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The Na^+ gradient and D-galactose accumulation in epithelial cells of bullfrog small intestine

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SUMMARY

Extracellular and intracellular Na^+ and K^+ activities in epithelial cells of isolated bullfrog small intestine maintained at 25 °C in an oxygenated isotonic sodium sulfate Ringer solution (containing 102.4 mM Na^+ and 5 mM K^+) were used to compute transmucosal Na^+ chemical and electrochemical gradients ($\Delta\mu_{Na}$ and $\Delta\bar{\mu}_{Na}$) and the corresponding K^+ electrochemical gradient ($\Delta\bar{\mu}_K$) in these cells. These were compared with the reversible work ($\Delta\mu_{Gal}$) required to achieve a steady state intracellular concentration of D-galactose in the same solution containing 2 mM of this sugar. The average value found for $\Delta\mu_{Gal}$ was 3700 $J \cdot mole^{-1}$. Corresponding values (in $J \cdot equiv^{-1}$ of Na^+ or K^+) for $\Delta\mu_{Na}$, $\Delta\bar{\mu}_{Na}$ and $\Delta\bar{\mu}_K$ were 2700, 5800 and 5700. These results indicate that, under the conditions of our experiments, $\Delta\mu_{Na}$ alone cannot account for galactose accumulation unless the Na^+/galactose coupling ratio is significantly greater than 1. The energy available from $\Delta\bar{\mu}_{Na}$ is theoretically adequate but requires an efficiency of the order of 60% for a coupling ratio of unity. $\Delta\bar{\mu}_{Na}$ and $\Delta\bar{\mu}_K$ together require about 30% efficiency.

The Na^+ gradient hypothesis[1,2] has been widely invoked to explain the dependence on luminal Na^+ concentration of accumulative sugar transfer across the brush border of epithelial cells of small intestine[3]. According to this hypothesis, intracellular sugar accumulation is a consequence of the chemical or electrochemical Na^+ potential gradient between the luminal medium and the cell interior, the energy needed for uphill sugar transport being supplied, through appropriate coupling mechanisms, by a concomitant downhill movement of Na^+ from lumen to cell. In contrast to mechanisms which require that all or part of the energy for active sugar transport be obtained through a direct link to metabolism[3–5], the Na^+ gradient hypothesis, *pur sang*, does not necessitate any direct investment of metabolic energy in sugar accumulation. It envisages, rather, an indirect role for metabolism in which the Na^+ gradient is conserved by the maintenance of a low steady state intracellular Na^+ activity through the operation of a metabolically linked Na^+ pump in the lateral serosal membrane of the epithelial cell[3].

A necessary though not sufficient criterion for the validity of the Na^+ gradient hypothesis is that adequate energy be available from the chemical or electrochemical Na^+ potential gradient under a given set of conditions to account for the steady state cell/lumen sugar distribution ratio observed under the same conditions. Until now, estimates of the energy available from this source have been complicated by serious uncertainties about the distribution and activity of intracellular Na^+ in epithelial cells of small intestine[7]. This uncertainty stemmed from the fact that in cells where direct measurement of intracellular Na^+ activity was achieved[8–12], the apparent Na^+ activity coefficient was usually much lower than would be predicted from the apparent intracellular Na^+ concentration, assuming that all the intracellular Na^+ are osmotically active.

The development of cation selective glass microelectrodes capable of measuring accurately the intracellular activities of Na^+ and K^+ in intestinal epithelial[13] permits a more rigorous experimental definition of transmucosal chemical and electrochemical Na^+ and K^+ potential gradients in this tissue under various conditions. We have used a combination of extracellular and intracellular activity measurements together with mucosal membrane potentials to determine these gradients in isolated bullfrog (*Rana catesbeiana*) small intestine and to compare the energy available from them with the reversible work required to achieve a steady state cell/medium distribution of D-galactose under the same conditions.

Strips of tissue were maintained at 25 °C in an oxygenated Ringer solution containing 102.4 mM Na^+ and 5.0 mM K^+ and in which Cl^- was replaced by sulfate[14]. The solution contained 67.5 mmoles/l mannitol and had an osmolality of 204.3 ± 9.1 mosM (mean ±S.D.; $n = 12$). Osmolality was measured with an Advanced Instruments Inc. Model 64-31 osmometer.

Galactose uptake was determined following 2-h incubation at 25 °C in the same medium in which 2 mM D-galactose replaced an equivalent amount of mannitol. A trace amount of D-[1-^{14}C]galactose (New England Nuclear) was also added. Galactose was selected as an example of an actively transported sugar which is poorly metabolized by intestinal epithelia[15]. 2 mM was chosen on the basis of previous studies[16–18] which indicated that, at this concentration, galactose induced changes in cell volume, intracellular electrolyte concentrations, and the electrical parameters of the tissue should be relatively trivial. Following incubation, the mucosa were scraped off, weighed while wet, dried to constant weight, and extracted in 0.1 M HNO_3 as previously described[19]. Aliquots of the extract were taken for counting in a Packard Series 3375 liquid Scintillation counter. Aliquots of the bathing medium were brought through the same "extraction" procedure with HNO_3 and counted at the same time as the tissue extracts. Blanks prepared from the same medium, without radioactive galactose, were similarly prepared and counted.

Mucosal extracellular volume was estimated in a parallel set of experiments using inulin, labelled with a trace amount of [*carboxyl*-^{14}C]inulin (New England Nuclear) as an extracellular marker. The incubation time and general procedure adopted were as described above for galactose uptake.

The Na$^+$ activity of the bathing medium ($a^o{}_{Na}$) was determined by two methods. In one a single Na$^+$ selective electrode drawn from NaS 11-18 glass[20] and filled with 3 M NaCl was used in each experiment. These electrodes were calibrated in standard Na_2SO_4 solutions. The mean activity coefficients ($f_\pm$) of the calibrating solutions were calculated from the Debye–Hückel equation in the form

$$\log f_\pm = -\frac{A/Z_1Z_2/\sqrt{I}}{1+\sqrt{I}} + bI \qquad (1)$$

where I is the ionic strength, Z_1 and Z_2 are the valences of the cation and anion respectively, and A and b are constants at a given temperature. For our conditions[21], A was taken as 0.512,and b as 0.16/ mole^{-1}. It was found by direct measurement that, at the Na$^+$/K$^+$ concentration ratio of the medium employed (102.4/5.0), K$^+$ had no significant effect on the potential registered by the Na$^+$ electrodes. Hence $a^o{}_{Na}$ was determined from the equation

$$E = E_o + S \ln a_{Na} \qquad (2)$$

where E was the electrode potential in the medium and S was the slope of E as a function of $\ln a_{Na}$ in the calibrating solutions. E_o was determined by extrapolation to $\ln a_{Na} = 0$.

In the second method $a^o{}_{Na}$ was measured with a pair of NAS 27-4 glass[20] K$^+$ selective electrodes, filled with 3 M KCl and having K/Na selectivity coefficients (k_{KNa}). These electrodes were calibrated in standard Na_2SO_4 solutions and $a^o{}_{Na}$ was estimated from the equations

$$E_1 = E_o + S \ln (a_K + k_{ij}a_{Na} \qquad (3)$$

$$E_2 = E'_o + S' \ln (a_K + k'_{ij}a_{Na} \qquad (4)$$

As the outset, Eqns 3 and 4 were used to determined $a^o{}_K$ as well as $a^o{}_{Na}$. Since the values obtained were not significantly different from $a^o{}_K$ (2.5 mM) calculated from Eqn 1, the latter value was subsequently used to solve Eqns 3 and 4 for $a^o{}_{Na}$ and for calculating the transmucosal electrochemical K$^+$ potential gradient ($\Delta\bar{\mu}_K$). Mucosal membrane potentials (E_m) of the epithelial cells were measured as previously described[18].

The results obtained were as follows. $a^o{}_{Na}$ calculated from Eqn 1 was 50.2 mM. Its measured values (mean ± S.D. $n = 10$ in each case) were 41.2 ± 5.6 mM with Na$^+$ selective electrodes and 45.9 ± 3.6 mM with K$^+$ selective electrodes. These values did not differ significantly at the 0.05 confidence level and the overall observed average (42.6 mM) was used to calculate the transmural Na$^+$ chemical ($\Delta\mu_{Na}$) and electrochemical ($\Delta\bar{\mu}_{Na}$) potential gradients (Table I).

In 9 experiments total mucosal water (mean ± S.D.) was found to be 3.61 ± 0.29 g/g tissue dry weight. Assuming that the extracellular volume is equal to the volume of distribution of inulin at the end of 2-h incubation[17], this gave 3.09 ± 0.25 g/g dry

TABLE I

COMPARISON OF THE REVERSIBLE WORK ($\Delta\mu_{Gal}$) REQUIRED TO ACHIEVE A STEADY STATE CELL/MEDIUM GALACTOSE CONCENTRATION IN EPITHELIAL CELLS OF BULLFROG SMALL INTESTINE WITH THE MAXIMUM REVERSIBLE WORK OBTAINABLE FROM THE CHEMICAL ($\Delta\mu_{Na}$) AND ELECTROCHEMICAL ($\Delta\bar{\mu}_{Na}$) TRANSMUCOSAL Na^+ GRADIENTS AND THE CORRESPONDING K^+ ELECTROCHEMICAL GRADIENT ($\Delta\bar{\mu}_K$) UNDER THE SAME CONDITIONS

All values in joules/mole galactose or joules/equiv. Na^+ or K^+ transferred across the mucosal membrane. Parenthetical values for $\Delta\bar{\mu}_{Na}$ and $\Delta\bar{\mu}_K$ are based on an average E_m of 44 mV[18].

$\Delta\mu_{Gal}$	$RT \ln C_i/C_o$	3400
$\Delta\mu_{Na}$	$RT \ln a^i_{Na}/a^o_{Na}$	2700
$\Delta\bar{\mu}_{Na}$	$\Delta\mu_{Na} + E_m F$	5800 (6900)
$\Delta\bar{\mu}_K$	$RT \ln a^i_K/a^o_K - E_m F$	5700 (4500)

weight for total intracellular water. The average tissue/medium galactose concentration after 2-h incubation was 3.45 ± 0.41 ($n = 6$). When combined with the above values for total tissue and cell water this gave a mean intracellular galactose concentration (C_i) of 7.8 mM.

The average (± S.D.) mucosal membrane potential (E_m) found during this investigation was 32 ± 12 mV (number of animals 32: total number of acceptable penetrations 98). This was lower than the average E_m determined previously[18] in a more extensive study (44 ± 7 mV; $n = 266$). The difference may be of seasonal origin. The present experiments were done during winter and early spring. The earlier data were collected mainly between late spring and late fall.

When the data obtained in this investigation are combined with the average values previously found, under the same conditions, for intracellular Na^+ (a^i_{Na}) and K^+ (a^i_K) activities in epithelial cells of bullfrog small intestine (14.4 and 85.4 mM, respectively)[13] the results shown in Table I are obtained. It is apparent from Table I that, if one assumes a coupling ratio of unity (as found by Goldner *et al.*[22] for Na^+ coupled transport in isolated rabbit ileum) for Na^+ dependent galactose accumulation in bullfrog small intestine $\Delta\mu_{Na}$ alone cannot provide sufficient energy to account for the apparent steady-state value of C_i attained in our experiments. The energy deficit involved may in fact be greater than appears from Table I since C_i in these experiments was computed on the assumption that all the mucosal cell water is available as a solvent for galactose. This may lead to an underestimate of C_i since earlier studies in our laboratory[13] suggest that a significant fraction (about 15%) of the cell water is not available as a solvent for intracellular ions.

In principle (Table I) $\Delta\bar{\mu}_{Na}$ could provide sufficient energy for galactose accumulation but, for a 1:1 Na^+/galactose coupling ratio, the efficiency required is in excess of 50%. If, as has been suggested[3], $\Delta\bar{\mu}_{Na}$ and $\Delta\bar{\mu}_K$ can function together as a source of energy for intestinal sugar accumulation, the required efficiency of the coupling mechanism is, according to the data of Table III, about 30%. If the coupling ratio for Na^+ dependent galactose transport in bullfrog small intestine is greater than unity the required efficiencies would be correspondingly less.

There is as yet little direct information concerning the thermodynamic efficiency of coupled transport processes. Geck *et al.*[23] investigated the coupling efficiency between Na^+ and α-aminoisobutyrate influxes in Ehrlich cells and concluded that the maximum efficiency attainable is 7%. If this value is representative of coupling efficiencies for Na^+ dependent transport of nonelectrolytes our results (Table I) might be interpreted as indicating that the Na^+ gradient hypothesis for intestinal sugar transport is energetically implausible. However, Ronquist *et al.*[24] have presented data which suggest that the degree of coupling between Na^+ and α-aminoisobutyrate transport in Ehrlich cells may not be typical of Na^+ dependent amino acid transport in general. Thus, at this time, maximal coupling efficiencies significantly greater than 7% cannot be ruled out for Na^+ dependent amino acid and/or sugar transport in small intestine.

The results reported herein point up the desirability of combined estimates of ionic activity gradients and coupling efficiencies in the same tissue under the same conditions. Such studies cannot of course provide proof that ionic gradients are in fact the sole driving forces for specific coupled transport processes but they can at least furnish reasonably accurate information concerning a *sine qua non* of all ion gradient hypotheses: the energetic adequacy of the designated ionic gradient for the task assigned to it by the hypothesis.

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