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EVIDENCE FOR AN INTESTINAL Na^+: SUGAR TRANSPORT COUPLING STOICHIOMETRY OF 2.0

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Summary

Membrane potentials maintained by normally-energized intestinal epithelium interfere with an accurate determination of the Na^+: sugar coupling stoichiometry associated with Na^+-dependent transport systems. The interference is due to the fact that basal Na^+ influx is itself a potential-dependent event, and sugar transport induces a membrane depolarization which therefore modifies basal Na^+ entry. New information obtained under circumstances in which the membrane potential is maintained near 0 indicates that the true coupling stoichiometry is 2:1 rather than the commonly-accepted value of 1:1. A 2:1 stoichiometry means that cellular electrochemical Na^+ gradients are adequate to account for recently observed 70-fold sugar gradients maintained by these cells under certain conditions.

Intestinal absorption of solute molecules represents the sequential function of transport systems located at the mucosal and serosal boundaries of the intestinal epithelial cells. For sugars, net transport is accomplished by an Na^+-dependent concentrative system localized in the brush border membrane [1—3] followed by a passive 'downhill' flow of sugar via a serosally-localized facilitated diffusion system [4—6]. Under physiological conditions, the full gradient-forming capability of the Na^+-dependent system is precluded due to activity of the serosal carrier-mediated 'leak'. This constraint on optimal gradient formation is particularly significant because the capacity, V, of the serosal system (approx. 40 nmol · min^{-1} · mg $protein^{-1}$) [6] is greater than that of the mucosal carrier (approx. 15 nmol · min^{-1} · mg $protein^{-1}$) [7]. Recently, a number of agents have been found which limit the activity of the serosal carrier such as theophylline [8], phloretin [5, 8—10], bio-

flavanoids [11] and cytochalasin B [12]. The most potent of these are cytochalasin B and phloretin although the latter is less desirable because it induces secondary metabolic effects [8].

When serosal transport is limited with cytochalasin B, isolated intestinal epithelial cells prepared from chickens are able to maintain sugar gradients as large as 70-fold rather than the 10- to 15-fold gradients observed for control cells [12]. Gradients of this magnitude raise important questions regarding the energetics of the Na^+-dependent transport system. If the transmembrane difference in electrochemical potential for Na^+ is the sole driving force for Na^+-dependent sugar uptake, as is commonly believed, then the following relationship must be obeyed:

$$RT \cdot \ln \frac{[S]_i}{[S]_o} \leqslant \left(RT \cdot \ln \frac{[Na^+]_o}{[Na^+]_i} + F\Delta\psi\right)n$$

which indicates that the difference in chemical potential for sugar between the cell interior and medium must be no greater than the difference in electrochemical potential for Na^+. The coefficient, n, represents the number of sodium ions coupled to the flow of each sugar molecule. The mean value determined by a variety of techniques for $[Na^+]_o/[Na^+]_i$ for intestinal epithelium is approximately 4-fold [13—19]. A similar value is observed even when Na^+-selective microelectrodes are employed in order to determine Na^+-activity gradients [18]. When mucosal membrane potentials are monitored by microelectrodes, mean values of 36 mV (interior negative) are recorded for mammalian intestine [20]. The total electrochemical potential for Na^+ maintained by intestinal epithelium in situ is, therefore, approx. 77 mV. It is unlikely that isolated intestinal epithelial cells maintain a higher potential gradient for Na^+. However, the 70-fold sugar gradient which these cells maintain [12] represents a sugar potential gradient of 110 mV. This implies that either more than one sodium ion is coupled to the flow of each sugar molecule ($n > 1$) or that an energy input for sugar transport exists in addition to that represented by the electrochemical Na^+ gradient. There is no compelling evidence for the latter possibility. On the other hand, other investigators have reported that the Na^+: sugar coupling stoichiometry is 1:1 for rabbit intestine [1] and this is the value commonly assumed to apply to the transport mechanism, regardless of the species considered. Recent work with brush border membrane vesicles prepared from rabbit kidney was also considered consistent with a 1:1 stoichiometry [21].

A serious drawback to measuring the coupling stoichiometry successfully is the electrogenic nature of the transport process. Sugar-dependent Na^+ entry creates a diffusion potential which significantly disturbes the transmural and transmucosal electrical potential [20, 22—24]. To the extent that Na^+ entry via other routes is also electrogenic, this disturbance of the membrane potential represents a change in driving force for its entry. Earlier workers did not take the possibility of a sugar-induced change in basal (absence of sugar) Na^+ entry into account so that their estimates of sugar-induced Na^+ entry may be underestimated owing to having used too high a value for

basal Na^+ entry. This possibility is confirmed by the data presented in Fig. 1 in which basal Na^+ influx (absence of sugar) was monitored under conditions in which valinomycin was used to modify the membrane potential. Note that the basal Na^+ entry is markedly dependent on the potential, becoming larger as the membrane is hyperpolarized.

In order to circumvent this problem, we have measured the coupling stoichiometry under conditions in which a membrane potential is absent. Isolated chicken intestinal cells were depleted of Na^+ and ATP by incubation in an Na^+-free medium containing 20 μM rotenone [25]. The preincubation medium also contained 132 mM Tris-HCl (pH 7.4), 30 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mg/ml bovine serum albumin, valinomycin (1 μg/ml) and 200 μM phloretin. Valinomycin confers a high permeability to K^+ on the cell membrane, so that in a subsequent incubation, outward transfer of K^+ can occur in response to an influx of Na^+ and thereby preclude formation of a diffusion potential due to sugar-induced flow of Na^+. Phloretin was included to selectively inhibit the Na^+-independent serosal transport system [8]. These cells were then transferred to a medium containing 112 mM NaCl (with 1 μCi/ml $^{22}Na^+$), 30 mM KCl, 20 mM 3-*O*-[*Me*^{14}C]methylglucose (0.3 μCi/ml), 20 mM Tris-HCl (pH 7.4) and the same amounts of rotenone, phloretin and valinomycin as in the preincubation medium. Unidirectional influx of $^{22}Na^+$ and 3-*O*-[^{14}C]methylglucose were monitored for a 1-min interval in order to obtain values for total influx of each solute. In a parallel set, the incubation medium also included 200 μM phlorizin in order to inhibit that part of the

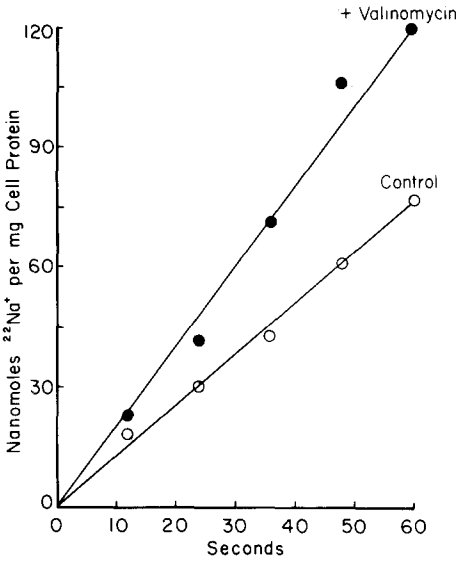

Fig. 1. The effect of a valinomycin-induced membrane potential on unidirectional $^{22}Na^+$ influx into isolated chicken intestinal cells. Cells were loaded with K^+ by pre-incubation for 20 min at 37°C in a medium containing 140 mM KCl, 20 mM Tris-HCl (pH 7.4), 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mg/ml bovine serum albumin and 20 μM rotenone. The pre-incubated cells were chilled to 4°C, centrifuged and resuspended in a K^+-free medium Tris-HCl substituted for KCl. At time zero, aliquots of the cells were transferred to a medium containing 120 mM NaCl, 40 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mg/ml bovine serum albumin and $^{22}Na^+$ (1.0 μCi/ml) in order to create an outwardly directed K^+ gradient. Influx of $^{22}Na^+$ was monitored ± valinomycin (1 μg/ml).

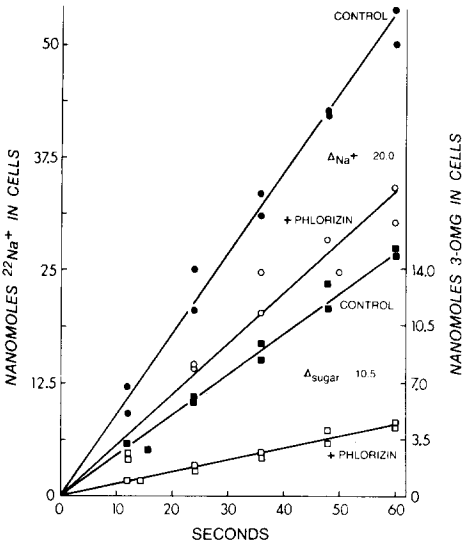

Fig. 2. Unidirectional influx of $^{22}Na^+$ and 3-*O*-[^{14}C]methylglucose into ATP-depleted isolated intestinal epithelial cells. The cells were depleted of Na^+ and ATP and equilibrated with K^+ as described in the text and Ref. 25. Each experimental run was performed in duplicate. Circles refer to $^{22}Na^+$ influx (left hand ordinate) and squares to 3-*O*-[^{14}C]methylglucose (3-OMG) influx (right hand ordinate). Solid symbols relate to control values, and open symbols to data obtained with phlorizin present in order to inhibit the Na^+-dependent sugar transport system. The indicated Δ values are equal to the difference in observed rates for Na^+ or sugar ± phlorizin. Phlorizin has no effect on Na^+ influx in the absence of sugar.

total flux which is mediated by the Na^+-dependent sugar carrier. The difference in flux (± phlorizin) for each solute expressed as a ratio ($\Delta Na^+/\Delta S$) therefore represents the coupling stoichiometry (n) for the sugar carrier. A representative data set is shown in Fig. 2 for which n = 1.9. There is considerable variability in total flux for each solute from one cell preparation to the next as shown in Table I, but the phlorizin-sensitive portion of each flux shows parallel changes such that the calculated coupling stoichiometry is always near 2.0. It is important to recognize that to the extent efflux of K^+ in reponse to Na^+ entry is unable to fully compensate for net charge-transfer, then one would expect this procedure to yield an underestimate of the coupling ratio. Indeed, when a similar experiment is performed using normally-energized cells rather than a preparation which has been ATP-depleted, the measured coupling ratio drops to approx. 1.0 (last line of Table I). A low value would be expected for energized cells if the Na^+ influx due to 20 mM 3-*O*-[^{14}C]methylglucose depolarizes the plasma membrane to an extent which disturbs basal Na^+ entry. That this might be the case is indicated from studies with intact tissue which show that high concentrations of transported sugars can decrease the membrane potential to an average value near −26 mV [13].

A coupling stoichiometry of 2.0 raises new conceptual ideas regarding the mechanism of intestinal transport. Until now, models have been considered in which either the free carrier or Na^+ sugar carrier complex bears a net electrical charge and is responsive to the membrane potential [18]. With two sodium ions involved, an anionic carrier (C^-) could load with sugar and

TABLE I

UNIDIRECTIONAL INFLUX RATES FOR Na^+ AND 3-O-[^{14}C]METHYLGLUCOSE (3-OMG)INTO VALINOMYCIN-TREATED, ATP-DEPLETED INTESTINAL EPITHELIAL CELLS

Expt. No.	$^{22}Na^+$ influx[a]			3-O-[^{14}C]methylglucose influx[a]			$\Delta Na^+/\Delta S$
	Control	+phlorizin[b]	ΔNa^+[c]	Control	+phlorizin	ΔS[c]	
1	46.6	33.7	12.9	12.0	4.8	7.2	1.8
2	67.2	44.5	22.7	17.0	5.2	11.8	1.9
3	76.0	50.3	25.7	19.5	8.4	11.1	2.3
4	101.2	72.8	28.4	28.2	11.5	16.7	1.7
5	81.0	52.0	29.0	24.2	6.8	17.4	1.7
6	50.2	40.0	10.2	8.7	3.2	5.5	1.9
7	44.2	33.0	11.2	8.9	3.9	5.0	2.2
						mean =	1.93
8[d]	110.0	68.8	41.2	43.5	10.7	32.2	1.3

[a]Flux values are given in nmol • min^{-1} • mg^{-1} cell protein; $[Na^+]$ = 112 mM, [3-OMG] = 20 mM.
[b]Phlorizin was added to a final concentration of 200 μM in order to inhibit the Na^+-dependent sugar transport system.
[c]The Δ values are the differences between control influxes and that observed with phlorizin present. It is therefore an estimate of that part of the total flux which is carried by the Na^+-dependent transport system.
[d]This experiment was performed with normally-energized isolated cells.

two Na^+ and be converted to a cationic form (Na_2CS^+) so that the membrane potential is important for driving the free carrier to the outer membrane face, and the loaded carrier to the inner membrane face. If the carrier form bearing a single Na^+ (NaCS) is completely impermeable, the transport system could bring about a theoretical equilibrium (in the absence of other sugar flux pathways) for which:

$$\frac{[S]_i}{[S]_o} = \left\{ \frac{[Na^+]_o}{[Na^+]_i} \cdot e^{\frac{VF}{RT}} \right\}^2$$

In this case, a 5-fold Na^+ gradient and membrane potential of −36 mV, would establish a maximal sugar gradient of nearly 400-fold. Our observed steady state gradients of 70-fold [12] are thus well within the capacity of a transport system driven solely by the electrochemical Na^+ potential and in which two Na^+ ions participate per carrier cycle.

It is interesting to note that Smith and Sepulveda [28] have recently reported indirect kinetic evidence that the alanine transport system in rabbit ileum may also exhibit an Na^+: solute stoichiometry of 2.0. Given these precedents, stoichiometry values greater than 1.0 may prove to be the rule rather than the exception for this tissue.

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