Transport of sugars or amino acids increases potassium efflux from isolated enterocytes

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Received 8 September 1983

A technique to isolate epithelial cells from rabbit jejunum using hyaluronidase is described. The cells obtained retained their abilities to accumulate sugars and potassium (^{86}Rb) against concentration gradients. Potassium efflux was monitored using cells preloaded with ^{86}Rb and the rate constant of efflux was seen to increase when actively transported sugars or amino acids are added to the bathing medium. The increase is related to the transport of the non-electrolyte, but not to volume regulatory events.

Rabbit *Enterocyte* *Sugar transport* *Amino acid transport* *Potassium transport* *Small intestine*

1. INTRODUCTION

Sugars or amino acids are cotransported with sodium ions into mature enterocytes across their brush border membrane [1,2]. Intracellular concentrations are maintained by the removal of sodium via the sodium-potassium pump located at the basolateral membrane, whilst the organic substrates are transported across the same membrane by virtue of their concentration gradients. Recent electrophysiological evidence, obtained mainly from tight epithelia, suggests that increased sodium entry across the brush border membrane may effect an increase in the potassium permeability of the basolateral membrane [3]. As early as 1962 [4] it was demonstrated that actively transported sugars do cause a decrease in the potassium content of intestinal tissue and more recently it has been shown [5] that the potassium permeability of the membranes of isolated rat hepatocytes is increased in the presence of alanine and other neutral amino acids.

Using isolated mature enterocytes from rabbit jejunum we have been able to demonstrate directly that sugars and amino acids which are transported across the brush border membrane cause an increase in potassium efflux, possibly through the operation mechanisms that are similar to calcium-dependent potassium channels.

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2. METHODS

2.1. *Animals*

Adult New Zealand White Rabbits (2.5–3.0 kg) of both sexes were used throughout.

2.2. *Cell isolation*

Cells were isolated from rabbit jejunum using hyaluronidase in a manner similar to that described for chicken intestine [6,7]. A rabbit was killed by an intravenous injection of sodium pentobarbitone, and a section of jejunum, about 20 cm long, was quickly removed, everted, tied at both ends and washed in phosphate-buffered saline containing 0.1 mM DL-dithiothreitol. The washed loop was then transferred to a buffer containing 1.5 mg/ml hyaluronidase (Sigma, type III) and 1 mg/ml bovine serum albumin (Sigma, fraction V) and incubated at 37°C for 20 min in a shaking water bath. The buffer used in this and subsequent incubations had the following composition (mM): NaCl, 75; $NaHCO_3$, 25; $CaCl_2$, 1.3; $MgCl_2$, 0.5;

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00145793/83/$3.00

K_2HPO_4, 0.36; KH_2PO_4, 0.44; KCl, 5; β-hydroxybutyrate, 0.5; D-mannitol, 63; HEPES, 10, pH 7.2. After the 20-min incubation the intestinal loop was transferred to hyaluronidase-free buffer where cells were released by gentle agitation with a plastic pipette tip. The cells were filtered first through gauze and then through a nylon sieve (200 μm mesh) before being washed twice.

2.3. *Uptake measurements*

Potassium or sugar uptake were measured at 37°C by mixing a prewarmed cell suspension with prewarmed incubation medium (final concentration 2–3 mg cell protein/ml) containing ^{14}C-labelled α-methyl-D-glucoside or ^{86}Rb, a suitable radioactive tracer for potassium (final radioactive concentrations of 0.4 μCi/ml and 0.5 μCi/ml, respectively), and [^{3}H]inulin (1.5 μCi/ml) as an extracellular space marker. Uptake was terminated by diluting 500 μl cell suspension in 500 μl ice-cold buffer and cells separated by centrifugation (10000 × g, 20 s) through a layer of oil (250 μl of di-*n*-butyl phthalate:dinonyl phthalate 3:2, v/v). The cell pellets were lysed in 0.5% (v/v) Triton X-100 and counted by liquid scintillation after protein precipitation. The amount of potassium or α-methyl-D-glucoside taken up was calculated taking into account the trapped extracellular volume estimated from the [^{3}H]inulin counts.

The intracellular volume of the cells was estimated by measuring the uptake of another sugar, 3-*O*-methyl glucose in the presence of 0.1 mM phloridzin. Under these conditions it was assumed that the cells only take up the sugar until its concentration in the intracellular water is equal to that of the bathing medium [8]. Knowing the concentration of 3-*O*-methyl glucose in the medium and the amount of sugar in the cells the intracellular volume was calculated.

2.4. *Efflux measurements*

To measure the release of ^{86}Rb from rabbit jejunum-isolated intestinal cells, a concentrated (60–120 mg cell protein/ml) suspension of enterocytes was preloaded by incubating at 37°C for 20 min in the presence of ^{86}Rb (10–15 μCi/ml). The rate of ^{86}Rb loss from the cells was then measured by diluting aliquots of this suspension of preloaded cells 100-fold into radioisotope-free buffer and incubating at 37°C. Samples (500 μl) were taken and the incubation terminated as in the uptake measurements. Under these conditions the extracellular ^{86}Rb carried with the pellet is negligible. Cell pellets were counted, after cutting the tips of the centrifuge tubes, in a scintillation spectrometer. Results are expressed as percent ^{86}Rb remaining in the cells. 100% is the initial radioactivity obtained by extrapolation on the assumption that ^{86}Rb loss is described by a single decreasing exponential.

3. RESULTS

Preparations of epithelial cells from mammalian small intestine have not previously been successfully employed in transport studies. The cells used here accumulated α-methyl-D-glucoside (fig.1b), a

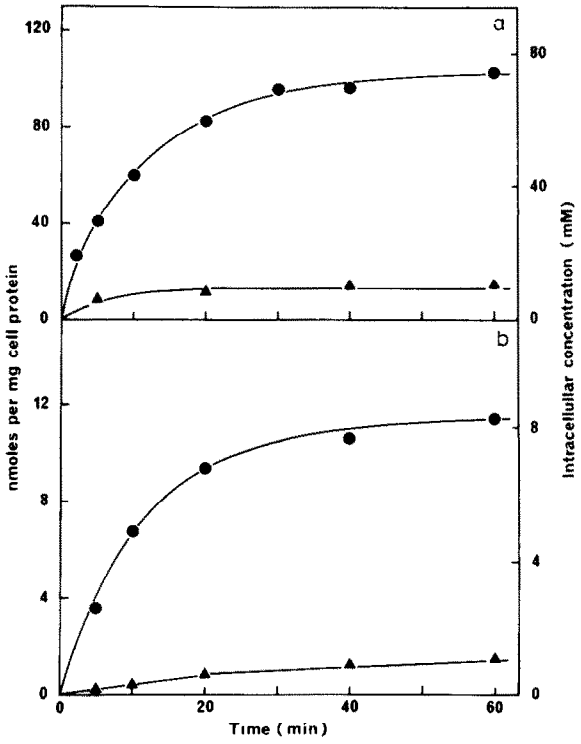

Fig.1. Potassium and α-methyl-D-glucoside accumulation by isolated rabbit enterocytes. (a) Potassium [^{86}Rb] uptake (●), external potassium concentration, 5.8 mM, and potassium uptake in the presence of 0.1 mM ouabain (▲). (b) The uptake of α-methyl-D-glucoside in the presence (▲) or absence (●) of the competitive inhibitor phloridzin (0.1 mM), the external sugar concentration was 0.1 mM.

non-metabolisable sugar which is transported at the brush border mainly by a sodium-dependent hexose transport system [9] and this uptake was greatly depressed by phloridzin, a competitive inhibitor of this system [10]. Accumulation of sugar in the intracellular space took place with an intracellular concentration, about 80-times that in the bathing medium. These findings suggest that the cells retain their active transport capabilities as does the result obtained when they were incubated in the presence of ^{86}Rb; the enterocytes accumulated the isotope (fig.1a) and a steady state was approached after about 20 min. Ouabain, an inhibitor of the sodium-potassium pump, reduced this accumulation by about 85%.

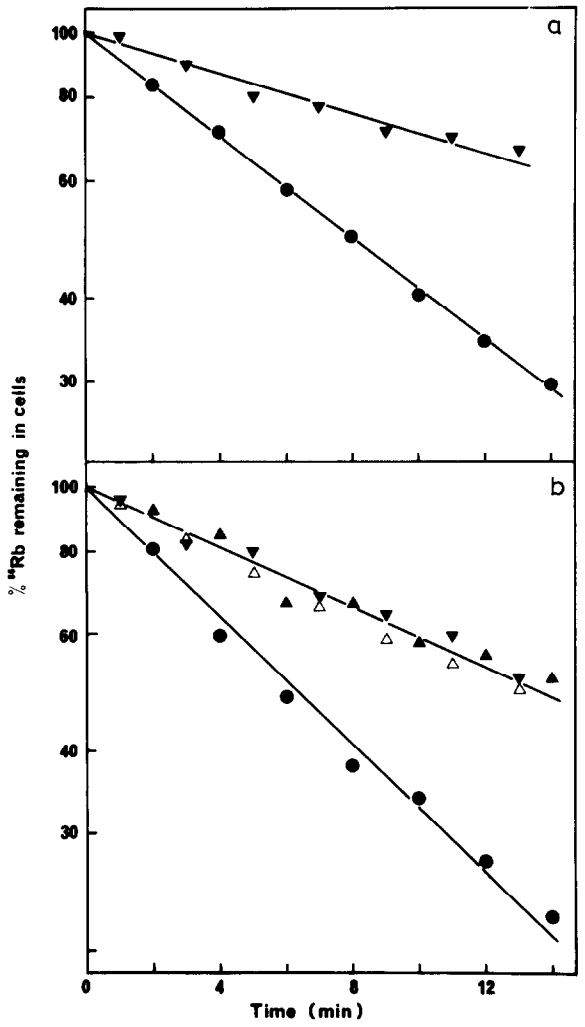

Fig.2. Release of ^{86}Rb from rabbit jejunum-isolated intestinal cells. (a) Loss of ^{86}Rb in the presence of 20 mM D-mannitol (▼) or 20 mM L-alanine (●). (b) Loss of ^{86}Rb from isolated enterocytes in the presence of 5 mM D-mannitol (▼), 5 mM α-methyl-D-glucoside (●), 0.1 mM phloridzin + 5 mM D-mannitol (△) or 0.1 mM phloridzin + 5 mM α-methyl-D-glucoside (▲).

Cells loaded with ^{86}Rb were used to monitor potassium efflux; fig.2 shows that the efflux apparently follows first order kinetics. The calculated rate constant for efflux ranged from 0.013 to 0.059 min^{-1} (0.035 ± 0.013 mean ± SD) in 29 different cell preparations although there was little variation between replicate determinations in a single batch of cells.

The addition of L-alanine (fig.2a) or α-methyl-D-glucoside (fig.2b) to the bathing medium produced substantial increases, of up to 240%, in the rate of potassium release. Phloridzin abolished the sugar-induced stimulation of potassium release (fig.2b), illustrating that the increase is related to the transport of the sugar and not merely to its presence in the extracellular fluid. The intracellular volume of the cells, as measured with 3-*O*-methyl glucose, does not appear to increase in the presence of 20 mM alanine, ruling out the possibility that the increase of potassium efflux is a result of cellular swelling [11]. A value of 1.38 μl/mg cell protein for intracellular volume was used to calculate intracellular concentrations in fig.1a,b.

4. DISCUSSION

Previous attempts to isolate viable epithelial cells from mammalian small intestine have been unsuccessful. We here show that rabbit enterocytes can be isolated by hyaluronidase treatment and that they are capable of active transport of nutrients and ions for at least 1 h after isolation. Accumulations of α-methyl-D-glucoside of the magnitude reported here have been obtained before, only in isolated enterocytes from chicken intestine [9]. We have demonstrated a direct effect of actively transported sugars and amino acids upon release of potassium from intestinal epithelial cells. The cotransport of such nutrients with sodium is known to depolarize the membrane by about 6 mV from a membrane potential of about −36 mV [12,13]. Such a decrease could be expected to yield an increase in efflux of only 15% if the membrane permeability to potassium re-

mains constant [14,15]. Since the size of the increases in rate constant we observed are of a much greater magnitude, a change in the potassium permeability of the membrane must be envisaged.

The presence of calcium-dependent potassium channels has been postulated in many cell types [16–18] where an elevation of cytoplasmic levels of calcium brings about an increase in membrane permeability to potassium ions. The presence of such channels has not been demonstrated in the small intestine, although they are known to exist in other transporting epithelia [19,20]. Preliminary experiments with isolated rabbit enterocytes show that large and sustained increases in the rate of potassium release are provoked by the calcium ionophore A23187 (10 μM). This increase in efflux is abolished by the bee venom neurotoxin apamin (0.5 μM, Sigma) suggesting that these cells possess calcium-dependent potassium channels, similar to the apamin-sensitive channels described in hepatocytes [16]. Apamin also abolishes the effects of L-alanine and α-methyl-D-glucoside on potassium efflux, indicating perhaps a role for these calcium-dependent potassium channels in these processes.

The results reported here show that uptake of sugars of amino acids by rabbit enterocytes invokes an increase in their membrane permeability to potassium ions. The nature of the cellular signals that couple entry of organic substrate and sodium to changes in permeability to potassium remains to be determined, as has the importance of the described phenomena in the partial repolarization of the membrane of intestinal cells following the initial amino acid or sugar-induced depolarization [21,22]. The increase in potassium permeability observed here may have a physiological role in maintaining an electrical potential favourable to non-electrolyte transport in the small intestine.

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