Effect of ouabain on Na,K-ATPase and electrolyte transport in the dog ileum *in vivo*

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SUMMARY It is currently believed that the rate and direction of sodium transport in the small intestine may be regulated by the activity of Na,K-ATPase in the basolateral cell membrane. We tested this hypothesis by selectively infusing ouabain, a known inhibitor of Na,K-ATPase, into the mesenteric artery supplying a perfused loop of ileum in 18 dogs. Before ouabain infusion there were significant correlations between the activity of Na,K-ATPase and net and lumen to plasma fluxes of sodium and chloride. After ouabain, there was no significant change in sodium and chloride transport, unidirectional fluxes or transmucosal potential difference, despite a 50% reduction in Na,K-ATPase activity. Furthermore, there was no significant correlation between Na,K-ATPase activity and sodium or chloride transport after ouabain. The only statistically significant effect of ouabain infusion was a reduction in the rate of bicarbonate secretion. Thus, the results of our experiments suggest that mucosal Na,K-ATPase is not a rate-limiting step in the absorption of sodium and chloride in the dog ileum, though it may be an important facilitative factor.

The normal small intestine *in vivo* usually absorbs physiological electrolyte solutions, although the rate of absorption is highly variable from subject to subject, and in the same subject on different days (Turnberg *et al.*, 1970). In some apparently normal people and animals, the small intestine even secretes fluid, rather than absorbs it (Florey *et al.*, 1941; Scott, 1965; Turnberg *et al.*, 1970). In some animals, secretion is more common than absorption (Powell *et al.*, 1968).

One of the plausible explanations of variable rates of absorption-secretion is the activity of the basolateral membrane concentration of sodium and potassium-dependent ATPase (Na,K-ATPase) (Schultz *et al.*, 1974). This enzyme is believed to be the biochemical counterpart of a sodium pump, which transports sodium in exchange for potassium across the basolateral membrane (Hokin, 1976). It is postulated that the Na,K-ATPase pumps more sodium out of the cell than potassium into the cell (Glynn *et al.*, 1971; Rose and Schultz, 1971). Thus, this ATPase is thought (1) to cause a net trans-epithelial absorption of cations against an electrochemical gradient; (2) to cause the cellular concen-

tration of sodium to be low and the cellular concentration of potassium to be high; and (3) to generate an electrical gradient (mucosa negative to serosa) across the intestinal epithelium (Koefoed-Johnsen and Ussing, 1958; Schultz *et al.*, 1974).

That the activity of this enzyme in the basolateral membrane might control the rate of intestinal absorption is suggested by several recent experiments. For example, the increase in sodium and water absorption in rat ileum produced by glucocorticoids is associated with increased activity of Na,K-ATPase (Charney *et al.*, 1975; Charney and Donowitz, 1976). Furthermore, the increased sodium absorption and the raised transmucosal potential difference (PD) in the rat colon after corticosteroids are associated with an increase in the activity of Na,K-ATPase (Thompson and Edmonds, 1974; Charney *et al.*, 1975).

If this enzyme is an important regulator of intestinal absorption, as these experiments suggest, then it follows that a reduction in its activity should be associated with a reduced rate of sodium absorption. Therefore, we designed experiments to study the effects of ouabain, an inhibitor of Na,K-ATPase, on the transport of electrolytes, the transmucosal PD, and the activity, of Na,K-ATPase in the dog ileum *in vivo*. To avoid systemic toxicity, ouabain was infused directly into the artery supplying the ileal loop. An abstract of this work has been presented to the

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British Society of Gastroenterology (Read *et al.*, 1978).

Methods

CONSTRUCTION OF LOOPS

Experiments were carried out in 18 healthy, dewormed mongrel dogs weighing between 15 and 30 kg. The animals were fasted for 24 hours before surgery. Anaesthesia was administered with intravenous sodium pentobarbital, and respiration was controlled by means of a large volume animal respirator (model 613, Harvard Apparatus, Millis, Mass.). Laparotomy was performed, and a loop of ileum 25 cm in length was isolated between 15 and 40 cm proximal to the ileocaecal valve. Teflon spools, attached to wide bore polyethylene tubing (inside diameter = 4 mm), were tied in place at the ends of the loop (Fig. 1). The gut adjacent to the loop was resected for at least 50 cm proximally and as far as the ascending colon distally. The remaining small intestine was drained externally through a Teflon spool and polyethylene tubing, while the proximal colon was closed.

Fig. 1 Experimental design for in vivo perfusion of a loop of dog ileum and measurement of transmembrane potential difference (PD). Ouabain was infused via a fine catheter inserted into the main artery supplying the loop.

For selective arterial infusion of ouabain into the ileal loop, a fine polyethylene catheter (inside diameter = 0.011 mm) was inserted through a distal arterial radical and advanced proximally until its tip was situated in the main trunk supplying the loop. The position of the catheter was ascertained by infusing bromosulphthalein (BSP 35 mg/ml) at a constant rate (0.76 ml/minute) by means of a Harvard pump (model 931, Harvard Apparatus, Millis, Mass.), and estimating the BSP concentration in samples taken from arteries (that had supplied the resected bowel) immediately proximal and distal to the loop, as well as from arteries supplying the remaining small bowel and ascending colon and from the femoral artery and femoral vein. If necessary, the position of the catheter was adjusted so that the concentration of BSP was high in the arteries immediately adjacent to the loop, but remained very low at the other sites. (The dilution of BSP reaching the arterial radicals immediately adjacent to the loop was also used to calculate the dose of ouabain reaching the loop.) Thereafter, the arterial catheter was used to infuse saline (154 mM NaCl) or a solution of ouabain in saline.

EXPERIMENTAL PROTOCOL

In all experiments, 175 ml of an electrolyte solution ($\text{Na}^+ = 135 \text{ mM}$, $\text{K}^+ = 5 \text{ mM}$, $\text{Cl}^- = 115 \text{ mM}$, and $\text{HCO}_3^- = 25 \text{ mM}$, polyethylene glycol = 5 g/l, $^{22}\text{Na} = 1 \mu\text{Ci/l}$, $^{36}\text{Cl} = 1 \mu\text{Ci/l}$, pH = 7.4, and osmolality = 275 mosmol/kg) bubbled with a mixture of 95% O_2 and 5% CO_2 (yielding a pCO_2 of about 40 mm Hg) was continuously recirculated (16 ml/min) through the loop from glass reservoirs (maintained at 38°C) by means of a peristaltic pump (Desaga, Heidelberg). Intraluminal pressure in the loops was kept constant between 4 and 6 cm of H_2O by adjusting the fluid level in the reservoir (Fig. 1). After an equilibration period of 15 minutes, an initial 12-ml sample of perfusate was removed for analysis. The perfusion was continued thereafter for a period of one hour before a final sample was withdrawn, and the loop was then drained. After the initial control period, ouabain was infused into the mesenteric artery at a constant rate for one hour, during which the loop was perfused continuously with the electrolyte solution. After ouabain infusion, a second one-hour perfusion period was carried out. Full thickness biopsies (1.5 cm $\times$ 1.5 cm) were taken after the control period, after ouabain infusion and after the second perfusion period. After each biopsy, the intestinal defect was repaired by everting the mucosa and suturing the cut ends together. Haemostasis was secured before continuing the study.

In all experiments, arterial pH and pCO_2 were

monitored hourly, while serum concentrations of Na^+ , K^+ , and Cl^- were determined in blood drawn at the beginning and end of each period. The ECG, arterial blood pressure and rectal temperature were monitored continuously. In order to prevent plasma volume depletion, sufficient Ringer's lactate solution was infused intravenously to maintain a diuresis of 20–40 ml/hour. A thermal blanket and a heating lamp were used to maintain body temperature at 38°C.

MEASUREMENT OF THE TRANSMUCOSAL POTENTIAL DIFFERENCE

The transmucosal PD was recorded using the method described previously (Read *et al.*, 1977). Briefly, the perfusate itself acted as a flowing intraluminal electrode, while the reference electrode was the intravenous infusion of Ringer's lactate. These electrodes were connected *via* salt agar bridges (2 M KCl in 3% agar) and balanced calomel half cells to the input terminals of a battery powered electrometer (model 602, Keithley, Cleveland, Ohio), the output of which was displayed on a chart recorder (model B-261, Rikadenki Kogyo Co., Tokyo, Japan). The asymmetry of the recording system was determined at the beginning and end of each experiment and never exceeded 1 mV.

ANALYSIS OF PERFUSATE

Samples were analysed for polyethylene glycol (PEG) and electrolytes (Cooper *et al.*, 1966), and the data were then used to calculate net electrolyte movements using methods previously described (Fordtran, 1966; Krejs *et al.*, 1977). The total recovery of PEG was $97 \pm 1\%$ during the control period and $98 \pm 1\%$ after ouabain. Lumen to plasma unidirectional fluxes of Na^+ and Cl^- were calculated using the equation described by Berger and Steele (1958). Samples of perfusate were also analysed for protein (Lowry *et al.*, 1951), and the output of protein during each period was calculated and used as an index of mucosal integrity.

For statistical analysis, the results from the two perfusion periods were compared using the paired *t* test.

ANALYSIS OF BIOPSY SAMPLES

Each specimen was washed thoroughly in ice cold isotonic saline and then scraped with a glass slide to remove the mucosa, which was then washed in ice cold saline and divided into two equal portions (120–220 mg). These were immersed briefly in ice cold buffer solution (pH 6.8) containing 130 mM NaCl, 5 mM Na_2EDTA and 30 mM imidazole before being stored at -70°C . The samples were subsequently thawed and analysed for Na,K, and Mg-ATPase activities by the method described by Charney *et al.*

(1974).

Briefly, the mucosal scrapings were homogenised by hand using iced Tenbroeck homogenisers filled with 4 ml of the imidazole Na_2EDTA buffer solution containing 2.4 mM sodium deoxycholate. The membrane-rich pellet obtained after successive centrifugations at 770 *g* and 10 000 *g* for 10 minutes at 0°C was suspended in the homogenising solution without sodium deoxycholate. ATP hydrolysis was measured by incubating an aliquot of suspension containing approximately 100 μg protein (Lowry *et al.*, 1951) for 15 minutes at 37°C in a solution containing 100 mM NaCl, 20 mM KCl, 10 mM imidazole, 5.4 mM MgCl_2 , and 5.4 mM disodium ATP (pH 7.6) and in a similar solution in which the KCl was omitted but the NaCl concentration was 120 mM. The inorganic phosphate liberated was measured spectrophotometrically (Taussky and Shorr, 1953), and the enzyme activity was expressed as micromoles of inorganic phosphate (P_i) liberated per mg protein per hour after a correction was made for nonenzymatic hydrolysis determined in blank tubes containing no tissue extract. The activity of Na,K-ATPase was calculated as the difference in activity between potassium-containing reactions (representing combined Na,K-ATPase, and Mg-ATPase) and potassium-free reactions (representing only Mg-ATPase activity).

Experiments were performed to compare activities of biopsies prepared from fresh tissue and assayed immediately with those of biopsies obtained at the same time, but then frozen for varying periods. Our results showed that biopsies could be preserved up to 120 days with no significant loss of Na,K-ATPase activity. As it was sometimes inconvenient to assay Na,K-ATPase on the same day, we routinely ran the assay 24–72 hours after each experiment.

Results

DATA OBTAINED DURING CONTROL PERIOD

Transport rates

Twelve of the 18 dogs absorbed sodium, while six secreted (Fig. 2). The mean result was absorption of $17 \mu\text{mol cm}^{-1} \text{h}^{-1}$ (Table). Data for other measured electrolytes and for the unidirectional sodium and chloride fluxes are given in Fig. 2 and the Table.

CORRELATION OF TRANSPORT RATE WITH Na,K-ATPASE

There was a significant correlation between the Na,K-ATPase activity and the net movement of sodium during the control period ($P < 0.001$, $r = 0.79$). As shown in Fig. 3, at activities above 20 units there was net absorption of sodium, whereas

Fig. 2 The distribution of the net transport rates for sodium, chloride, and bicarbonate from the ileum of 18 dogs during the control period. For conversion to SI units, $1 \mu\text{Eq}/\text{cm}/\text{h} = 1 \mu\text{mol cm}^{-1} \text{h}^{-1}$.

Fig. 3 Net sodium movement in each ileal loop during the control period plotted against mucosal Na,K-ATPase activity ($\mu\text{mol P}_i$ liberated/ $\text{mg protein}^{-1}\text{h}^{-1}$). Absorption is above the broken line and secretion below. The distribution of points suggests a significant correlation ($r = 0.79$, $P < 0.001$, $n = 18$). For conversion to SI units, $1 \mu\text{Eq}/\text{cm}/\text{h} = 1 \mu\text{mol cm}^{-1} \text{h}^{-1}$.

Table Experimental data obtained during control period and after ouabain infusion (mean $\pm$ SEM)

	Control	Post-ouabain	P
Na-K ATPase activity ($\mu\text{mol P}_i$ liberated $\text{mg protein}^{-1}\text{h}^{-1}$)	23 ± 1	12 ± 1	<0.001
Mg ATPase activity ($\mu\text{mol P}_i$ liberated $\text{mg protein}^{-1}\text{h}^{-1}$)	17 ± 1	16 ± 1	>0.05
Na (net) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	-17 ± 23	-5 ± 19	>0.6
Na (L $\rightarrow$ P) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	133 ± 18	144 ± 26	>0.6
Na (P $\rightarrow$ L) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	116 ± 15	139 ± 18	>0.3
K (net) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	$+1 \pm 1$	$+3 \pm 1$	>0.1
Cl (net) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	-50 ± 17	-22 ± 17	>0.1
Cl (L $\rightarrow$ P) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	104 ± 15	118 ± 18	>0.4
Cl (P $\rightarrow$ L) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	54 ± 9	96 ± 16	>0.05
HCO ₃ (net) ($\mu\text{mol cm}^{-1}\text{h}^{-1}$)	$+29 \pm 7$	$+5 \pm 4$	<0.01
Protein leakage ($\text{mg cm}^{-1}\text{h}^{-1}$)	1.4 ± 0.3	2.3 ± 0.6	>0.1
PD (mV)	-0.2 ± 0.6	-0.3 ± 0.7	>0.1
Plasma electrolyte concentration			
Na (mmol l^{-1})	153 ± 1	152 ± 1	>0.6
Cl (mmol l^{-1})	114 ± 2	119 ± 2	<0.02
K (mmol l^{-1})	4.3 ± 0.2	5.4 ± 0.2	<0.001
Lumen electrolyte concentration			
Na (mmol l^{-1})	140 ± 1	140 ± 1	>0.7
Cl (mmol l^{-1})	111 ± 1	114 ± 1	<0.001
K (mmol l^{-1})	5.4 ± 0.1	5.8 ± 0.1	<0.001

For the net transport data, positive values indicate secretion, negative values absorption. The polarity of the PD is that of the lumen with respect to blood. The electrolyte concentrations are the mean of values obtained before and after each perfusion period. The P values in the last column indicate the probability that the observed difference between data obtained during the control period and data obtained after ouabain infusion was due to chance alone. Statistically significant differences (95% confidence limits) are in *italics*.

below this value there was net sodium secretion. There were also significant correlations between Na,K-ATPase activity and net transport of chloride ($P < 0.001$, $r = 0.81$), bicarbonate ($P < 0.01$, $r = 0.57$), and potassium ($P < 0.005$, $r = 0.66$). Na,K-ATPase activity was correlated with the unidirectional lumen to plasma flux of sodium ($P < 0.001$, $r = 0.71$) and chloride ($P < 0.001$, $r = 0.72$), but not with the opposing plasma to lumen fluxes. There was no correlation between Na,K-ATPase activity and transmucosal PD.

There was no correlation between net transport or unidirectional fluxes of any measured electrolyte and the activity of Mg-ATPase.

EFFECT OF OUABAIN

Preliminary experiments

Ouabain was infused in three consecutive doses (10, 30, and 50 $\mu\text{g/kg/h}$) into the artery supplying the ileal loop in three dogs. Each infusion lasted one hour and was separated from the next infusion by a gap of one hour, during which saline was infused into the mesenteric artery. Biopsies were taken for ATPase activity before, immediately after, and one hour after each ouabain infusion was discontinued. A dose of 10 $\mu\text{g/kg/h}$ produced a decline in Na,K-ATPase activity, but this recovered 60 minutes after the infusion. With 30 $\mu\text{g/kg/h}$, the activity of Na,K-ATPase fell but did not recover, while with 50 $\mu\text{g/kg/h}$ two of the dogs died shortly after the infusion as a

result of cardiac arrhythmias. For all additional experiments, we used a dose of 30 $\mu\text{g/kg/h}$.

To decide when to carry out the test perfusion, we determined the Na,K-ATPase activity in biopsies taken at short intervals after starting the ouabain infusion in two dogs. In both animals there was an initial increase in Na,K-ATPase activity. After 30 minutes this fell below the initial value, reaching its lowest activity at one hour (Fig. 4). The activity of Mg-ATPase did not show any consistent decline during this period (Fig. 4).

Because Na,K-ATPase activity was not stable at any time during ouabain infusion, the 30- to 90-minute period after the completion of ouabain infusion was selected as the test period for measurement of ion transport.

Results after ouabain infusion

After infusion of ouabain (30 $\mu\text{g/kg/h}$), the activity of Na,K-ATPase was reduced to 50% of the control value ($P < 0.001$) and remained at this level for at least three hours (Fig. 5). The activity of Mg-ATPase was unchanged by ouabain (Fig. 5).

Despite the profound and sustained reduction of Na,K-ATPase, there was no significant change in the net or unidirectional lumen to plasma flux of sodium and chloride (Fig. 6 and the Table). This was true whether all animals were considered or whether only dogs who absorbed in the control period were considered. As shown in Fig. 6, the distribution of net absorption-secretion rates of sodium and chloride

Fig. 4 Change in activity of Na,K-ATPase (left) and Mg-ATPase (right) ($\mu\text{mol P}_i \text{ liberated/mg protein}^{-1} \text{ h}^{-1}$) in two dogs during infusion of 30 $\mu\text{g/kg}$ ouabain over a period of one hour. Biopsies were taken before and at 1, 3, 5, 10, 15, 20, 30, and 60 minutes after ouabain infusion.

Fig. 5 The activity of Na,K-ATPase and Mg-ATPase ($\mu\text{mol P}_i$ liberated/mg protein⁻¹ h⁻¹) before and after infusion of ouabain ($30 \mu\text{g/kg}^{-1} \text{ h}^{-1}$). Results are mean $\pm$ SEM of 18 dogs.

Fig. 6 A comparison of the distribution of the net transport rates for sodium, chloride, and bicarbonate from the ileum of 18 dogs during the control period (solid symbols) and in the test period after ouabain infusion (open symbols). For conversion to SI units, $1 \mu\text{Eq/cm/h} = 1 \mu\text{mol cm}^{-1} \text{ h}^{-1}$.

were similar to those observed in the control period. However, ouabain infusion did result in a significant reduction in bicarbonate secretion ($P < 0.01$), as shown in the Table.

There was no significant difference between the PD during the perfusion period after ouabain compared with that recorded during the control period (Table). There was no significant change in the protein output from the loops before and after ouabain (Table), suggesting that the perfusion data after ouabain were not affected by any nonspecific toxic or ischaemic damage to the mucosa.

The mean concentration of ouabain reaching the

loop was estimated indirectly by dividing the concentration of ouabain infused by a dilution factor, obtained by infusing BSP at a known concentration (35 mg/ml) and measuring its concentration in the arterial blood supplying the loop. Thus, assuming that there was no disappearance of ouabain or BSP from the plasma in the precapillary vascular bed we estimated that when ouabain was infused at a rate of $30 \mu\text{g/kg/h}$ the mean concentration reaching the loop was $3 \times 10^{-7} \text{ M}$ (range 2 to $9 \times 10^{-7} \text{ M}$).

We could not detect any significant alteration in pulse rate, blood pressure, or ECG during or after local arterial infusion of ouabain. The pH and sodium

concentration in peripheral blood also remained constant, although there were slight rises in plasma concentrations of potassium and chloride (Table) after ouabain infusion.

Correlation of transport rates with Na,K-ATPase after ouabain infusion

Unlike the findings in the control period, there were no significant correlations between Na,K-ATPase activity and net transport of sodium (Fig. 7), chloride, bicarbonate, or potassium after ouabain infusion. Inspection of Figs 3 and 7 shows that, in the control period, activities of Na,K-ATPase below 20 were associated with secretion of sodium, while, after ouabain, activities below 20 accompanied absorption of sodium as often as sodium secretion. In several dogs, relatively high rates of sodium absorption occurred in spite of relatively low concentrations of Na,K-ATPase, and, in all instances where sodium was absorbed, the absorption was against both electrical and chemical gradients.

Fig. 7 Net sodium movement during the perfusion period after ouabain infusion plotted against mucosal activity of Na,K-ATPase ($\mu\text{mol P}_i$ liberated/mg protein $\cdot$ h $^{-1}$) in ileal loops from 18 dogs. Absorption is above the broken line, while secretion is below. Linear regression analysis revealed a correlation coefficient of 0.39 ($P > 0.05$). For conversion to SI units, 1 $\mu\text{Eq/cm/h} = 1 \mu\text{mol cm}^{-1} \text{h}^{-1}$.

There were no correlations in the post-ouabain period between Na,K-ATPase and the unidirectional fluxes of sodium or chloride.

Discussion

The results obtained in the control period demonstrate the problem which prompted these experiments. The ileal loops of these normal animals displayed a wide range of transport activity. Two-thirds of the animals absorbed sodium, and the rest secreted. The primary purpose of our experiments was to seek evidence bearing on the hypothesis (Charney and Donowitz, 1976) that the rate and direction of sodium movement in the intestine are determined by the activity of Na,K-ATPase in the basolateral cell membrane.

The data obtained in the control period show a significant correlation between the activity of Na,K-ATPase and absorption of sodium and chloride. This is consistent with the hypothesis that Na,K-ATPase is a rate-limiting step in the absorption of salt from the ileum. Furthermore, since secretion was noted when the mucosal Na,K-ATPase activity was less than 20, these results are also consistent with the notion that observed net transport rate is the sum of an absorptive function (usually ascribed to villous cells) and a secretory function (usually ascribed to the crypts) (Hendrix and Bayless, 1970). For example, it might be speculated from our control data that villous absorption is progressively reduced toward zero as ATPase falls, and that crypt secretion exceeds villous absorption when mucosal ATPase falls below 20, resulting in an overall secretory mucosa.

However, before accepting a cause and effect relationship between mucosal Na,K-ATPase activity and net transport rate of sodium and chloride, it needs to be shown that an imposed reduction in Na,K-ATPase is associated with a fall in absorption rate or an enhanced secretion rate. To test this, we studied the changes in electrolyte transport caused by deliberately reducing the activity of Na,K-ATPase with ouabain. To avoid systemic toxicity, ouabain was administered selectively into an artery supplying the ileal loop in a dose which caused significant reduction of Na,K-ATPase activity without causing obvious damage to the loop or cardiac arrhythmias. Using this method, we found that the activity of Na,K-ATPase was significantly and selectively reduced from a mean of 23 units to a value of 12 units after ouabain infusion. According to the relationship observed in the control period, such a reduction in Na,K-ATPase activity should be associated with profound secretion of sodium if Na,K-ATPase were the rate-limiting factor for sodium absorption. However, there was still a mean

net sodium and chloride absorption from the ileal loops during the post-ouabain period, and the individual data showed a similar distribution of absorption-secretion rates to that in the control period (Fig. 6). The mean unidirectional sodium and chloride fluxes out of the lumen were also unchanged after ouabain infusion. Nine of the 18 ileal loops absorbed sodium in the post-ouabain period in spite of the reduction in Na,K-ATPase, and in each instance sodium absorption occurred against an electrochemical gradient. Moreover, unlike what obtained in the control period, there was no correlation after ouabain infusion between Na,K-ATPase activity and net or lumen to plasma unidirectional flux of sodium or chloride.

Experiments *in vitro* (Schultz and Zalusky, 1964) have shown that inhibition of sodium transport by ouabain is accompanied by a decrease in PD (lumen becoming less negative). In our present experiments, the PD was the same after ouabain infusion as it was before. Although changes in PD can arise from mechanisms other than electrogenic sodium absorption, these results nevertheless support our conclusion that ouabain did not decrease the activity of the transepithelial sodium pump.

Infusion of ouabain into the mesenteric artery in similar doses to those used in the present study has been shown to cause a reduction of mesenteric blood flow (Pawlik *et al.*, 1974). Although we observed a slight pallor of the ileal loop during ouabain infusion (consistent with some reduction in blood flow), this had recovered completely by the beginning of the test perfusion; and there was no significant increase in protein output, which suggests that ouabain did not cause ischaemic damage to the mucosa. Moreover, it seems unlikely that our failure to show an effect of ouabain on electrolyte transport could be related to a transient decrease in blood flow, since a period of ischaemia in the canine small intestine is immediately followed by profound secretion of fluid and electrolytes (Mirkovitch and Menge, 1976). Indeed, it is remarkable that, despite the possibility of ischaemia together with considerable reduction in the activity of Na,K-ATPase, the ileal epithelium of half of the dogs absorbed sodium after ouabain infusion.

If we assume that a possible decrease in blood flow did not somehow facilitate absorption, then the only way to explain our findings that is still compatible with a rate-limiting role for Na,K-ATPase is by invoking two Na,K-ATPases, one subserving secretion and the other absorption. The inhibition of cholera-induced secretion by ouabain (Field, 1976) suggests the existence of a secretory Na,K-ATPase. If ouabain inhibits both absorption and secretory Na,K-ATPases *in vivo*, then the expected mean result in a number of loops would be little or no change in

net sodium transport, but a significant reduction in the mean values for both unidirectional fluxes of sodium. In our experiments, however, there was no reduction in either the observed unidirectional flux of sodium out of the lumen or the calculated flux of sodium into the lumen. Thus, it is unlikely that the absence of a change in the net sodium transport after ouabain infusion can be explained by suppression of opposing Na,K-ATPases.

The only significant alteration in ion fluxes we observed after ouabain infusion was a reduction in bicarbonate secretion. The mechanism underlying this observation is open to speculation, but it might be somehow mediated by a change in intracellular sodium or potassium concentration.

In our opinion, the results of these experiments suggest that the mucosal activity of Na,K-ATPase is not a rate-limiting step in the absorption of sodium and chloride in the dog ileum. Our studies do not conclusively disprove such a role for this enzyme, since there may be several Na,K-ATPases within the intestinal epithelium, whereas only the basolateral membrane ATPase of the enterocyte is believed to be rate-limiting for salt absorption. However, we used essentially the same ATPase assay as previous workers whose results support the concept that Na,K-ATPase activity controls the rate of sodium absorption in the small intestine (Charney *et al.*, 1975; Charney and Donowitz, 1976). Furthermore, most experts agree that the Na,K-ATPase method used in this experiment does in fact measure mainly the enzyme concentration of the basolateral membranes (Douglas *et al.*, 1972; Fujita *et al.*, 1972; Charney *et al.*, 1974). It is possible, of course, that ouabain binds to sites other than Na,K-ATPase during infusion *in vivo* and inhibits the enzyme only later during the homogenisation procedure. Although this could explain the lack of change in sodium transport during ouabain infusion, it seems an unlikely explanation, because ouabain entering the epithelial layer during infusion would be much more avidly and irreversibly bound to ATPase than to any other site. Moreover, if this were a valid criticism of our results, it would also apply to all other studies involving ouabain inhibition of ATPase, *in vitro* as well as *in vivo*.

Our conclusions are compatible with the recent experiments of Nellans and Schultz (1976), whose results suggest that *in vitro* the sodium-potassium exchange mechanism at the basolateral membrane functions independently of transepithelial sodium transport. Also, our data do not conflict with the observation that ouabain applied to *in vitro* gut preparations will inhibit sodium transport (Schultz and Zalusky, 1964), since the epithelium would appear to be exposed to a much higher ouabain concen-

tration in the *in vitro* experiments than in the present study (2×10^{-4} vs. 3×10^{-7} M). For example, if there was a reduction in Na,K-ATPase of 100% *in vitro* but only 50% *in vivo*, the possible discrepancy would be resolved by postulating that Na,K-ATPase is an important facilitative factor for sodium absorption but not the rate-limiting step. Unfortunately, we are unaware of any *in vitro* study which measured the effect of ouabain on both transport and Na,K-ATPase activity concomitantly in intestinal mucosa.

Our results are in disagreement with a recent report that ouabain applied to the luminal surface of the mucosa reduced mucosal Na,K-ATPase by 32% and reduced absorption or caused secretion in the rabbit ileum *in vivo* (Charney and Donowitz, 1978). The explanation for this apparent discrepancy is unknown, but may be related either to the very high luminal concentrations of ouabain (19.2 mM) used in that study, or to the possibility that different enzyme or transport systems may be affected when ouabain is delivered to the luminal rather than the serosal side of the epithelium, or to species differences.

If our conclusion that Na,K-ATPase is not a rate-limiting step for sodium and chloride absorption is correct, then the relationship between Na,K-ATPase activity and transport rate observed in the control period must either indicate that the sodium transport rate determines the level of basal Na,K-ATPase activity instead of the reverse, or that both sodium transport and basal Na,K-ATPase activity are regulated by some other, as yet undetermined, factor. By the same token, if our conclusions are correct, the rise in absorption rate after glucocorticoid hormones (Charney *et al.*, 1975; Charney and Donowitz, 1976) cannot be taken as definitive evidence that Na,K-ATPase controls the rate of sodium absorption. In this respect, it is interesting that two groups of workers have observed that corticosteroids increased sodium transport and PD in the rat colon at a time when there was no detectable increase of Na,K-ATPase activity (Thompson and Edmonds, 1974; Binder, 1978).

It should be emphasised that our results do not argue against a role of Na,K-ATPase in mediating the coupled transport of sodium and potassium across the cell membrane (Hokin, 1976). Neither do they contradict the possibility that Na,K-ATPase may have an important or even a critical facilitative role in active absorption of sodium. The data do suggest that Na,K-ATPase is not the rate-limiting step for transepithelial sodium transport in dog ileum during perfusion of a balanced electrolyte solution.

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