Biochimica et Biophysica Acta, 339 (1974) 291–302
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BBA 76587

SODIUM ION TRANSPORT IN ISOLATED INTESTINAL EPITHELIAL CELLS

THE EFFECT OF ACTIVELY TRANSPORTED SUGARS ON SODIUM ION EFFLUX

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(Received October 22nd, 1973)

SUMMARY

A technique to measure Na^+ efflux from isolated intestinal epithelial cells has permitted us to examine the mechanisms responsible for Na^+ transport in absorptive cells without contamination by other cell types. We examined the effect of actively transported sugars on Na^+ efflux from isolated rat jejunal epithelial cells to evaluate the mechanism by which actively transported non-electrolytes stimulate Na^+ absorption. Glucose, galactose and 3-*O*-methylglucose, sugars known to be actively transported by the small intestine, stimulate total Na^+ efflux from isolated epithelial cells. This stimulation results from an increase of active Na^+ transport, since it is inhibited by ouabain. Glucose stimulation is significantly greater than that produced by galactose or 3-*O*-methylglucose. 2-Deoxyglucose, a sugar that is not actively transported, has no effect on total Na^+ efflux from isolated cells. Phloridzin, which has no effect on Na^+ efflux in a sugar-free medium, completely abolishes the effect of galactose. These findings (a) support the hypothesis that the increase in intestinal absorption of Na^+ in the presence of actively transported non-electrolytes occurs by a transcellular route; and (b) are consistent with the ion-gradient model. The results are not compatible with the direct energy-coupling model.

INTRODUCTION

The multiple transport processes of the mucosal epithelial cell of the small intestine have long been recognized [1–5]. Isolated intestinal epithelial cells offer a unique preparation for studying transport processes in the primary absorptive cell. These cells obtained by mechanical [6, 7] or enzymatic methods [8–10] are structurally intact, possess the morphologic structure characteristic of intestinal epithelial cells before isolation and retain their metabolic and transport capabilities [10–13].

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Measurement of Na^+ transport in isolated intestinal epithelial cells has not been reported previously. Current concepts of Na^+ transport across the epithelial cell from the gut lumen to extracellular space are as follows [14]. Net Na^+ entry from the lumen across the brush border into the epithelial cell takes place down an electrochemical gradient, does not require an energy input and is not inhibited by ouabain. Na^+ exit from the cell into the extracellular solution is an active transport process inhibited by ouabain and localized to the lateral and basal cell membrane.

Actively transported sugars and amino acids in the solution bathing the mucosal surface of the cell are known to stimulate Na^+ absorption in a variety of in vitro and in vivo preparations of small intestine. Fordtran et al. [15] suggested that this increase in Na^+ absorption may be the result of solvent drag through tight junctions and lateral intercellular spaces, secondary to the sugar-induced increase in water absorption. Crane [16, 17] and Schultz and Curran [18] proposed a transcellular route. In later studies using indirect methods, Frizzell et al. [19] obtained results consistent with the concept of a transcellular pathway. Controversy also exists concerning the possible cellular mechanisms by which active absorption of non-electrolytes might occur and interact with Na^+ absorption. In Crane's [16, 17] ion-gradient model, interaction between Na^+ and brush-border carrier mechanisms for sugar and amino acids results in an increased influx of Na^+ and absorption of solutes from the mucosal solution into the cell, across the mucosal membrane. The driving force for active solute absorption is the Na^+ gradient across the mucosal membrane. The increased influx of Na^+ into the cell is then followed by active extrusion of Na^+ out of the cell into the extracellular fluid. This extrusion utilizes the ouabain-sensitive mechanism responsible for active Na^+ absorption in the absence of sugars and amino acids, located in the lateral and basal membrane [14]. The results of Barry and Eggenton [20] in studies of transmural potential across epithelial cells, agree with the concept of a serosally located electrogenic pump which is stimulated by actively transferred hexoses and amino acids, and that the Na^+-dependent entry mechanism at the mucosal membrane is non-electrogenic. Kimmich and coworkers have challenged the validity of the ion-gradient hypothesis and have instead proposed a model with direct energy-coupling between Na^+ and actively absorbed non-electrolytes at the brush border [13, 21, 22]. The experiments in the present paper, which deal with the effect of actively transported sugars on Na^+ transport from isolated rat jejunal cells, support a transcellular pathway and favour the ion-gradient model.

METHODS

Animals

Male Wistar rats weighing 180–300 g were used in all experiments.

Materials

Plastic containers were used for in vitro manipulations of the epithelial cells since the usual glassware caused lysis.

Isolation procedure

Epithelial cells were isolated from the gut by a procedure similar to that

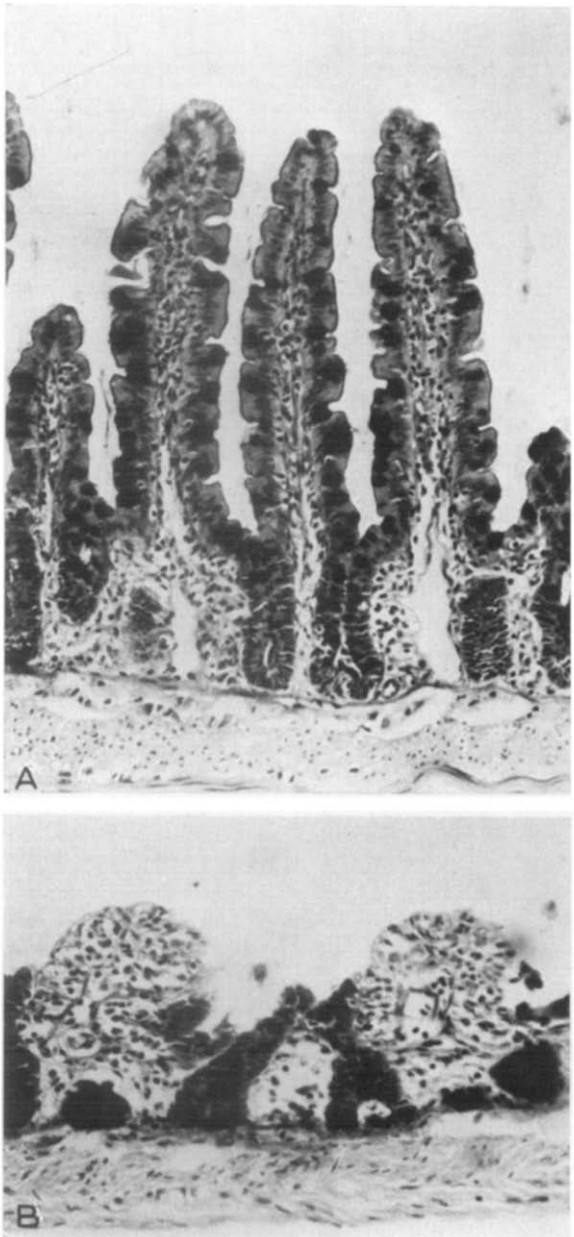

Fig. 1. Changes in the histology of intestinal mucosa following cell isolation (×40). A. (Top) Normal rat mucosa. B. (Bottom) After completion of the isolation procedure there is complete loss of epithelial cells from the villi, but no shedding of crypt cells.

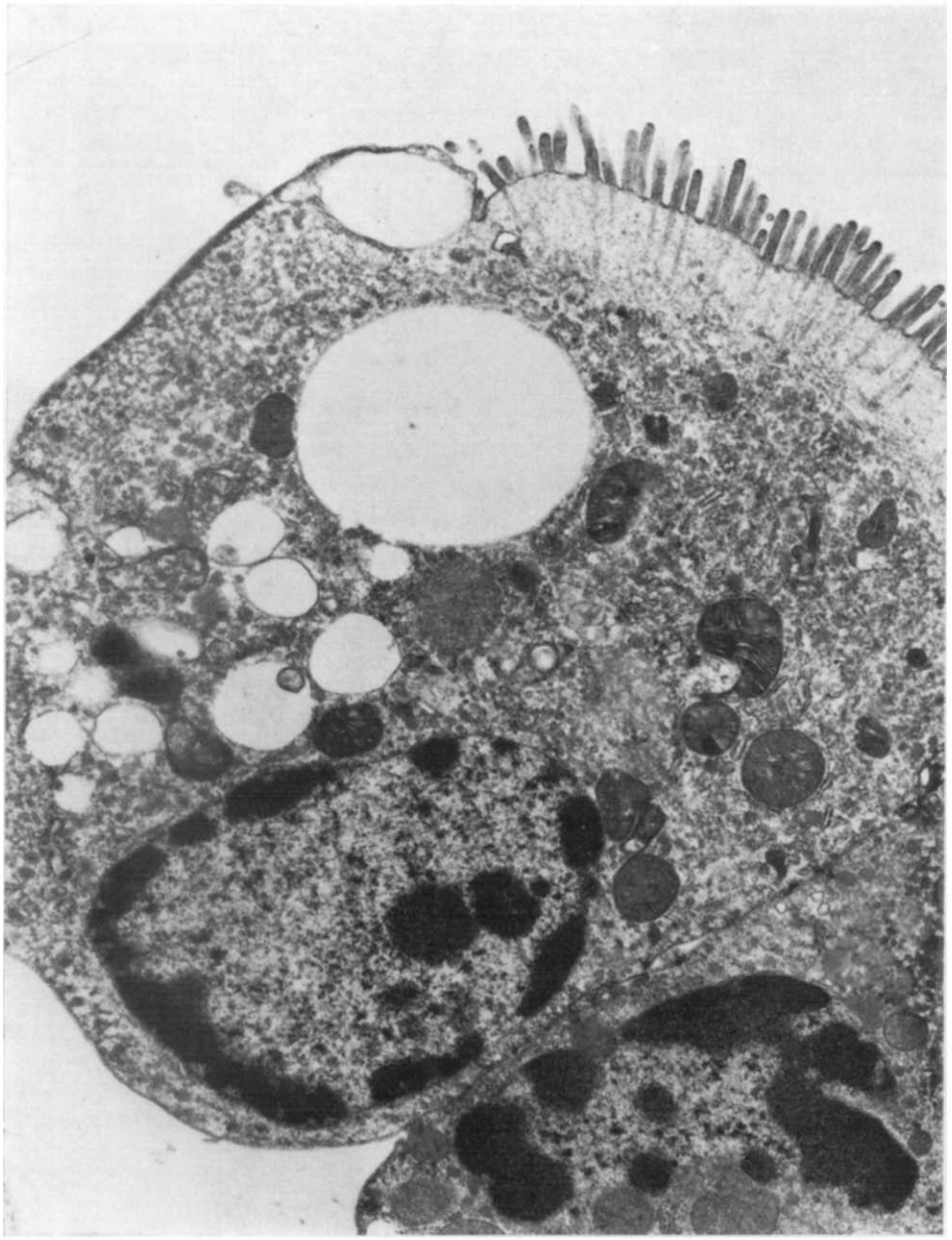

Fig. 2. Electron micrograph of an isolated epithelial cell (rat jejunum), demonstrating intact brush border and lateral and basal membranes ($\times$ 14 500).

described by Levine and Weintraub [7]. Non-fasted rats were decapitated and bled and 15-cm segments of upper jejunum were removed, flushed and soaked for 20 min in an isolation solution. The segments were everted over a metal spiral with a diameter of 5 mm and connected to a Vibro Mixer, Model E1. The segments were then immersed in 200 ml of fresh isolation solution and vertically vibrated 100 times per s over an amplitude of 1 mm for 20 min. The cell pellet obtained by centrifugation at $500 \times g$ was washed twice with the isolation solution. The temperature was kept constant at 37 °C throughout the isolation procedure and the procedure was carried out in room air.

Fig. 1 demonstrates that the cells obtained with the above isolation procedure are epithelial cells from the villi and not crypt cells. The isolated cells retain their normal triangular shape, basal nucleus and intact brush border and lateral and basal membranes. Electron microscopy confirms the light microscopic findings of structural integrity of these isolated cells (Fig. 2).

Na^+ efflux procedure

The method of measuring Na^+ efflux was similar to that described for red cells [23]. 1 ml of the cells was incubated in a Dubnoff Metabolic Shaker at 37 °C in 6 ml of incubation medium containing 10 μCi of $^{22}Na^+$ for 20 min. The cell suspension was centrifuged and the cells washed twice with $MgCl_2$–Tris buffer solution at 4 °C. The cells were returned to fresh tracer-free medium at a dilution of 1 : 30 by vol. and $^{22}Na^+$ efflux was ascertained by measuring the rate of appearance of radioactive Na^+ in the medium. The procedure was carried out in room air as no beneficial effect had been seen with 100% oxygen. 0.5 ml samples of cell suspension were obtained at the beginning and completion of the efflux period. 0.5 ml samples of

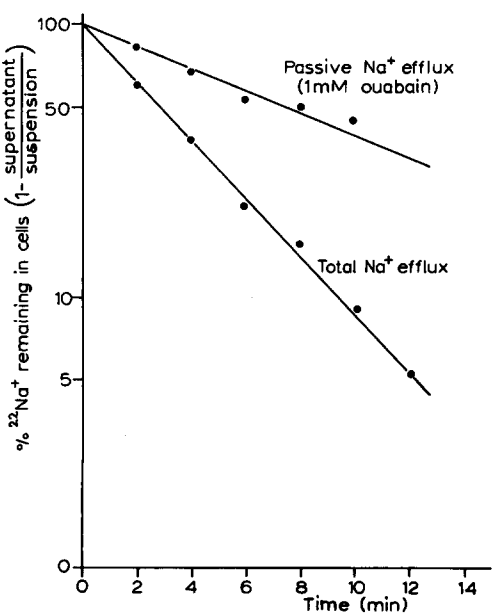

Fig. 3. A semilogarithmic plot of total, active and passive Na^+ efflux from one experiment.

supernatant were obtained at 0, 2, 4, 6, 8, 10 and 12 min by centrifugation of 1 ml aliquots of total suspension for 1 min at 4 °C. The outflux rate constant ${}^{0}K_{Na}$ (that portion of the intracellular Na^+ extruded per unit of time) was calculated by Hoffman's method [24]. The basic presumption underlying the method is that the Na^+ outflux can be looked upon as a first-order occurrence and calculations are made with the stipulation that the cell concentration by vol. is less than 1–30. The values of (1−supernatant/suspension)×100, the percent of radioactive Na^+ remaining within the cell at a given time, are plotted against time on a semilogarithmic scale (Fig. 3). The rate constant, described by the equation: ${}^{0}K_{Na}$ = ln (1−supernatant/suspension)/time-unit, is determined from the straight line obtained and is reported as the amount of Na^+ exchanged per h. Passive efflux is defined as that portion of the efflux constant remaining after maximal inhibition with ouabain (Fig. 3). The difference between the total Na^+ efflux rate constant and the passive efflux rate constant (Fig. 3) is a measure of the ouabain-sensitive efflux of Na^+ and can be considered active Na^+ transport.

Solutions

The isolation solution contained 154 mM NaCl, 2 mM Tris buffer, 3 mM K_2HPO_4, 10 mM sucrose and 1 mg albumin per ml with an osmolality of 310 mosM and a pH of 7.4 at 37 °C. The $MgCl_2$ wash-solution contained 110 mM $MgCl_2$ and 2 mM Tris buffer per ml with an osmolality of 292 mosM and a pH of 7.4 at 4 °C. The incubation medium used in the flux procedure contained 120 mM NaCl, 20 mM Tris–HCl, 3 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mg albumin per ml with an osmolality of 280 mosM and a pH of 7.4 at 37 °C.

Sugars, when examined, were present in the incubation medium during both the loading and efflux periods. The concentration in the incubation medium of all monosaccharides and sucrose was 10 mM, while maltose and lactose were used in a concentration of 5 mM. Initial experiments with sugar-free medium were done without osmotic equivalent mannitol replacement. All subsequent experiments with sugar-free medium contained 10 mM mannitol. The efflux rate constants obtained with or without mannitol were not significantly different and were therefore combined. 1 mM ouabain and 0.5 mM phloridzin when used, were present during both loading and efflux periods. In assessing the effect of ouabain, we allowed a 2-min equilibration

TABLE I

THE EFFECT OF OUABAIN ON Na^+ EFFLUX

To rule out any variation between animals, three concentrations of ouabain were evaluated simultaneously on cells isolated from a single rat in three separate experiments. The results are compared with the total sodium efflux rate constant derived from other experiments.

Ouabain concentration (mM)	Efflux rate constant h^{-1}*	$P<$
0	13.7±1.9 (32)	
0.01	7.4±1.3 (18)	0.0005
0.1	5.7±1.0 (18)	0.0005
1	5.4±1.3 (18)	0.0005

* Each value represents mean±S.D. Number of observations are in parentheses.

period after adding the cells to the efflux medium. We assessed the effect of increasing concentrations of ouabain on Na^+ efflux. As demonstrated in Table I, all concentrations of ouabain examined significantly inhibited Na^+ efflux. 0.01 mM ouabain caused significantly less inhibition than either 1 or 0.1 mM ($P < 0.0005$). Between 0.1 and 1 mM ouabain the amount of inhibition did not change significantly, indicating that maximal inhibition had occurred.

Assay procedures

Na^+ and K^+ concentrations of the isolation solution and incubation medium were routinely measured by direct analysis in a flame photometer. The osmolality of all solutions was determined by freezing-point osmometer (Precision Systems). The osmolality of incubation medium plus 10 mM of sugar or mannitol was 290 mosM and incubation medium plus 5 mM disaccharide was 285 mosM. Samples of total cell suspension and supernatant obtained during the efflux period were assayed for $^{22}Na^+$ using a liquid scintillation spectrometer (Beckman). Invertase and total lactase and maltase activities were measured on homogenized whole mucosa, obtained immediately after animal death, and homogenized isolated cells, obtained after a sham flux procedure. The assay procedures were carried out using the conventional method of Dahlqvist [25] and also in the presence of 20 mM Tris–HCl. Data were compared by the standard Students "*t*" test.

RESULTS

Effect of actively transported sugars

Table II compares total and passive Na^+ efflux rate constants obtained in the presence of actively transported sugars with those obtained with sugar-free incubation medium. Actively transported sugars, glucose, galactose and 3-*O*-methylglucose [2], at a 10 mM concentration, significantly stimulated the total Na^+ efflux rate constant from isolated jejunal epithelial cells. The stimulation by glucose was significantly

TABLE II

EFFECT OF MONOSACCHARIDES ON Na^+ EFFLUX FROM ISOLATED JEJUNAL EPITHELIAL CELLS

	Total*	$P<$**	Passive* (1 mM ouabain)	$P<$**
Sugar-free medium				
mannitol 10 mM	9.2±0.2 (53)		5.3±0.1 (41)	
Monosaccharides actively transported				
10 mM glucose	13.7±0.3 (32)	0.0005	5.2±0.2 (20)	N.S.
10 mM galactose	11.6±0.5 (19)	0.0005	5.4±0.1 (17)	N.S.
10 mM 3-*O*-methylglucose	11.0±0.4 (23)	0.0005	5.2±0.1 (38)	N.S.
Monosaccharide not actively transported				
10 mM 2-deoxyglucose	9.8±0.6 (11)	N.S.	4.5±0.2 (23)	0.0025

* Na^+ efflux rate constants h^{-1}. Each value represents mean±S.E. with the number of observations in parentheses.

** Compared with sugar-free medium. N.S., not significant.

greater than that produced by galactose or 3-*O*-methylglucose ($P < 0.0005$). Passive efflux rate constants in the presence of these actively transported sugars were not significantly different from that in the sugar-free medium. Since the passive efflux rate constant was unchanged, the stimulation of total Na^+ efflux rate constant from isolated epithelial cells by these sugars was due to an increase in active Na^+ efflux.

Effect of a sugar not actively transported

Table II also includes results for 2-deoxyglucose, a sugar that is not actively transported by intestinal epithelial tissue [2]. The total Na^+ efflux rate constant in the presence of 2-deoxyglucose was not significantly different from that in the sugar-free medium, but was significantly less than that in medium containing glucose ($P < 0.0005$), galactose ($P < 0.025$) and 3-*O*-methylglucose ($P < 0.05$). The passive efflux rate constant in the presence of 2-deoxyglucose was, however, significantly less than the constant obtained in the presence of actively transported sugars or in sugar-free medium ($P < 0.025$).

Effect of phloridzin

Because phloridzin competitively inhibits active hexose transport at the brush border [26], we investigated the effect of phloridzin on galactose stimulation of Na^+ efflux from isolated cells. As shown in Table III phloridzin had no effect on the total Na^+ efflux rate constant from cells incubated in a sugar-free solution. However, the stimulation of this rate constant produced by galactose was completely abolished by phloridzin. The total efflux rate constant for galactose plus phloridzin was not significantly different from the rate constant derived in a sugar-free medium.

TABLE III

EFFECT OF 0.5 mM PHLORIDZIN ON Na^+ EFFLUX FROM ISOLATED CELLS IN THE ABSENCE OF AND IN THE PRESENCE OF 10 mM GALACTOSE

	Phloridzin*		$P <$**
	0	0.5 mM	
10 mM mannitol	9.0±0.3 (23)	9.0±0.4 (23)	N.S.
10 mM galactose	11.3±0.4 (24)	8.9±0.3 (26)	0.0005

* Total Na^+ efflux rate constant h^{-1}. Each value represents the mean±S.E. with the number of observations in parentheses.

** N.S., not significant.

Effect of disaccharides

The effects of disaccharides on Na^+ efflux from isolated cells are shown in Table IV. Maltose and lactose, in concentrations equivalent to those used for monosaccharides, significantly increased the total Na^+ efflux rate constant over that of the sugar-free medium. The passive efflux rate constant found with maltose or lactose was not significantly different from that obtained with sugar-free medium. Therefore, as with monosaccharides, the increase in the total Na^+ efflux rate constant was a result of increased active Na^+ efflux. The effect of maltose on the total Na^+ efflux rate constant was not significantly different from that produced by glucose, but was

TABLE IV

EFFECT OF DISACCHARIDES ON Na^+ EFFLUX FROM ISOLATED CELLS

	Total*	$P<$**	Passive* (1 mM ouabain)	$P<$**
10 mM mannitol	9.2±0.2 (53)		5.3±0.1 (41)	
5 mM maltose	13.3±0.5 (18)	0.0005	5.5±0.2 (17)	N.S.
5 mM lactose	11.6±0.5 (18)	0.0005	5.3±0.2 (18)	N.S.
10 mM sucrose	9.6±0.5 (23)	N.S.	4.7±0.1 (27)	0.0025

* Na^+ efflux rate constants h^{-1}. Each value represents mean ±S.E. with the number of observations in parentheses.

** Compared with sugar-free medium. N.S., not significant.

significantly greater than that seen with lactose, galactose and 3-*O*-methylglucose ($P < 0.01$). The effects of lactose, galactose and 3-*O*-methylglucose were not significantly different. The presence of sucrose, however, did not result in stimulation of the total Na^+ efflux rate constant as there was no significant difference between the constants in sucrose-containing medium and sugar-free medium. There was a significant difference between the constants in mediums containing sucrose and the other disaccharides and actively transported monosaccharides ($P < 0.05$). The passive Na^+ efflux rate constant in the presence of sucrose was significantly lower than (a) the constant in the sugar-free medium and (b) the constants obtained in the presence of all the examined sugars except 2-deoxyglucose ($P < 0.01$).

The surprising results obtained with sucrose led us to investigate invertase and total lactase and maltase activities in the isolated cells. We found no significant difference between the activity of these enzymes in homogenates of isolated cells and of whole mucosa (Table V). However in the presence of 20 mM Tris–HCl, as compared to Tris-free solutions, the activities of these enzymes were all significantly reduced ($P < 0.0005$) with almost complete inhibition of invertase and total lactase activity (Table V).

TABLE V

DISACCHARIDASE ACTIVITIES

Disaccharidase activity is expressed in units per g tissue protein. 1 unit = 1 μmole substrate hydrolyzed per min. Each value represents the mean±S.E.

	0 Tris–HCl		P*	20 mM Tris–HCl		P*
	Whole mucosa	Isolated cells		Whole mucosa	Isolated cells	
Lactase	11.5±1.7	13.6±1.6	N.S.	0.7±0.4	0.2±0.1	N.S.
Maltase	315.3±40.5	286.7±20.8	N.S.	68.3±16.2	56.3±8.3	N.S.
Sucrase	64.9±9.5	49.2±5.3	N.S.	2.5±0.4	1.5±0.9	N.S.

* N.S., not significant.

DISCUSSION

The method described for measuring Na^+ efflux rate constants from isolated epithelial cells of the small intestine is a valid technique for at least two reasons. Firstly, inhibition of Na^+ transport by ouabain demonstrates that we are examining an active process occurring in intact cells and not passive release of Na^+ from cell membrane fragments [27]. Secondly, the efflux rate constants follow first-order kinetics, as has been found in other cell types.

$Na^+ + K^+$-dependent ATPase activity of rat tissues has been demonstrated to be less sensitive to ouabain inhibition than ATPase activity of tissues from other species [28, 29]. Maximal inhibition can be obtained, however, with higher concentrations of ouabain [28]. Rate constants in rat epithelial cells for total, active and passive Na^+ efflux were 13.7, 8.5 and 5.2 h^{-1}, respectively, against 0.49, 0.41 and 0.08 h^{-1}, respectively for human erythrocytes [30]. Since the percentage inhibition of Na^+ efflux produced by ouabain is much less in rat epithelial cells (62%) than in human erythrocytes (83.7%), we were concerned that maximal inhibition was not occurring in these cells. However, Table I demonstrates that maximal inhibition occurs with 1.0 mM, the concentration used in the experiments reported here. Also previous studies [31] of sodium efflux in epithelial cells isolated from pig small intestine have shown a total Na^+ efflux rate constant of 7.2 ± 1.8 h^{-1}, which was inhibited to 3.1 ± 1.3 h^{-1} with 1 mM ouabain (a percentage inhibition of 57%), which is similar to the inhibition obtained in rat cells (62%). These results imply that epithelial cells are proportionately more permeable than erythrocytes. If this is the case, one would expect the intracellular concentration of Na^+ to be greater in intestinal epithelial cells than erythrocytes. The intracellular Na^+ concentration in erythrocytes is 8.7 mequiv per l of cell water [30] while estimates of Na^+ concentration in epithelial cells is 40 mequiv per l [32]. Comparison of Na^+ efflux data similarly derived from epithelial cells and erythrocytes demonstrates the relatively large capacity of epithelial cells to transport Na^+.

We have demonstrated that sugars known to be actively transported by the small intestine increase the total Na^+ efflux rate constant from isolated jejunal epithelial cells of the rat. The increase in the Na^+ efflux rate constant is inhibited by ouabain, indicating that this increase results from an increase of the active Na^+ efflux rate constant. This increase cannot be attributed to supplying metabolic energy as it occurs with galactose, which is metabolized to a limited extent by rat jejunal tissue, and with 3-*O*-methylglucose, which is not metabolized [17]. Also the increase cannot be attributed to movement of these sugars through the lateral and basal membrane as Bihler and Cybulsky [33] have demonstrated that the transport of sugars through this membrane is independent of Na^+ and insensitive to phloridzin. The fact that phloridzin blocks the increase in the Na^+ efflux rate constant produced by galactose confirms that we are measuring a specific process identical to that seen with intact tissue.

The failure to find an increase in the activity of disaccharidases, when expressed as units of activity per g of protein, is somewhat surprising in that there should be less extraneous protein in the isolated cells than in whole tissue homogenate. However, homogenate of isolated cells was obtained after 80 min of in vitro manipulation at 37 °C while whole tissue homogenate was obtained immediately after animal death.

The stimulation of the total Na^+ efflux rate constant by lactose is unexpected since total lactase activity, at least in homogenates of mucosa and isolated cells, is non existent in the presence of 20 mM Tris–HCl. The reason why sucrose and 2-deoxyglucose result in a significantly lower passive Na^+ efflux rate constant requires further study.

Our findings support the hypothesis that the increase in intestinal absorption of Na^+ when actively transported sugars and amino acids are present, can occur by a transcellular route; and that this increase need not be attributed to solvent drag through extracellular pathways. The results are consistent with the ion-gradient model for active absorption of non-electrolytes as proposed by Crane [17] and Schultz and Curran [18]. The results are not compatible with the direct energy-coupling hypothesis. In this model,Kimmich and co-workers [13, 21, 22] predict that sugar transport would interfere with Na^+ extrusion. One would expect this interferenceto be associated with a decrease or at least no change in the total Na^+ efflux rate constant of epithelial cells. Our results demonstrate just the opposite, a significant increase in the total Na^+ efflux rate constant. Our investigations have not enabled us to delineate the mechanism by which actively transported sugars stimulate a serosally located electrogenic Na^+ pump.

Since the intestinal epithelial cell is polarized as regards morphologic structure and function, one can question whether the isolated-cell technique is suitable for investigating transport processes. The ion-gradient model visualizes a physical separation between (a) the locus for the interaction of Na^+ and actively transported non-electrolytes responsible for generating a sugar gradient and increased Na^+ influx (located in the brush border) and (b) the Na^+ pump responsible for maintaining the low intracellular Na^+ concentration and Na^+ absorption (located in the lateral and basal membrane). The evidence for this separation is that actively transported sugars have a stimulatory effect on Na^+ absorption only when present in the mucosal solution [34], that phloridzin inhibition only occurs at the brush border [26], and that ouabain inhibition only occurs at the serosal side of the epithelial cell [35]. The inhibition by phloridzin of the stimulatory effect of an actively transported sugar on Na^+ efflux and the inhibition by ouabain of Na^+ efflux indicate that isolated cells and cells aligned in a membrane function similarly.

ACKNOWLEDGEMENTS

These studies were supported by funds from the Pathfinder Foundation, Grant PR390C of the Ontario Ministry of Health, the Medical Research Council of Canada, and the Faculty of Medicine, University of Calgary, Calgary, Alberta. Dr Gall was supported by a McLaughlin Travelling Fellowship during the conduct of many of these experiments. We are indebted to Dr K. G. Simon, University of Toronto, for the electron microscopic studies.

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