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THE EFFECTS OF REMOVAL OF SODIUM IONS FROM THE MUCOSAL SOLUTION ON SUGAR ABSORPTION BY RABBIT ILEUM

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Summary

Net absorption and accumulation of D-galactose, β-methyl D-glucose and low concentrations of 3-*O*-methyl-D-glucose by sheets of rabbit ileum are observed even when Na^+ in the mucosal solution is replaced by choline This indicates that active sugar transport can occur in the direction opposite to the brush-border Na^+ gradient

There is considerable disagreement concerning the effects of Na^+ replacement in the solution bathing the mucosal surface of the small intestine on subsequent sugar absorption Perfusion studies in vivo [1] suggest that replacement of NaCl in the mucosal solution by either isotonic KCl or mannitol has no effect on glucose absorption by human, dog or rat ileum With suspensions of chick intestinal epithelial cells, removal of Na^+ from the external solution does not prevent active accumulation of D-galactose by cells which have been preincubated with Na^+ [2] On the other hand, several studies do suggest that the presence of a Na^+ gradient across the brush-border from mucosa-cell is essential for active sugar accumulation and absorption [3, 4]

Rinaldo et al [5] who re-examined this question recently, measured transmural flux from mucosal-serosal solution and from serosal-mucosal solution of 20 mM 3-*O*-methyl D-glucose across an in vitro preparation of rabbit ileum and concluded that when Na^+ is unilaterally replaced by choline in the mucosal solution, no significant active sugar absorption occurs

Since it has been shown that the asymmetry of brush-border sugar transport is decreased at higher sugar concentrations [6] and that asymmetry of the brush-border sugar transport system is a function of sugar affinity for the brush-border, (Holman, G D and Naftalin, R J , in preparation) it was

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TABLE I

Fluxes of sugars were measured at 30, 60 and 90 min after addition of the radioisotope as previously described [6,9]. The Ringer solutions were gassed continuously with 95% O_2, 5% CO_2 and were maintained at 37°C. M-S fluxes were measured with ^{3}H-labelled sugar, s-m fluxes with ^{14}C-labelled sugars. Identical sugar concentrations were placed in mucosal and serosal chambers. Following incubation, the tissues were washed in ice-cold choline chloride Ringer and then extracted for 4 h in 0.1 M HNO_3. The ratio R was obtained from the ratio of dpm, ^{3}H/dpm ^{14}C-labelled sugar extracted from the tissues. Tissue sugar concentration is calculated on the basis that it is present entirely within the cell space, cell water is obtained from the wet-dry weight of tissue less 30% for the extracellular fluid [9,14]. Ringer solution contains 140 mM NaCl, 10 mM $KHCO_3$, 0.4 mM KH_2PO_4, 2.4 mM K_2HPO_4, 1.2 $CaCl_2$, 1.2 mM $MgCl_2$; in choline/Ringer, choline chloride is substituted for NaCl. 3-*O* Methyl glucose and β-methyl glucose were obtained from Koch Light Ltd. ^{3}H, ^{14}C-labelled D-galactose, ^{14}C-labelled 3-*O* methyl glucose and ^{3}H-labelled glucose (used as starting material for synthesis of labelled β-methyl glucose) were all obtained from the Radio-Chemical Centre, Amersham. ^{3}H-labelled 3-*O* methyl glucose and ^{14}C-labelled β-methyl glucose were obtained from New England Nuclear Ltd., all the other chemicals were Analar grade. The rabbits used were 2–3 kg New Zealand white rabbits which were killed by intravenous injection of Nembutal. The terminal ileum was dissected immediately and stripped of its serosa and outer muscle layers before mounting as a flat sheet in the flux chambers. Statistics: the significance levels were calculated using Student's t test (unpaired means solution). * = $P < 0.05$, ** = $P < 0.02$, *** = $P < 0.001$ indicate the significance of the difference between the figure and that in the row above.

Mucosal solution Na^+ mequiv	Serosal solution Na^+ mequiv	Sugar	n	J_{ms} (μmol cm^{-2} h^{-1}) ± SEM	J_{sm} (μmol cm^{-2} h^{-1}) ± SEM	R ± SEM	Acc (mM) ± SEM	J_{net} (μmol cm^{-2} h^{-1})
140	140	20 mM 3-*O* methyl glucose	5	0.87 ± 0.19	0.206 ± 0.035	1.63 ± 0.67	38.4 ± 1.7	0.66 ± 0.17
0	140	20 mM 3-*O* methyl glucose	5	0.335 ± 0.38*	0.17 ± 0.031	0.507 ± 0.053	33.2 ± 0.965*	0.164 ± 0.025††
0	0	20 mM 3-*O* methyl glucose	4	0.334 ± 0.031	0.27 ± 0.033	0.30 ± 0.033	24.1 ± 1.3***	0.07 ± 0.015
140	140	2 mM 3-*O* methyl glucose	5	0.315 ± 0.05	0.009 ± 0.0012	2.73 ± 0.56	8.08 ± 0.63	0.305 ± 0.05
0	140	2 mM 3-*O* methyl glucose	6	0.067 ± 0.019***†	0.017 ± 0.002†	0.88 ± 0.08**	3.92 ± 0.27***	0.05 ± 0.02†††
0	0	2 mM 3-*O* methyl glucose	6	0.023 ± 0.003†	0.021 ± 0.004	0.32 ± 0.057†††	2.35 ± 0.14***†	0.0016 ± 0.0026†
140	140	2 mM galactose	5	0.553 ± 0.038	0.0068 ± 0.0008	3.85 ± 0.50	11.78 ± 0.69	0.55 ± 0.038
0	140	2 mM galactose	5	0.101 ± 0.021†**	0.01 ± 0.002	1.55 ± 0.20††	5.51 ± 0.98***	0.09 ± 0.02**
0	0	2 mM galactose	5	0.029 ± 0.005††	0.024 ± 0.005†	0.48 ± 0.085††	2.92 ± 0.32*	0.005 ± 0.001†
140	140	1 mM β-methyl glucose	7	0.347 ± 0.026	0.0027 ± 0.0004	12.43 ± 0.085	13.11 ± 1.3	0.34 ± 0.026
0	140	1 mM β-methyl glucose	5	0.072 ± 0.0156†	0.0048 ± 0.0011	4.89 ± 0.613	6.27 ± 0.626*	0.067 ± 0.015
0	0	1 mM β-methyl glucose	7	0.011 ± 0.0016††	0.0098 ± 0.0012†	1.05 ± 0.213†††	1.73 ± 0.264***†	0.0018 ± 0.0015 †††

TABLE Ib

The Na^+ concentration in the mucosal incubation bath was measured following 90 min incubation [Na^+] was estimated by flame photometry Tissue [Na^+] was calculated from the HNO_3 extracts From the mucosal [Na^+], net s-m Na^+ flux was calculated and found to be constant for all sugars tested and for all [sugar] tested

Initial concentration of Na^+ mequiv			Concentration of Na^+ mequiv in mucosal solution following 90 min incubation
Mucosal solution	Serosal solution	*n*	
0	0	16	0 95 ± 0 073
0	140	25	3 44 ± 0 17
			Tissue [Na^+] mequiv /litre cell water after 90 min incubation
0	0	16	4 55 ± 0 4
0	140	26	22 37 ± 0 92
140	140	13	44 61 ± 2 47

Calculated net S-M Na^+ flux 5 81 $\mu mol \cdot cm^{-2} \cdot h^{-1}$

considered worthwhile examining the effects of mucosal deprivation of Na^+ on the absorption of 3-*O*-methyl glucose at both high and low concentrations and on D--galactose and β-methyl-D-glucose (methyl β-D-glucopyranoside) transport (both these last sugars have higher affinities for the brush-border than 3-*O*-methyl glucose [7])

Table I shows that removal of Na^+ from the mucosal solution alone causes a significant reduction in m-s flux of 3-*O*-methyl glucose, at both 2 and 20 mM, 1 mM methyl glucose and 2 mM D-galactose It can also be seen that ratios of $^3H/^{14}C$-labelled sugar entering the tissue from the mucosal and serosal solutions respectively are all reduced by replacement of mucosal Na^+ by choline Except in the case of 20 mM 3-*O*-methyl glucose removal of mucosal Na^+ increases the serosal-mucosal sugar fluxes

Removal of Na^+ from both mucosal and serosal solution causes a further significant decrease in J_{ms} and net flux in all conditions except with 20 mM 3-*O*-methyl glucose and reductions in tissue accumulation and the specific activity ratio occur in all conditions

Additionally, there is a further increase in serosal-mucosal fluxes of ^{14}C-labelled sugars in all cases shown in Table I when Na^+ is removed from both serosal and mucosal solutions, compared with the effect of unilateral removal of Na^+ from the mucosal solution

The similarities between these results and those reported by Rinaldo et al [5], are that no changes in J_{ms}, net flux or J_{sm} of 20 mM 3-*O*-methyl glucose are observed when Na^+ is removed from both mucosal and serosal solutions, compared with fluxes observed when the Na^+ in the mucosal solution is unilaterally replaced However, significant reductions in J_{ms}, J_{net}, tissue accumulation and specific activity ratio of $^3H/^{14}C$-labelled sugars are observed in all conditions shown in Table I except with 20 mM 3-*O*-methyl glucose It can be seen (Table II) that no measurable effect of serosal Na^+ on 20 mM 3-*O*-methyl glucose transport is observed because the brush-border transport system is partially saturated and furthermore the optimal brush-border permeability asymmetry towards 3-*O*-methyl glucose is considerably less than for galactose or β-methyl glucose

The results indicate that, except with 20 mM 3-*O*-methyl glucose, when

TABLE II

The unidirectional fluxes were calculated according to the following relationship $P_{ij} = J_{ij}/C_i$ It is assumed that the fluxes are at steady-state and that the tissue behaves kinetically as a single compartment On this basis it can be calculated that $J_{12} = J_{31}$ $R + J_{13}, J_{21} = J_{31}(1 + R), J_{23} = J_{13}(1 + 1/R)$ and $J_{32} = J_{31} + J_{13}/R$ [9] where compartments 1, 2 and 3 are the mucosal, tissue and serosal fluid respectively

Mucosal solution Na^+ mequiv	Serosal solution Na^+ mequiv	Sugar	n	$cm \cdot h^{-1} \pm S\ E\ M$				$\frac{P_{12}}{P_{21}}$
				P_{12}	P_{21}	P_{23}	P_{32}	
140	140	20 mM 3-*O*-methyl glucose	5	0 064 ± 0 02	0 0156 ± 0 0063	0 040 ± 0 0064	0 044 ± 0 006	4 1
0	140	20 mM 3-*O* methyl glucose	5	0 021 ± 0 0026*	0 0076 ± 0 0012	0 030 ± 0 0022	0 042 ± 0 004	2 76
0	0	20 mM 3-*O*-methyl glucose	4	0 021 ± 0 0022	0 015 ± 0 0026*	0 062 ± 0 007**	0 071 ± 0 005**	1 4
140	140	2 mM 3-*O* methyl glucose	5	0 17 ± 0 023	0 0042 ± 0 0006	0 058 ± 0 01	0 073 ± 0 014	40 5
0	140	2 mM 3-*O* methyl glucose	6	0 041 ± 0 01***	0 008 ± 0 001*	0 034 ± 0 007	0 044 ± 0 007	5 1
0	0	2 mM 3-*O* methyl glucose	6	0 0147 ± 0 002*	0 0136 ± 0 003	0 042 ± 0 009	0 048 ± 0 007	1 08
140	140	2 mM galactose	5	0 289 ± 0 019	0 0027 ± 0 0003	0 050 ± 0 002	0 078 ± 0 0063	113 3
0	140	2 mM galactose	5	0 058 ± 0 011***	0 0048 ± 0 0009*	0 031 ± 0 0031***	0 036 ± 0 0025***	16 3
0	0	2 mM galactose	5	0 020 ± 0 0038*	0 012 ± 0 0017**	0 034 ± 0 005	0 045 ± 0 008	1 68
140	140	1 mM β-methyl glucose	7	0 38 ± 0 027	0 0028 ± 0 0005	0 030 ± 0 0028	0 031 ± 0 003	167 5
0	140	1 mM β-methyl glucose	5	0 096 ± 0 021***	0 0046 ± 0 0012	0 013 ± 0 0014**	0 019 ± 0 0026*	24 2
0	0	1 mM β-methyl glucose	7	0 022 ± 0 002**	0 012 ± 0 0016**	0 019 ± 0 0047	0 025 ± 0 005	1 95

the normal Na^+ gradient is reversed, significant net sugar transport against the direction of the Na^+ gradient remains.

The evidence for this statement is based on two independently measured variables, net flux and tissue accumulation, of three different non-metabolized sugars [8,9,10]

Table II shows the calculated unidirectional permeabilities obtained from the observed data according to the equations derived previously [9]

The data show, contrary to the predictions of the Na^+ gradient hypothesis, that the exit permeability of 3-*O*-methyl glucose, β-methyl glucose and galactose are all increased when intracellular [Na^+] is lowered, first by replacing mucosal Na^+ and increased still further by removing both mucosal and serosal Na^+. The permeability ratio of β-methyl glucose P_{12}/P_{21} is 24 when the distribution of Na^+ between the tissue water and mucosal solution is approximately 10 The Na^+ gradient hypothesis predicts that in steady-state, the distribution ratio of sugars, or the permeability ratio P_{12}/P_{21}, should not exceed the distribution ratio of [Na^+] (mucosal)/[Na^+] (cell) [11] Here it is observed that that the brush-border unidirectional permeability ratio to β-methyl glucose exceeds the predicted ratio by at least two orders of magnitude when the [Na^+] in the mucosal solution is replaced by choline

We observe in Table Ib that alteration of the sugar concentration, or type does not affect s-m Na^+ movement, yet in some cases the unidirectional permeability ratios of sugars across the brush-border are so high (Table II) that in order to satisfy the requirements of the Na^+ gradient hypothesis the [Na^+] in the mucosal unstirred layer would have to be higher than is, in fact, present in the serosal solution If this were the case, then, according to the Na^+ gradient hypothesis, 20 mM 3-*O*-methyl glucose should be accumulated, since 20 mM 3-*O*-methyl glucose is not accumulated when Na^+ is unilaterally removed from the mucosal solution, and since it is unlikely that the [Na^+] in the brush-border region will be very much higher than is present within the cell fluid, it can be inferred that latent reversal of the imposed Na^+ gradient across the brush-border following unilateral removal of Na^+ from the solution bathing the mucosal surface is an improbable explanation of the results presented here

It may be concluded from these results that although the presence of Na^+ in the mucosal solution accelerates sugar influx across the brush border, it is not essential for net sugar absorption and accumulation by the small intestine on the other hand, Na^+ is required to maintain the activity of the tissue Na^+ pump The reciprocal rise and fall of sugar entry and exit permeability on activation of the tissue Na^+ pump by increasing cell Na^+ is consistent with the view that actively transported sugars cross the brush-border by convective-diffusion the force generating this convective flow may arise from osmotic pressure gradients across the lateral-based border These are formed due to the action of the Na^+ pump which deposits hypertonic NaCl in the extra-cellular space [6,12,13,14]

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