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SODIUM ION TRANSPORT IN ISOLATED INTESTINAL EPITHELIAL CELLS

II COMPARISON OF THE EFFECT OF ACTIVELY TRANSPORTED SUGARS ON SODIUM ION EFFLUX IN CELLS ISOLATED FROM JEJUNUM AND ILEUM

D GRANT GALL* and DIANNE CHAPMAN

Department of Pediatrics, University of Toronto and the Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8 (Canada)

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SUMMARY

Na^+ transport studies in intestinal epithelial cells indicate that enterocytes from different regions of the small intestine differ in their response to actively transported sugars

1 Compared with sugar-free medium total Na^+ efflux rate constants from isolated rat jejunal cells were significantly increased when medium contained actively transported sugars, glucose and galactose, but not when medium contained fructose

2 In contrast total Na^+ efflux rate constants from isolated rat ileal cells did not respond to actively transported sugars, glucose and galactose

3 Similar results for the effect of actively transported sugars on Na^+ efflux were obtained for isolated rabbit jejunal and ileal epithelial cells

4 Passive Na^+ efflux rate constants for isolated jejunal and ileal enterocytes are not significantly different, indicating similar permeability characteristics

INTRODUCTION

Although the ileum possesses several specialized functions including bile salt [1] and B-12 absorption [2], the effect of actively transported sugars and amino acids on Na^+ movement is thought by most to be similar in jejunum and ileum [3, 4] However, several authors have reported significant differences between jejunum and ileum concerning the interaction of actively transported non-electrolytes and Na^+ transport Barry et al [5] reported that water absorption, which is thought to be passive and secondary to active solute transport [4], in the jejunum was glucose dependent, whereas, in the ileum the majority of total water absorption was glucose independent Fordtran et al [6] in in vivo perfusion studies of human intestine found

* To whom correspondence should be sent

that Na^+ absorption in the jejunum was stimulated by glucose and galactose but not in the ileum The experiments in the present paper, which deals with the effect of actively transported sugars on Na^+ transport from isolated jejunal and ileal epithelial cells, demonstrates significant differences between jejunal and ileal cells

MATERIALS AND METHODS

Animals used were male Wistar rats weighing 150–300 g and New Zealand white rabbits weighing 1 5–4 0 kg Epithelial cells were isolated from a 30-cm segment of jejunum starting 10 cm distal to the ligament of Treitz and from a 30-cm segment of ileum, in rats immediately proximal to the ileocecal valve, in rabbits proximal to the end of the mesenteric attachment of the appendix, by mechanical vibration as previously described [7] Enterocytes isolated by this technique are mature cells from the villi and not crypt cells [7] The composition of the solutions and the technique used for measuring Na^+ efflux from isolated small intestinal epithelial cells were identical to that previously described except the isolation solution and $MgCl_2$ wash solution contained 5 mM Tris/Tris HCl buffer instead of 2 mM Tris buffer [7] Briefly, harvested epithelial cells were loaded with radioactive Na^+ by suspending them in incubation medium containing $^{22}Na^+$, then washed with $MgCl_2$ wash solution and returned to trace-free medium Na^+ efflux was ascertained by measuring the rate of appearance of radioactive Na^+ in the medium The rate constants are reported as the amount of Na^+ exchange per h The efflux constant remaining after inhibition with 1 mM ouabain is defined as passive efflux Sugars, ouabain and phloridzin, when used were present in the incubation medium during both loading and efflux periods

RESULTS

Effect of actively transported sugars Table I compares total and passive Na^+ efflux rate constants obtained in the presence of actively transported sugars with those obtained in sugar-free medium for jejunal and ileal epithelial cells from rats We confirmed our previous findings that actively transported sugars, glucose and galactose at a 10 mM concentration, significantly stimulated the total Na^+ efflux rate constant from isolated jejunal epithelial cells [7] In contrast total Na^+ efflux rate constants obtained in the presence of these same sugars at identical concentration for isolated ileal epithelial cells were not significantly different from that found for sugar-free medium The total rate constant for jejunal cells in sugar-free medium was significantly less than that found for ileal cells in sugar-free medium and in medium containing glucose or galactose ($P < 0\,0005$) Passive efflux rate constants for jejunal and ileal cells in the presence of actively transported sugars were not significantly different from that found in sugar-free medium Passive rate constants in sugar-free medium for ileal and jejunal cells were also not significantly different

Effect of fructose Table I includes results for the effect of fructose on Na^+ efflux from isolated rat jejunal epithelial cells Fructose is not actively transported by the Na^+-dependent glucose-galactose carrier mechanism [8] but can be utilized by rat jejunal tissue as an energy source for ion transport [9] In isolated jejunal cells the total Na^+ efflux rate constant in the presence of fructose was not significantly different

TABLE I

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

	Jejunum	P **	Ileum	P **
	Total Na^+ efflux rate constant (h^{-1})*			
Sugar-free medium				
10 mM mannitol	9 6±0 2 (100)		14 6±0 6 (51)	
Monosaccharides actively transported				
10 mM glucose	14 0±0 4 (84)	0 0005	14 3±0 5 (44)	n s
10 mM galactose	11 5±0 4 (42)	0 0005	13 5±0 7 (35)	n s
Monosaccharide not actively transported				
10 mM fructose	10 3±0 3 (38)	n s		
	Passive (1 mM ouabain) Na^+ efflux rate constant (h^{-1})*			
Sugar-free medium				
10 mM mannitol	6 7±0 2 (52)		7 1±0 5 (26)	
Monosaccharides actively transported				
10 mM glucose	6 9±0 3 (47)	n s	8 0±0 3 (39)	n s
10 mM galactose	7 0±0 3 (23)	n s	6 5±0 5 (23)	n s
Monosaccharide not actively transported				
10 mM fructose	7 1±0 3 (37)	n s		

* Each value represents mean ± S E with the number of observations in parentheses
** Compared with sugar-free medium
n s , not significant

TABLE II

EFFECT OF 0 5 mM PHLORIDZIN ON Na^+ EFFLUX FROM ISOLATED CELLS IN THE ABSENCE OF AND IN THE PRESENCE OF 10 mM GALACTOSE

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

	Jejunum			Ileum		
	Phloridzin (0 mM)	Phloridzin (0 5 mM)	P	Phloridzin (0 mM)	Phloridzin (0 5 mM)	P
Mannitol 10 mM	9 0±0 3 (23)	9 0±0 4 (23)	n s	13 8±0 5 (78)	13 6±0 8 (22)	n s
Galactose 10 mM	11 3±0 4 (24)	8 9±0 3 (26)	0 0005	12 7±0 8 (17)	12 9±1 2 (15)	n s

n s , not significant

from that in sugar-free medium, but was significantly less than that obtained in medium containing glucose ($P < 0\ 0005$) or galactose ($P < 0\ 025$) The passive efflux rate constant in the presence of fructose was not significantly different from that in sugar-free medium or in medium containing glucose or galactose

Effect of phloridzin Table II compares the effect of phloridzin on Na^+ efflux from rat ileal epithelial cells to our previous findings for jejunal cells [7] In contrast to jejunal cells, in which galactose stimulates the total efflux rate constant and this increase is completely abolished by phloridzin, the presence of galactose does not produce an increase in the total rate constant from ileal cells and phloridzin has no inhibitory effect

TABLE III

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

	Jejunum	$P <$**	Ileum	$P <$**
	Total Na^+ efflux rate constant (h^{-1})*			
10 mM mannitol	15 8±0 6 (41)		14 6±0 5 (49)	
10 mM glucose	18 8±0 7 (38)	0 001	14 5±0 6 (34)	n s
	Passive Na^+ efflux rate constant (h^{-1})*			
10 mM mannitol	7 2±0 3 (30)		7 5±0 3 (23)	
10 mM glucose	8 0±0 3 (33)	n s	6 7±0 5 (23)	n s

* Each value represents the mean ±S E with the number of observations in parentheses
** Compared with sugar-free medium

n s not significant

Effect of actively transported sugars on Na^+ efflux from rabbit enterocytes The surprising lack of stimulation of Na^+ efflux from rat ileal epithelial cells by actively transported sugars led us to investigate Na^+ transport in isolated enterocytes from another species Table III demonstrates that as seen in rat intestinal cells, the presence of glucose, at a 10 mM concentration significantly stimulated the total Na^+ efflux rate constant from isolated rabbit jejunal epithelial cells as compared to sugar-free medium, but had no effect on this rate constant from isolated rabbit ileal cells The passive Na^+ efflux rate constant in the presence of glucose was not significantly different from that found with a sugar-free medium for both jejunal and ileal cells and there was no significant difference between the rate constants for jejunal and ileal cells

DISCUSSION

We have demonstrated that actively transported sugars, which increase the total Na^+ efflux rate constant from isolated jejunal epithelial cells, have no effect on this rate constant from isolated ileal epithelial cells Although there are marked differences in substrate requirements for ion transport between in vitro jejunum and ileum, the difference in the effect of actively transported sugars on Na^+ efflux from isolated jejunal and ileal epithelial cells cannot be attributed to the supplying of metabolic energy to jejunal cells Galactose, which is metabolized to a limited extent by rat jejunum [10], and as previously shown 3-*O*-methylglucose [7], which is not metabolized [10], result in significant stimulation of Na^+ efflux from rat jejunal cells,

while fructose, which can be utilized as a source of metabolic energy for ion transport by rat jejunum [9], has no effect on Na^+ efflux from jejunal cells Thus the increased active Na^+ efflux from isolated jejunal cells is dependent on whether or not the sugar is actively transported and not on its subsequent metabolic fate Also the failure of glucose to stimulate the total efflux rate constant from ileal cells cannot be due to a lack of metabolic substrate since glucose is utilized by ileal epithelial tissue as a source of metabolic energy for ion transport [11] The difference between jejunal and ileal cells cannot be attributed to an unusual characteristic of the rat intestine since identical results were obtained for isolated rabbit small intestinal epithelial cells Previous studies, using intact tissue from these species, have demonstrated that actively transported non-electrolytes stimulate Na^+ absorption in the ileum [4] Our results indicate that actively transported sugars, at least, do so by a different mechanism than that occurring in the jejunum This conclusion is further supported by the effect of phloridzin which blocks the stimulatory effect of galactose on the total Na^+ efflux rate constant from jejunal cells, but has no effect on the total rate constant from ileal cells in the presence of galactose

Fordtran et al [12] demonstrated that permeability of the upper small bowel was significantly greater than the lower small bowel in human subjects and related this difference to an effective pore size in the jejunum of approximately twice that of the ileum Their investigations apply to the intestinal mucosa as a single barrier and do not allow conclusions to be drawn concerning permeability of the intestinal epithelial cell itself The passive sodium efflux rate constant is determined by maximally inhibiting the $(Na^+ + K^+)$-ATPase sodium pump [7] and since there is no compelling evidence to indicate another mechanism for active Na^+ transport in the intestine [13] this rate constant can be interpreted as a measure of the passive outward leak or permeability of the cell membrane Our results show that passive efflux rate constants for isolated jejunal and ileal epithelial cells are not significantly different, suggesting that the variation in permeability between the proximal and distal small intestine documented by Fordtran et al [12] is a function of extracellular pathways and tight junctions rather than epithelial cell membrane permeability

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