Biochimica et Biophysica Acta 901 (1987) 209–216
Elsevier

BBA 73597

Na^+, K^+ and Cl^- transport in isolated small intestinal cells from guinea pig. Evidences for the existence of a second Na^+ pump

Jesús R. Del Castillo and Guillermo Whittembury

Instituto Venezolano de Investigaciones Cientificas (IVIC), Centro de Biofisica y Bioquímica, Caracas (Venezuela)

(Received 20 March 1987)

Key words: Ion transport; Sodium ion transport; Sodium pump, second; Epithelial cell; Small intestine; (Guinea-pig)

Isolated small intestinal epithelial cells, after incubation at 4°C for 30 min, reach ion concentrations (36 mM K^+, 113 mM Na^+ and 110 mM Cl^-) very similar to those of the incubation medium. Upon rewarming to 37°C, cells are able to extrude Na^+, Cl^- and water and to gain K^+. Na^+ extrusion is performed by two active mechanisms. The first mechanism, transporting Na^+ by exchanging it for K^+, is inhibited by ouabain and is insensitive to ethacrynic acid. It is the classical Na^+ pump. The second mechanism transports Na^+ with Cl^- and water, is insensitive to ouabain but is inhibited by ethacrynic acid. Both mechanisms are inhibited by dinitrophenol and anoxia. The second Na^+ extruding mechanism could be the $Na^+/K^+/2Cl^-$ cotransport system. However, this possibility can be ruled out because the force driving cotransport would work inwards, and because Na^+ extrusion with water loss continues after substitution of Cl^- by NO_3^-. We propose that enterocytes have a second Na^+ pump, similar to that proposed in proximal tubular cells.

Introduction

In the small intestine, the transcellular movement of sodium is known to depend on cellular energy and to involve the participation of carriers. Sodium ion may enter the enterocyte following its electrochemical gradient in three ways. (a) As an electrogenic movement of sodium ions across the apical pole of the cell, without any direct coupling to the movement of other solutes [1,2]. In this case, the sodium movement would be associated with a passive absorption of chloride. (b) Coupled with the entry of a large variety of organic molecules [3,4] and (c), coupled to the movement of Cl^- across the brush-border membrane [5].

Sodium ions are pumped out of the epithelial cell across the basolalteral membrane against an electrochemical gradient by a process that requires the supply of energy. This energy is derived from the hydrolysis of ATP. There exists at this site at least one enzyme responsible for such hydrolysis, namely the $(Na^+ + K^+)$-ATPase.

There are several indications that the active transport of sodium across the basolateral plasma membrane of the intestinal epithelial cell is not uniquely dependent on the Na^+–K^+ exchange pump. When intracellular sodium is depleted and its transepithelial movement is abolished by removal of this cation from the mucosal medium, there is no change in the intracellular concentration of potassium nor in cell water, and the *trans*-serosal flulx of potassium is unaltered [6]. This observation indicates that the transepithelial fluxes of sodium and potassium entry into the cell from the serosal side are not tightly coupled and that the transepithelial transport of sodium, as well as the regulation of cell water, are not entirely dependent on Na^+–K^+ exchanges.

Correspondence: J.R. del Castillo, IVIC, Centro de Biofisica y Bioquímica, P.O. Box 21827, Caracas 1020A, Venezuela.

In addition, it is known that solutes such as D-glucose and L-alanine strongly enhance the transcellular movement of sodium by stimulating the entry of the cation across the apical pole of the cell [7]. When the effect of these organic solutes on potassium exchange kinetics is examined, it is found that they do not influence the rate of exchange of $^{42}K^+$ across the basolateral membrane [6]. These observations agree with the findings of Lee and Armstrong [8] who determined the intracellular activities of sodium and potassium in bullfrog small intestine using cation-selective microelectrodes and observed that in the presence of 3-*O*-methylglucose, the ion activities were significantly reduced, despite the stimulation of transcellular sodium transport caused by this sugar. If there existed an absolute relationship between the transport of sodium and Na^+–K^+ exchange, an increase in cell potassium would be predicted, if the leak of K^+ out of the cells does not increase. Indeed, these observations have been confirmed with isolated cells [9] where it was found that non-metabolizable hexoses elicit no rise in cell potassium.

Recently, we have found that basolateral plasma membrane vesicles from enterocytes possess two distinct Mg^{2+}-ATP-dependent Na^+ transport mechanisms. One transports sodium actively in the absence of potassium, is insensitive to ouabain, it is inhibited by ethacrynic acid and furosemide. In this study experimental conditions were used which ruled out the involvement of the cation/Cl^- cotransport system in the movement of Na^+ just described. The second Na^+ trnasport mechanism requires potassium, is strongly inhibited by ouabain, weakly inhibited by ethacrynic acid and is insensitive to furosemide [10]. These transport mechanisms have been found associated with two different ($Mg^{2+} + Na^+$)-dependent ATPases [11]. Small intestinal basolateral plasma membranes have an ATPase activity stimulated by the simultaneous addition of sodium and potassium, which is inhibited by ouabain and to a small extent by ethacrynic acid, but is refractory to furosemide. This corresponds to the classical ($Na^+ + K^+$)-ATPase [11]. In addition, another enzyme activity has been characterised. It is stimulated by sodium alone (it does not require potassium), is insensitive to ouabain, but is inhibited by ethacrynic acid and furosemide [11]. The possible existence of these additional mechanism has not been directly tested in the intestinal epithelium. To investigate whether a second sodium transport mechanism exists in addition to the Na^+/K^+ pump in the small intestinal mucosa, we decided to use isolated enterocytes.

This preparation is free of inconveniences related to diffusion delays in the intestinal space which can result in incomplete access of transport inhibitors to their sites of action. For examples, persistance of sodium extrusion in the presence of ouabain could be due to limited accessibility of ouabain for its sites of action when whole intestine, or rings or mucosal sheets are used. We decided then to study net ion movements between cells and bathing medium trying to differentiate whether the Na^+ movements described above can be characterized. Due to the ease of ion substitution in the isolated cell preparation conditions were used which allowed to sort out whether the $K^+/Na^+/2Cl^-$ cotransport system could explain part of the experimental results.

In the present work, we demonstrate the existence of two distinct Na^+-transport mechanisms unrelated to a cation/Cl^- cotransport system.

Part of this work was presented at the 30th Congress of International Union of Physiological Sciences, in Vancouver, Canada (1986).

Material and Methods

Materials. ^3H_2O and [^{14}C]inulin were purchased from New England Nuclear (Boston). Dulbecco's modified Eagle's medium and ouabain from Sigma Chemical Co. (St. Louis). Ethacrynic acid from Merck, Sharp and Dohme (Haarlem). Di-*n*-butylphthalate and di-*n*-nonylphthalate from Merck (Darmstadt). All other chemicals of analytical grade were obtained from Sigma Chemical Co. or Merck.

Preparative procedure for isolation of intestinal epithelial cells. Intestinal cells were prepared from 300–350 g guinea-pigs by the procedure described previously [12]. Briefly, the small intestine was excised from the duodenal flexure to the ileocecal junction and then rinsed, filled and incubated for 10 min at 37 °C with intracellular-like solution I (7 mM K_2SO_4, 44 mM K_2HPO_4, 9 mM $NaHCO_3$,

10 mM Hepes and 180 mM glucose (pH 7.4; 340 mosmol/l)). The luminal content was discarded and the intestinal segment was refilled and incubated for 3 min at 37 °C with intracellular-like solution II (0.5 mM dithiothreitol, 0.25 mM ethylenediaminotetraacetic acid (EDTA), 7 mM K_2SO_4, 44 mM K_2HPO_4, 9 mM $NaHCO_3$, 10 mM Hepes and 180 mM glucose (pH 7.4; 340 mosmol/l)). The intestine was then gently palpated and the luminal content, containing isolated cells, was collected on Dulbecco's modified Eagle's medium (100 ml) at 4 °C and then filtered through a nylon filter (60 μm pore diameter) and centrifuged twice at $100 \times g$ for 5 min. Isolated cells were resuspended in Dulbecco's modified Eagle's medium and stored at 4 °C. Dulbecco's modified Eagle's medium ion composition was modified to contain: 116 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM $NaHCO_3$, 1 mM NaH_2PO_4, 10 mM K_2SO_4, 10 mM Hepes (pH 7.4; 320 mosmol/l).

Centrifugal filtration. For analysis cells were separated from the incubation medium by centrifugation through an oil layer in a 400 μl microcentrifuge tube containing 50 μl of the mixture di-*n*-butylphthalate/ di-*n*-nonylphthalate (3 : 2 (v/v) density 1.019). Samples of cells containing 1–2 mg protein were placed on top of the oil layer. On centrifugation (20 s at $13000 \times g$) the cells were separated from the incubation medium by the oil layer. The supernatant was removed and the tubes were washed three times with distilled water. Then the oil was removed. Pellets were resuspended with 200 μl of water, they were strongly stirred for 3 min to lyse the cells and then 200 μl of 10% trichloroacetic acid was added to precipitate the proteins. The suspensions were centrifuged for 5 min at $13000 \times g$. Supernatants were used to determine intracellular contents. Pellets were resuspended with 1 M NaOH and cellular protein was determined by a modified Coomassie blue method [13].

Determination of intracellular water and trapped volume. Cells were exposed simultaneously to ^3H_2O, and [^{14}C]inulin, to measure intracellular water and trapped volume, respectively. The intracellular water was calculated as the difference between the total water content and the volume trapped in the pellet.

Cell electrolyte contents. Na^+, and K^+ contents, were meausred with a Zeiss PMQ II, flame photometer and Cl^- was mesured with a Buchler Digital Chloridometer. The amounts of sodium, potassium and chloride in the pellets were corrected for the sodium, potassium and chloride contents in the trapped volume, and were divided by the cellular water to obtain the intracellular concentration.

Oxygen consumption. Oxygen consumption was measured with a Clark electrode. Cells (1–2 mg cell protein) were incubated in 3 ml of incubation medium at 37 °C. Oxygen consumption was linear with time for at least 15 min. Experimental zero O_2 concentration in the chamber was obtained by adding sodium dithionite.

Results

Intracellular ion and water contents after cooling (4 °C) and on rewarming (37 °C)

At the end of the preparative procedure, the isolated cells were incubated for 30 min at 4 °C in Dulbecco's modified Eagle's medium and intracel-

TABLE I

INTRACELLULAR ION AND WATER CONTENTS OF ISOLATED SMALL INTESTINAL CELLS

Cells were collected in ice-cold Dulbecco's modified Eagle's medium at the end of the preparative procedure and washed twice by centrifugation. After this step, the cells were incubated for 30 min at 4 °C in the same medium. Part of the cell preparation was prepared for analysis (A). The rest was re-incubated at 37 °C for 30 min (B). Values presented are means ± S.E. of four experiments.

	Content (nmol/mg cell protein)	Concentration (mM)
At 4 °C (A)		
Na^+	714 ± 31.5	113
K^+	232 ± 17.2	36
Cl^-	697 ± 14.8	110
H_2O [a]	6.34 ± 0.097	–
At 37 °C (B)		
Na^+	163 ± 7	34
K^+	545 ± 12	114
Cl^-	435 ± 10	91
H_2O [a]	4.80 ± 0.16	–

[a] Water content expressed in μl/mg cell protein.

lular ion and water contents were determined. Cell have high Na^+ and Cl^- and low K^+ concentrations, similar to that of the incubation medium. However, upon rewarming to 37 °C in Dulbecco's modified Eagle's medium the cells extrude Na^+, Cl^- and water and gain K^+, reaching ion concentrations similar to those obtained 'in situ' [22]. These results are shown in Table I.

Fig. 1 shows the time course of cell ion contents. Na^+ and Cl^- are lost while K^+ is gained. Initial rates were estimated as suggested by Jacquez [14]. Mean rate of net flux for the first five timepoints were calculated and plotted as a function of the actual sampling time. These data were fitted with the equation $F = a_0 + a_1 t + a_2 t^2$ by standard regression techniques. The intercept, a_0 estimates the initial rate of ion movements. The values obtained were 98.6 ± 2.43 for Na^+, 45.4 ± 2.46 for K^+ and 51.3 ± 5.48 for Cl^- in nmol/mg cellular protein per min. The relationship between the initial fluxes for Na^+ and Cl^- extrusion and K^+ gain was about 2 : 1 : 1.

Effects of some inhibitors on Na^+, K^+, Cl^- and water transport in isolated small intestinal cells

Table II shows the effects of ouabain (1 mM) and ethacrynic acid (1.5 mM) on rewarming-induced ion and water movements in isolated enterocytes. Ouabain, a specific inhibitor of the Na^+/K^+ pump, only partially inhibited Na^+ extrusion, abolished K^+ gain and did not affect Cl^- and water movements.

Ethacrynic acid, a diuretic inhibitor of the proposed second Na^+ pump in the kidney [16], partially inhibited Na^+ extrusion, had no effect on K^+ gain and abolished Cl^- and water movements. The effects of ouabain and ethacrynic acid were additive. These results indicate the existence, in the enterocyte, of an ouabain-insensitive Na^+, Cl^- and water transport in addition to a ouabain-sensitive Na^+/K^+ transport.

When cells were rewarmed in the presence of dinitrophenol or in anoxia (which was achieved by using N_2 instead of O_2) both mechanisms were abolished as shown in Table III, indicating their dependence on cellular metabolism.

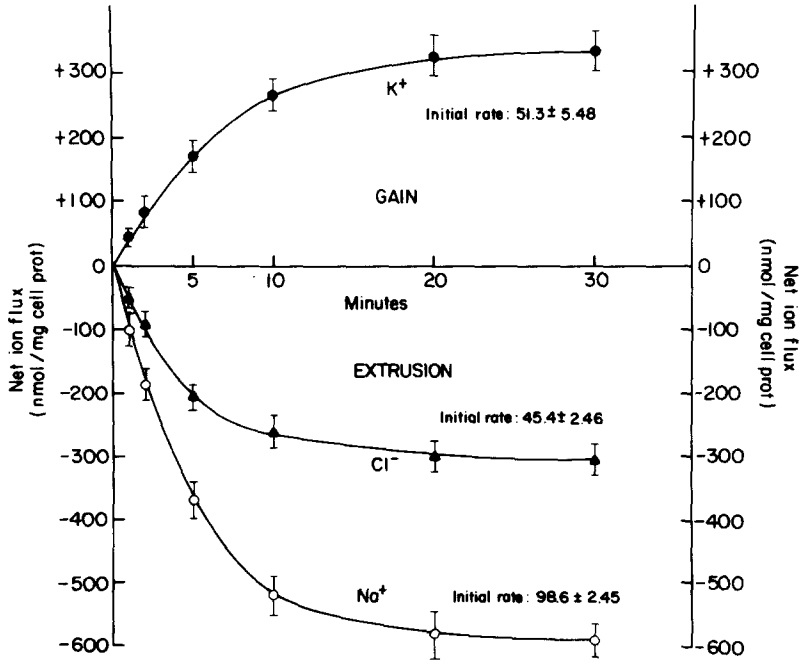

Fig. 1. Net ion movements in isolated small intestinal cells. Effect of rewarming (37 °C). Positive values indicate gain and negative ones loss. Initial rates are estimated as indicated in results. Values presented are means ± S.E. of four experiments

TABLE II

EFFECT OF OUABAIN (OU) (1 mM) AND ETHACRYNIC ACID (EA) (1.5 mM) ON INTRACELLULAR ION AND WATER CONTENTS OF ISOLATED SMALL INTESTINAL CELLS

Cells were preincubated at 4 °C for 30 min and then reincubated at 37 °C for 30 min. The differences, (B − A), (C − A), (D − A), and (E − A) are net ion movements. Values presented are means ± S.E. of four experiments.

Condition	Intracellular content			
	Ion (nmol/mg cell protein)			Water (μl/mg cell protein)
	Na^+	K^+	Cl^-	
A, 4 °C	739 ± 25	241 ± 15	720 ± 17	6.33 ± 0.03
B, 37 °C	155 ± 9	548 ± 17	415 ± 8	4.44 ± 0.09
C, 37 °C, + OU	405 ± 19	196 ± 9	426 ± 9	4.35 ± 0.06
D, 37 °C, + EA	480 ± 22	566 ± 20	741 ± 26	6.35 ± 0.07
E, 37 °C, + OU + EA	750 ± 30	217 ± 13	713 ± 20	6.48 ± 0.09
Rewarming				
(B − A)	−584	+307	−305	−1.89
+ OU (C − A)	−334	−45	−294	−1.98
+ EA (D − A)	−259	+325	+21	+0.02
+ OU + EA (E − A)	+11	−24	−7	+0.15

TABLE IV

OXYGEN CONSUMPTION OF ISOLATED SMALL INTESTINAL CELLS. EFFECT OF OUABAIN (1 mM) AND ETHACRYNIC ACID (1.5 mM)

Oxygen consumption was measured using a Clark oxygen electrode at 37 °C. Values presented are means ± S.E. of three experiments.

Conditions	Oxygen consumption (nmol/mg cell protein)
Control	11.7 ± 1.06
+ ouabain	5.9 ± 0.60
+ ethacrynic acid	5.3 ± 0.43
+ ouabain + ethacrynic acid	1.2 ± 0.07

Table IV shows the effects of ouabain and ethacrynic acid on oxygen consumption by isolated enterocytes. Ouabain reduces the oxygen consumption by 50 ± 5% and ethacrynic acid by 55 ± 4%. The effects of ouabain and ethacrynic acid were additive.

Effect of substitution of Cl^- by NO_3^-

One additional explanation for our results is that the observed ouabain-insensitive Na^+, Cl^- and water extrusion is due to the action of a $Na^+/K^+/2Cl^-$ cotransport mechanism as described in some tissues [5,15]. This cation/Cl^- cotransport system is able to transport Na^+ against its electrochemical gradient by obtaining energy

TABLE III

INTRACELLULAR CONTENT OF ISOLATED SMALL INTESTINAL CELLS. EFFECT OF ANOXIA (N_2) AND DINITROPHENOL (DNP) 1 mM

Cells were preincubated at 4 °C for 30 min and then incubated at 37 °C for 30 min. Values presented are means ± S.E. of four experiments.

Condition	Intracellular content			
	Ion (nmol/mg cell protein)			Water (μl/mg cell protein)
	Na^+	K^+	Cl^-	
A, 4 °C	739 ± 24.8	241 ± 15.4	720 ± 17.1	6.33 ± 0.031
B, 37 °C	155 ± 82	548 ± 16.8	415 ± 7.5	4.44 ± 0.094
C, 37 °C + N_2	723 ± 23.5	230 ± 11.7	719 ± 25.1	6.26 ± 0.075
D, 37 °C + DNP	712 ± 29.2	177 ± 8.9	738 ± 27.5	6.51 ± 0.098
(B − A)	−584	+307	−305	−1.89
(C − A)	−16	−11	−1	−0.07
(D − A)	−27	−64	+18	+0.18

from the K^+ and/or Cl^- downhill movement. In fact this is a mechanism passive for K^+ and/or Cl^- and 'secondary active' for Na^+. One characteristic of the cation/Cl^- cotransport mechanism is its strong dependence on Cl^-. When Cl is substituted by NO_3^- this mechanism is inhibited [15,21]. Table V shows ion and water movements when cells were prepared and incubated in NO_3^--containing media without Cl^-. After 30 min at 4°C, cells have similar Na^+ and K^+ contents to cells prepared and incubated with Cl^-. Upon rewarming, enterocytes incubated with NO_3^- extruded Na^+, and water and gained K^+. Ouabain partially inhibited Na^+ extrusion, abolished K^+ gain but did not affect water movements. Ethacrynic acid partially inhibited Na^+ extrusion, had no effect on K^+ gain and totally inhibited water transport. The effects of ouabain and ethacrynic acid were additive.

TABLE V

EFFECT OF SUBSTITUTION Cl^- BY NO_3^- ON INTRACELLULAR ION AND WATER CONTENTS OF ISOLATED SMALL INTESTINAL CELLS

Cells were prepared and resuspended in media without Cl^-, preincubated at 4°C for 30 min and then rewarmed at 37°C for 30 min. n.d., not detectable. Values presented are means ± S.E. of three experiments. OU, ouabain; EA, ethacrynic acid.

Condition	Intracellular content			
	Ion (nmol/mg cell protein)			Water (μl/mg cell protein)
	Na^+	K^+	Cl^-	
A, 4°C	750±32	219±20	n.d.	6.41±0.05
B, 37°C	60±10	500±10	n.d.	3.62±0.18
C, 37°C, +OU	360±20	160±11	n.d.	3.60±0.20
D, 37°C, +EA	350±25	520±11	n.d.	6.50±0.19
E, 37°C, +OU +EA	745±30	200±18	n.d.	6.35±0.11
Rewarming (B−A)	−690	+281	−	−2.79
+OU (C−A)	−390	+59	−	−2.81
+EA (D−A)	−400	+301	−	+0.09
+OU +EA (E−A)	−5	−19	−	−0.06

The substitution of Cl^- by NO_3^- had no effect on the characteristics of Na^+, K^+ and water transport. These results eliminate the possibility that the ouabain-insensitive Na^+, Cl^- and water extrusion would be due to the functioning of the $Na^+/K^+/2Cl^+$ cotransport system.

Discussion

To our knowledge this paper shows for the first time that isolated small intestinal cells extrude Na^+, Cl^- and water and gain K^+ upon rewarming, when they have been previously loaded with Na^+, Cl^- and water and depleted of K^+ by cooling at 4°C (Table I). This fact allows the study of the mechanisms involved in the iso-osmotic regulation of intracellular ionic and water contents. Examination of the initial rates for Na^+ and Cl^- extrusion and K^+ gain, obtained from the results shown in Fig. 1, indicates that two Na^+ are lost when one K^+ is gained. The electrical difference obtained by the net movement of one Na^+ in excess to one K^+ is probably compensated by the loss of one Cl^-. The net movement of NaCl outwards produces the loss of water. If the Na^+/K^+ pump, which exchanges intracellular Na^+ by extracellular K^+, were the single mechanism implicated in the movement of these ions in the enterocyte, ouabain, a specific inhibitor of this pump, should fully inhibit the Na^+, Cl^- and water extrusion and K^+ gain. However, ouabain inhibits only partially Na^+ extrusion and does not affect Cl^- and water transport, but it abolishes K^+ gain. On the other hand, ethacrynic acid mainly inhibits Na^+, Cl^- and water extrusion. These results suggest the existence of two mechanism for Na^+ transport in the enterocyte. One exchanges Na^+ by K^+, is inhibited by ouabain and insensitive to ethacrynic acid. The second mechanism extrudes Na^+ with Cl^- and water, is inhibited by ethacrynic acid and is insensitive to ouabain. The observation that the net Na^+/K^+ movements inhibited by ouabain keep a ratio of 1 does not imply Na^+/K^+ coupling rates of 1 for the functioning of the Na^+/K^+ pump. To evaluate the Na^+/K^+ coupling by the pump knowledge of the unidirectional (not of the net) ion movements is required. Willis [23] argued that some Na^+ pumps located in sites inaccessible to ouabain could explain the

persistence of Na^+ estrusion in the presence of ouabain. We believe that the use of isolated enterocytes and of ouabain concentration at least two orders of magnitude higher than the maximal inhibitory concentration of ouabain in the guinea-pig intestine rules out this possibility.

Brugnara et al. [21] have shown, in human red blood cells, that substitution of Cl^- by NO_3^- inhibits the $Na^+/K^+/2Cl^-$ cotransport mechanism. In the enterocyte, substitution of Cl^- by NO_3^- has no effect on Na^+, K^+ and water movements observed after rewarming (Table V). This fact rules out the possibility that the ouabain-insensitive Na extrusion were due to the functioning of the Na^+, K^+ Cl^- cotransport system.

In addition the following arguments also rule out that a cation/Cl^- cotransport mechanism may explain the extrusion of Na^+ observed in the presence of ouabain. Saier and Boyden [15] have shown, in cells where the $Na^+/K^+/Cl^-$ cotransport system is operative, that equilibrium is reached at the relative concentrations predicted by the following equation

$$\text{(net driving force) } \Delta\tilde{\mu}_{net} = \Delta\tilde{\mu}_K + \Delta\tilde{\mu}_{Na} + \Delta\tilde{\mu}_{Cl} = 0$$

$$= RT \ln[K]_{out}[Na]_{out}[Cl]^2_{out}/[K]_{in}[Na]_{in}[Cl]^2_{in}$$

where the $\Delta\tilde{\mu}$ terms denote the electrochemical potential differences for the given ions across the cell membrane, R is the gas constant, T the absolute temperature, [] are the ion concentrations in the cell (in) and outside it (out), respectively.

If we analyze our results with this equation we obtain the following values: at the end of incubation in the cold, that is the moment when the rewarming starts, the net driving force is 0.005 kcal/mol directed inwards, (i.e. it is virtually zero). Under these circumstances the cotransport system cannot proceed. After 30 min of rewarming the net driving force is 0.258 kcal/mol again directed inwards. Therefore under the conditions in which rewarming was carried out cotransport must work as an influx system. However, under these conditions, Na^+, Cl^- and water extrusion are obtained.

The present observations are in agreement with the results obtained with basolateral plasma membrane vesicles of small intestinal cells where two different Mg^{2+})-ATP-dependent Na^+ transport mechanisms were demonstrated [10]. One transported Na^+ in the presence of K^+, was inhibited by ouabain, it was weakly inhibited by ethacrynic acid and it was refractory to furosemide. The second mechanism transported Na^+ in the absence of K^+, was insensitive to ouabain but it was inhibited by ethacrynic acid and furosemide. Two different Na^+-dependent ATPases associated with these Na^+ transport mechanisms have been described [11]. Similar transport mechanisms and ATPase systems have been described in proximal tubular kidney cells [16–20], where it has been recently shown that the $(Na^+ + K^+)$-ATPase can be totally inhibited under conditions in which the $(Na^+ + Mg^{2+})$-ATPase continues working. Alternatively the latter ATPase can be totally inhibited while the former continues working, indicating that these two ATPase activities reflect different entities [24].

The ATP dependence of Na^+ transport mechanism in basolateral plasma membrane vesicles and the existence of two different ATPase systems associated with each one of them, explains the dependence of two modes of Na^+ extrusion on cellular metabolism (Tables III and IV).

On the other hand, furosemide which inhibits $Na^+/K^+/Cl^-$ cotransport [15], and which also inhibits the ouabain-insensitive Mg^{2+}-ATP-dependent Na^+ transport in basolateral plasma membrane vesicles of enterocytes [10], has no effect on ions and water transport in isolated enterocytes (data not shown). The difference between the effects of furosemide in basolateral plasma membrane vesicles and in isolated cells could be explained if furosemide does not enter the cells and therefore does not reach the site of action on the second Na^+ pump, which would be located inside the cells, whereas in the inside-out vesicles [10] the site of action would be directly in contact with the incubation medium where furosemide has been added.

The involvement of the Na^+–Ca^{2+} exchange or the Na^+–H^+ exchange in the ouabain-insensitive Na^+, Cl and water transport has been discarded since both the absence of extracellular Ca^{2+} and changes of the extracellular pH (6.5 to 7.5) did not affect these fluxes (data not shown).

Based on the present evidence obtained with isolated cells and the previously shown in basolateral plasma membrane vesicles and ATPase sys-

tems, we propose that enterocytes regulate their Na^+ content by two pumps located in the basolateral plasma membrane. One exchanges Na^+ for K^+, is inhibited by ouabain and insensitive to ethacrynic acid and furosemide. The second Na^+ pump transports Na^+ with Cl^- and water, is insensitive to ouabain but is inhibited by ethacrynic acid and furosemide.

Acknowledgements

We are extremely grateful to Dr. F. Michelangeli for his suggestions and constructive criticism of this manuscript. We would also like to thank Lic. H. Linares for his technical assistance and Mrs. Rebeca Godoy for word processing the manuscript.

References

1 Schultz, S.G. (1979) in Membrane Transport in Biology, Vol. IV-B (Giebish, G., Tosteson, D.C. and Using, H.H., eds.) pp. 749–780, Springer-Verlag, Heidelberg, New York
2 Schultz, S.G. and Frizzell, R.A. (1972) Gastroenterology 63, 161–170
3 Schultz, S.G. (1977) Am. J. Physiol. 233, E249–E254
4 Schultz, S.G. (1978) in Physiology of Membrane Disorders (Andreoli, T.E., Hoffman, J.F. and Fanestil, D.D., eds.), pp. 273–286, Plenum Press, New York
5 Frizzell, R.A., Field, M. and Schultz, S.G. (1979) Am. J. Physiol. 236, F1–F8
6 Nellans, H.N. and Schultz, S.G. (1976) J. Gen. Physiol. 68, 441–463
7 Schultz, S.G. and Curran, P.F. (1970) Physiol. Rev. 50, 637–718
8 Lee, C.O. and Armstrong, W.McD. (1972) Science 175, 1261–1264
9 Brown, P.D. and Sepúlveda, F.V. (1985) J. Physiol. 363, 271–285
10 Del Castillo, J.R. and Robinson, J.W.L. (1985) Biochim. Biophys. Acta 812, 402–412
11 Del Castillo, J.R. and Robinson, J.L.W. (1985) Biochim. Biophys. Acta 812, 413–422
12 Del Castillo, J.R. (1987) Biochim. Biophys. Acta 901, 201–208
13 Gadd, K.G. (1980) Med. Lab. Sci. 38, 61–63
14 Jacquez, J.A. (1978) in Physiology of Membrane Disorders, (Andreoli, T.E., Hoffman, J.F. and Fanestil, D.D., eds.), pp. 147–164, Plenum Press, New York
15 Saier, M.H. and Boyden, D.A. (1984) Mol. Cell. Biochem. 59, 11–32
16 Whittembury, G. and Proverbio, F. (1970) Pflügers Arch. 316, 1–25
17 Marín, R., Proverbio, T. and Proverbio, F. (1985) Biochim. Biophys. Acta 817, 299–306
18 Proverbio, F. and Del Castillo, J.R. (1981) Biochim. Biophys. Acta 646, 99–108
19 Del Castillo, J.R. Marín, R., Proverbio, T. and Proverbio, F. (1982) Biochim. Biophys. Acta 692, 61–68
20 Proverbio, F., Proverbio, T. and Marín, R. (1982) Biochim. Biophys. Acta 688, 757–763
21 Brugnara, C., Canessa, M., Cusi, D. and Tosteson, D.C. (1986) J. Gen. Physiol. 87, 91–112
22 Armstrong, W. McD., Musselman, D.L. and Reitzug, H.C. (1970) Am. J. Physiol. 219, 1023–1026
23 Willis, J.S. (1968) Biochim. Biophys. Acta 163, 516–530
24 Proverbio, F., Proverbio, T. and Marín, R. (1986) Biochim. Biophys. Acta 858, 195–202