	2·57	1·41	2·35	—	—	—	0·42	0·27	0·19
Mean	3·04	1·95	2·73	0·97	0·80	1·00	0·59	0·45	0·33
± S.E. of mean	0·47	0·27	0·41	0·11	0·06	0·06	0·04	0·04	0·04

* Eighth 30 min period; excluded from mean value.

for Period 6 compared to those for Periods 3 and 9 [i.e. Period 6 divided by $\frac{1}{2}$ (Period 3 and Period 6); see Table 1] were reduced to $72 \pm 4{\cdot}0\%$ (mean ± S.E. of mean; $n = 9$) from rumen to blood and $84 \pm 4{\cdot}0\%$ from blood to rumen. The short-circuit current fell slightly after treatment of the epithelium with Diamox and was not restored in the recovery periods. Treatment of the epithelium with only 1 mM Diamox caused a slight fall in net sodium flux in the second 30 min of treatment with a very slight recovery after treatment.

Chloride transport

In eight out of the thirte[illegible] *paired* experiments treated with 10 mM Diamox, the protocol was [illegible]at just described for the majority of the sodium measurements. The mean data for the unidirectional chloride fluxes and the over-all mean current fluxes for this group of eight experiments are illustrated in Fig. 2 and the measurements obtained in Periods

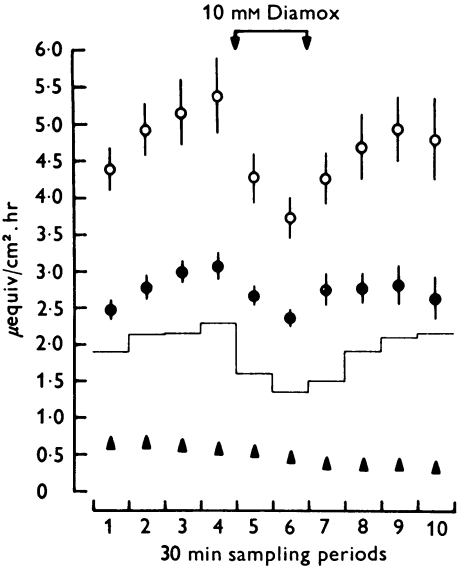

Fig. 2. The averaged results of eight *paired* experiments in which the chloride fluxes and short-circuit current were measured before, during and after treatment of both sides of the epithelium with Diamox (10 mM). Isotope was added to the rumen or blood side of the preparations at the start of the first 30 min period after equilibration. Symbols as in Fig. 1.

3, 6 and 9 after addition of isotope are summarized in Table 2. There was a sharp reduction in the unidirectional flux of chloride from rumen to blood and the opposite flux from blood to rumen was also reduced. The fluxes for Period 6 compared to those for Periods 3 and 9 (see above and Table 2) were reduced to $76 \pm 2{\cdot}3\%$ (mean $\pm$ S.E. of mean; $n = 8$) from rumen to blood and $83 \pm 2{\cdot}1\%$ from blood to rumen. Nevertheless, the mean net flux of chloride from rumen to blood was reduced during treatment with Diamox. When the mean net chloride fluxes for each period (Fig. 2) are plotted against the corresponding mean net fluxes of sodium (Fig. 1) obtained mainly in the same double-labelled experiments, there is a positive correlation (Fig. 3) (regression $y = 0{\cdot}406 + 0{\cdot}892x$; $r = 0{\cdot}95$;

TABLE 2. Chloride and current fluxes passing across isolated sheets of rumen epithelium during the third, sixth and ninth 30 min periods after the addition of isotope to the rumen side (*R–B*) or the blood side (*B–R*). *Paired* pieces of epithelium were taken from the same animal. Diamox (10 mM) was added to both sides of the epithelium for the fifth and sixth periods

	Chloride fluxes (μmole/cm^2.hr)						Short-circuit current fluxes (μequiv/cm^2.hr)		
	R–B Period			*B–R* Period			Period		
Expt. no.	3	6	9	3	6	9	3	6	9
306	—	—	—	3·26	2·46	3·25	0·91	0·73	0·64
307	7·11	4·75	6·52	—	—	—	0·77	0·71	0·60
318	—	—	—	3·42	2·48	(2·37)*	0·75	0·26	(0·18)*
319	4·38	3·41	(3·42)*	—	—	—	0·67	0·29	(0·33)*
322	—	—	—	2·99	2·33	2·56	0·69	0·61	0·44
323	5·16	3·68	4·34	—	—	—	0·78	0·62	0·46
324	—	—	—	2·39	1·85	1·48	0·59	0·43	0·25
325	4·81	3·72	4·00	—	—	—	0·78	0·67	0·45
326	—	—	—	3·58	3·04	3·79	0·60	0·59	0·37
327	4·85	3·71	4·92	—	—	—	0·52	0·42	0·31
328	—	—	—	2·77	2·14	2·65	0·41	0·36	0·26
329	7·04	4·98	6·60	—	—	—	0·16	0·21	0·11
330	—	—	—	2·62	2·22	2·87	0·60	0·44	0·29
331	3·94	2·72	3·84	—	—	—	0·51	0·34	0·27
332	—	—	—	2·99	2·48	3·17	0·72	0·55	0·35
333	3·99	2·86	4·23	—	—	—	0·42	0·27	0·19
Mean	5·16	3·73	4·92	3·00	2·37	2·82	0·62	0·47	0·36
± S.E. of mean	0·44	0·28	0·44	0·14	0·12	0·27	0·05	0·04	0·04

* Eighth 30 min period; excluded from mean value.

$n = 10$) between the changes produced in the two fluxes by Diamox treatment. In three *paired* experiments, chloride fluxes were measured during treatment of the epithelium with 1 mM Diamox and, as in the case of sodium, slight reductions in the fluxes were observed. The mean changes in the net fluxes of sodium and chloride were also correlated (regression $y = -0{\cdot}273 + 1{\cdot}164x$; $r = 0{\cdot}91$; $n = 6$) for the six periods with complete average data.

Potassium transport

Although observations of potassium fluxes were made in only three *paired* experiments using 10 mM Diamox treatment and two *paired* experiments with 1 mM Diamox, the mean flux data for the 10 mM treatment group illustrated in Fig. 4 show that Diamox treatment of the

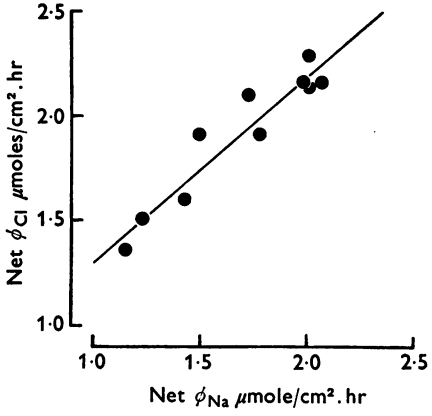

Fig. 3. Regression of mean net sodium flux and mean net chloride flux for all periods illustrated in Figs. 1 and 2.

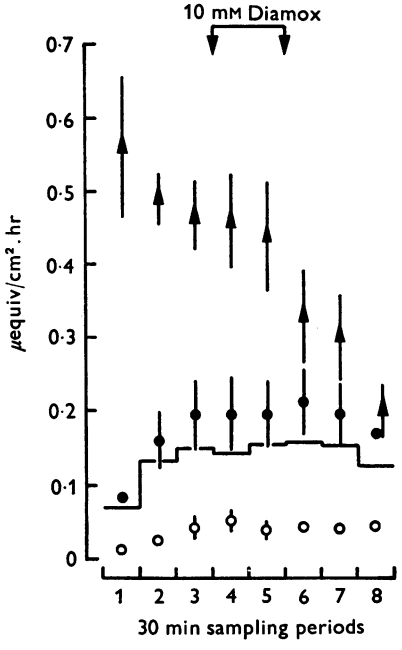

Fig. 4. The averaged results of three *paired* experiments in which the potassium fluxes and short-circuit current were measured before, during and after treatment of both sides of the epithelium with Diamox (10 mM). Isotope was added to the rumen or blood side of the preparations at the start of the first 30 min period after equilibration. Symbols as in Fig. 1.

epithelium had no obvious effect on the unidirectional or net fluxes of potassium. The mean data for the third 30 min after addition of isotope, the fifth period or second Diamox treatment period in these experiments and the eighth period or third recovery period are summarized in Table 3.

TABLE 3. Potassium and current fluxes passing across isolated sheets of rumen epithelium during the third, fifth and eighth 30 min periods after the addition of isotope to the rumen side (*R–B*) or blood side (*B–R*). *Paired* pieces of epithelium were taken from the same animal. Diamox (10 mM) was added to both sides of the epithelium for the fourth and fifth periods

	Potassium fluxes (μmole/cm^2.hr)						Short-circuit current fluxes (μequiv/cm^2.hr)		
	R–B Period			*B–R* Period			Period		
Expt. no.	3	5	8	3	5	8	3	5	8
278	—	—	—	0·23	0·25	(0·24)*	0·50	0·50	(0·34)*
279	0·04	0·02	(0·04)*	—	—	—	0·63	0·77	(0·55)*
281	—	—	—	0·10	0·11	0·11	0·41	0·33	0·23
282	0·07	0·06	0·05	—	—	—	0·29	0·24	0·12
291	—	—	—	0·25	0·22	0·23	0·50	0·38	0·16
292	0·02	0·04	0·05	—	—	—	0·51	0·40	0·28
Mean	0·04	0·04	0·05	0·19	0·19	0·17	0·47	0·44	0·20
±S.E. of mean	0·014	0·012	—	0·05	0·04	—	0·05	0·07	0·04

* Seventh 30 min period; excluded from mean value.

DISCUSSION

Acetazolamide (2-acetylamino-1,3,4 thiadiazole-5-sulphonamide) or Diamox is a substituted sulphonamide drug which has been shown to inhibit chloride transport in other tissues (see Hogben, 1955). Our preliminary observations (see Keynes & Harrison, 1970) indicated that some inhibition of chloride transport across isolated rumen epithelium of sheep could be produced by Diamox. Our present findings show that treatment of both sides of the isolated epithelium with Diamox reduces the net fluxes of sodium and chloride from the rumen to the blood side of the preparations and that these changes are reversed by removal of the Diamox. Hogben (1955) found that isolated gastric mucosa of the frog was unresponsive to 1 mM or less of Diamox whereas others had found that, *in vivo*, the dog stomach responded to these levels. However, 10 mM Diamox depressed H^+ secretion by the frog gastric mucosa and reduced the potential difference and net flux of chloride across the tissue. Although 1 mM Diamox appeared to affect the fluxes of sodium, chloride and current across isolated rumen epithelium, most of our results were

obtained with 10 mM Diamox where a definitive response was seen in all experiments.

In some experiments (see Methods) we did not correct for the elevation of the pH of the Ringer solution from 7·5 to 8 caused by the addition of 10 mM Diamox. However, in two recent experiments the fluxes of sodium and chloride were observed separately when the pH of the Ringer solution was changed from 7·4 to 8·4 and then back to 7·4. No changes in the net fluxes of sodium or chloride were seen in these experiments and we can conclude that the changes reported here were produced by Diamox and not the altered pH of some of the Ringer solutions. We have previously shown (Ferreira *et al.* 1972) that part of the net flux of sodium from rumen to blood is coupled to chloride ions and the good correlation found in the present experiments between the changes in sodium and chloride net fluxes (Fig. 3) suggests that Diamox was probably affecting this coupled flux.

Diamox is a drug with potent inhibitory effects on carbonic anhydrase activity (see Maren, 1967). Aafjes (1967) has reported the presence of carbonic anhydrase activity in epithelia from the forestomachs of cows, and Carter (1971) has recently identified two iso-enzymes of carbonic anhydrase with 'low-activity' in rumen epithelium of the ox. Assuming that sheep rumen epithelium contains these or similar enzymes and that they are inhibited by Diamox, it could be that the coupled flux of sodium and chloride is mediated through the activity of these enzymes. It seems unlikely that the HCO_3^--stimulated ATPase found by Hegner & Anika (1975) in the plasma membranes of the rumen epithelium of *Bos primigenius taurus* is concerned with the activity which we have now shown to be inhibited by Diamox.

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