ON THE EFFICIENCY OF ENERGY CONVERSION IN SODIUM-DRIVEN D-GLUCOSE TRANSPORT ACROSS SMALL INTESTINAL BRUSH BORDER MEMBRANE VESICLES: AN ESTIMATION

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1. Introduction

Crane's mechanism for Na^+–substrate cotransport [1,2] found strong support in kinetic and other studies (reviewed [3]). However, the accumulation of D-galactose observed in isolated enterocytes in the absence of a Na^+ concentration gradient across the membrane [4,5] appeared at first to question the validity of Crane's concept, until it was recognized that the Na^+–monosaccharide transport across the brush border membrane is an electrogenic, and thus electro-driven, process [6–8]. Thus, the $\Delta\tilde{\mu}_{Na^+}$, rather than the $\Delta\mu_{Na^+}$, should be regarded as being the driving force of monosaccharide transport (of the glucose type), in addition to the conjugated one (reviewed [9,10]).

The question is now: does the $\Delta\tilde{\mu}_{Na^+}$ across the brush border membrane indeed account for the accumulation of D-glucose?

The availability of a very stable preparation of vesicles from this membrane makes possible, in principle at least, a comparison between this postulated additional driving force (i.e., $\Delta\tilde{\mu}_{Na^+}$) and the obtained accumulation of D-glucose.

The minimum thermodynamic requirements in Na^+-driven, electrogenic, cotransport of D-glucose are:

$$\nu\left(\frac{RT}{F}\ln\frac{A_{Na^+out}}{A_{Na^+in}} - \Delta\psi\right) \geqslant \frac{RT}{F}\ln\frac{A_{Glc\,out}}{A_{Glc\,in}} \qquad (1)$$

where: ν is the flux ratio; A indicates the activity of Na^+ or glucose in the medium (out) or in the intravesicular space (in), respectively; the other symbols have their usual meanings.

We assume now that $\nu = 1$ (for which there are indications from both kinetic [11–13] and stoichiometric [3] measurements), that the activity coefficients are such that concentrations can be substituted for activities and, finally, that the conditions for Goldman's equation to be valid are fulfilled. For the case that $\Delta\psi$ is imposed across the membrane as a diffusion potential (e.g., via an out > in gradient of NaSCN) eq. (1) becomes:

$$\frac{Glc_{in}}{Glc_{out}} \leqslant \frac{Na^+_{out}}{Na^+_{in}} \cdot \frac{Na^+_{in} + SCN^-_{out}\cdot P_{SCN^-}/P_{Na^+}}{Na^+_{out} + SCN^-_{in}\cdot P_{SCN^-}/P_{Na^+}} \qquad (2)$$

where P is the electrogenic permeability coefficient of the ion indicated in the subscripts.

2. Experimental

Brush border membrane vesicles were prepared from rabbit small intestine by the Ca^{2+} precipitation method [18] as in [14]. Uptake measurements were carried out as in [14,19]. The details are reported in the legends to the figures.

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3. Results and discussion

Equation (2) relates the concentrations of D-glucose, Na^+ and SCN^- during the short-lived plateau of the D-glucose overshoot. In the experiment of fig.1 the maximum uptake of glucose was reached after 15 s and amounted to 600 pmol/mg membrane protein. In order to express this uptake as concentration of intravesicular glucose, the volume of the intravesicular space at 15 s must be known. This space is, under equilibrium conditions (i.e., as determined from either the glucose or Na^+ uptake-values at long incubation time, 90 min) 1.2 μl/mg membrane protein. The experiment was designed so as to minimize osmotic volume changes; however, the vesicles shrank during the incubation by 10–15%, which indicated that the intravesicular volume at short incubation times was ~1.4 μl/mg membrane protein. (Changes in the intravesicular volume were determined in vesicles loaded with traces of labelled fructose, a sugar which rapidly equilibrates across the membrane via a Na^+-independent pathway. Data not shown.) On the other hand, as 35% of the total glucose in the incubation mixture at 15 s has been transferred into the intravesicular space, the glucose concentration in the outer medium (initially 20 μM) was, at 15 s, ~13 μM. As a result, the Glc_{in}/Glc_{out} ratio at 15 s (left term in eq (2)) is ~33.

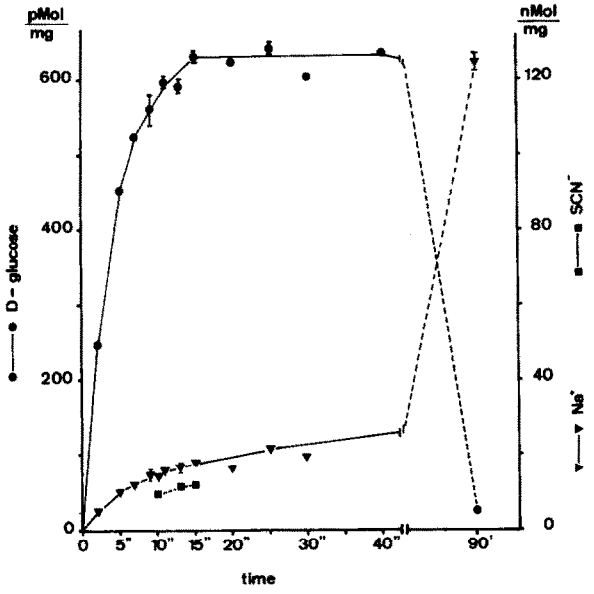

Fig.1. Uptake of Na^+ (▲), SCN^- (▪) and D-glucose (•) into membrane vesicles isolated from rabbit intestinal brush border. Vesicles were prepared by the calcium precipitation method [14,18] and suspended in 700 mM mannitol/20 mM Hepes/Tris (pH 7.5). At the start of the incubation, D-glucose and NaSCN were added to give the following final concentrations in the incubation medium: 500 mM mannitol; 20 mM Hepes/Tris (pH 7.5); 100 mM NaSCN; 20 μM D-glucose. Incubations were run at room temperature. At the indicated times, the incubation was stopped by injecting 100 vol. ice cold saline, followed by rapid filtration through 0.6 μm pore size nitrocellulose filters. The filter was washed with an additional 5 ml saline (details in [14]). D-glucose accumulation as a consequence of both the Na^+ concentration gradient and the potential (inside negative) initially present, reaches its maximum at 15 s. After 90 min all components have equilibrated. The values of SCN^- in the experiment shown were constantly below those of Na^+. This is at least partially explained by the findings that SCN^- is partially lost from the vesicles even if the washing is carried out at 0°C (experiments not shown).

By the same reasoning as in the above paragraph, the concentrations of Na^+ and of SCN^- in the intravesicular space could be estimated to be 13 ± 1 mM and 9 mM, respectively. The former figure (Na^+_{in}) is probably accurate, since the amounts of glucose and Na^+ associated with the vesicles at equilibrium (90 min) match closely: the Na^+_{out}/(Na^+ taken up at 90 min) and the Glc_{out}/(Glc taken up at 90 min) ratios in the experiment in fig.1 are nearly identical, and thus the loss during vesicle rinsing must be very small. The figure for intravesicular SCN^- concentration (9 mM) is on the other hand a lower limit, due to the probably significant losses of this highly permeant anion during vesicle rinsing: the SCN^-_{out}/(SCN^- taken up at 90 min) ratio is smaller than the corresponding ratios for Na^+ or glucose, data not shown. The actual intravesicular concentration of SCN^- at 15 s must therefore be > 9 mM, but < 13 mM, because not all of the Na^+ movement is electrogenic (see next paragraph). As to the concentrations of Na^+ and of SCN^- in the outer medium at 15 s, they can be considered to be identical with their original (high) values at time zero, i.e., 100 mM.

The ratio between the permeability coefficients P_{SCN^-}/P_{Na^+} could be estimated from the initial rates of uptake of Na^+ and of SCN^- under tracer exchange conditions with $\Delta\psi = 0$ (fig.2). The ratio between the two uptake rates tends to be an underestimate of the ratio to be inserted into eq. (2), because:

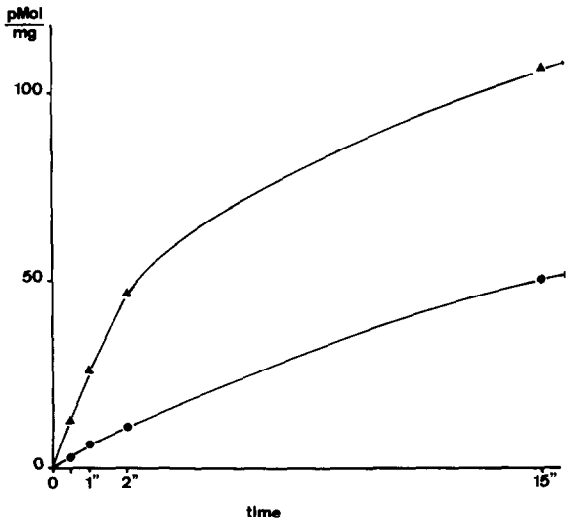

Fig.2. Determination of P_{Na^+} and P_{SCN^-}. The influx of radioactive tracers of Na^+ (•) and SCN^- (▪) was measured in vesicles which had been pre-equilibrated in 300 mM mannitol, 10 mM Hepes/Tris (pH 7.5) and 100 mM NaSCN. Uptake was measured with an automated incubator described in [19]. The tracer fluxes are virtually linear during the first 1 s. The ratio J_{SCN^-}/J_{Na^+} of the initial fluxes was 4.0 ± 0.2. Incubations were carried out at room temperature (~20°C). In parallel incubations (not shown) tracer influx was measured in vesicles containing no NaSCN inside at the start of the incubation. As expected from the principle of macroscopic electroneutrality, the two fluxes were then virtually identical.

(i) As pointed out above, the loss of SCN^- during vesicle rinsing leads to underestimation of SCN^- uptake and thus of P_{SCN^-}.

(ii) Only a part of Na^+ uptake is electrogenic, and obviously the electrogenic part only must be inserted in eq. (2).

This part can be grossly estimated by subtracting from the total Na^+ uptake the electroneutral Na^+/H^+ exchange, which may account $\lesssim 50\%$ of total Na^+ uptake (estimated from the data in [15]). The P_{SCN^-}/P_{Na^+} ratio thereby estimated is ~8.

Using this value one can now estimate the right-hand term in eq. (2) to be ~35–40, i.e., the observed glucose accumulation ratio (33, see above) is > 80% of the theoretical one. In terms of energy conversion, this would correspond to an energetic yield > 90%. Although care was taken to choose the most 'pessimistic' corrections, additional factors which may have escaped our attention, may lead to a lower yield. However, even a P_{SCN^-}/P_{Na^+} ratio much larger than that used in the calculation above, e.g., a ratio of 20, would lead to a high yield of energy conversion, i.e., of ~80%. Similarly high yields of energy transformation were arrived at if, rather than a constant electric field, linear concentration gradients of the individual ions across the membrane were assumed*. It seems clear, therefore, that the glucose–Na^+ cotransporter of the small intestinal brush border membrane is an efficient energy converter, at least under the conditions of the experiment in fig.1. The gradient of $\tilde{\mu}_{Na^+}$ fully accounts for the glucose accumulation observed in small-intestinal brush border vesicles. This conclusion adds one more piece of evidence supporting the validity of Crane's concept [1,2].

It should be mentioned that Heinz's group [16,17] has offered evidence, based on considerations of irreversible thermodynamics (rather than of classical thermodynamics, as here), of the validity of the Na^+–amino acid cotransport mechanism in Ehrlich ascites cells.

Our data allow one more calculation to be made. Thiocyanate is clearly an 'unphysiological' anion. The question can be raised, therefore, on how close the $\Delta\psi$ produced as a NaSCN diffusion potential (usually 100 mM in the medium, 0 in the vesicular space) compares with the $\Delta\psi$ normally present across the brush border membrane of surviving small intestine (i.e.,–35–40 mV [7,8]). At the beginning of the incubation, such as in the experiment of fig.1, Goldman's equation reduces to:

$$\Delta\psi = \frac{RT}{F} \ln \frac{P_{Na^+}}{P_{SCN^-}}$$

The initial $\Delta\psi$ produced by the NaSCN gradient is, therefore, ~–35–50 mV and compares favourably with the physiological membrane potential difference.

* If the $\Delta\psi$ is described by Henderson's equation, the accumulation ratio is given, rather than by eq. (2), by the following expression:

$$\frac{Glc_{in}}{Glc_{out}} \leqslant \frac{Na^+_{out}}{Na^+_{in}} \cdot \left(\frac{Na^+_{in} + SCN^-_{in} \cdot Q}{Na^+_{out} + SCN_{out} \cdot Q} \right)^{\left(\frac{Na^+_{in} - Na^+_{out} - Q\,(SCN^-_{in} - SCN^-_{out})}{Na^+_{in} - Na^+_{out} + Q\,(SCN^-_{in} - SCN^-_{out})} \right)}$$

where $Q = P_{SCN^-}/P_{Na^+}$

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