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Calcium movements accompanying the transport of sugar or amino acid by rabbit enterocytes

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Isolated rabbit enterocytes can be loaded with radioactive calcium most of which is presumably in intracellular stores. In the presence of sugar or amino acid there is a transient loss of calcium, followed by replenishment. It is suggested that this movement might be related in the signalling leading to increased potassium permeability observed in enterocytes transporting sugar and amino acids.

Recent radioisotope flux and patch-clamp studies have provided evidence for the presence in rabbit small intestinal epithelial cells of a potassium permeability pathway activated by intracellular calcium ions [1–3]. Increases in potassium permeability of enterocytes of *Necturus* and rabbit have also been observed during sodium-coupled sugar or amino acid transport [1,4]. Although it has been proposed that this change in permeability is mediated by calcium-activated potassium channels [2] the link between the two phenomena, which occur initially at opposite poles of the cell, remains obscure. The present report attempts to explore the hypothesis that either intracellular calcium mobilisation or entry of extracellular calcium might be the signal leading to the increase in potassium permeability occurring during sodium-coupled non-electrolyte transport in rabbit enterocytes.

It has often been reported that calcium accumulated within intracellular organelles can be observed to be released from cells stimulated by suitable agonists which raise cytoplasmic calcium ($[Ca_i^{2+}]$) [5,6]. In the present work we have preloaded isolated enterocytes with ^{45}Ca to see whether transport of sugars or amino acids would also be accompanied by a similar efflux of intracellular calcium.

A 20-min incubation of enterocytes with ^{45}Ca was sufficient to allow a complete exchange of the extracellular isotope with the intracellular pool of calcium, a high proportion of which is stored in cellular organelles [7]. When 20 mM alanine or 5 mM α-methyl-D-glucoside were added to these cells they caused a rapid loss of cellular ^{45}Ca (Fig. 1, curves a and b). This release, which was presumably due to the mobilisation of Ca from intracellular stores, seemed to be related to the transport of the amino acid or sugar since mannitol, a monosaccharide which is not cotransported with sodium, had little effect on ^{45}Ca distribution (curve c in Fig. 1). Although beyond the resolution of the present method, the mobilisation of calcium stores could be expected to elevate $[Ca_i^{2+}]$ by the submicromolar amounts thought necessary for the gating of potassium channels [8,9].

The intracellular stores of calcium also appeared to be mobilised by 10 μM A23187 (curve a in Fig. 2). The time-course was similar to that described by the same ionophore used with

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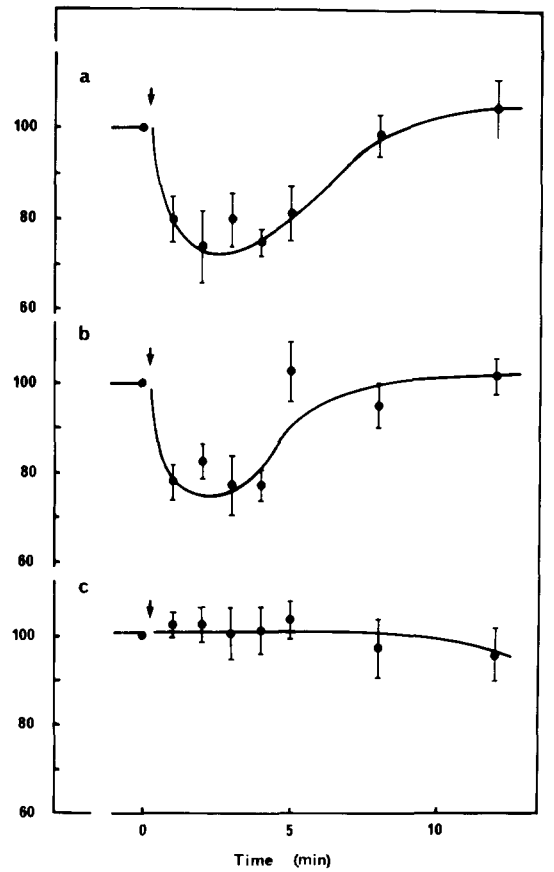

g. 1. ^{45}Ca content of isolated enterocytes in the presence of methyl-D-glucoside, alanine and mannitol. Curve a shows the fect of 5 mM α-methyl-D-glucoside, added from a 1 M stock the arrow, on the ^{45}Ca content of isolated enterocytes which ıd been equilibrated with the tracer for 20 min up to zero-time. ıe individual points expressed as a percentage of the initial ıunts in the cells give the mean ± S.E. of five experiments. ırve b shows the effect of the 20 mM L-alanine, the results e the mean ± S.E. of four experiments. Curve c shows the fect of 20 mM mannitol; mean ± S.E. of four experiments. ethod: a suspension of cells (1 mg cell protein/ml), isolated ı hyaluronidase treatment of rabbit jejunum [14], was loaded th ^{45}Ca (1 μCi/ml) for 20 min, in a modified Hanks' edium which also contained 2 μCi/ml [^{3}H]inulin. Duplicate 0-μl samples of suspension were mixed with 1 ml of ice-cold ıffer layered on top of 250 μl oil (di-*n*-butylphthalate and nonylphthalate, 3 : 2, v/v) in microcentrifuge tubes. The tubes ere immediately centrifuged (20 s at 10000 × *g*) and the nount of ^{45}Ca initially in the cells calculated from the counts the pellets. At zero time, α-methyl-D-glucoside, alanine or annitol were added to the cells as concentrated solutions, to ve final concentrations of 5, 20 and 20 mM, respectively. The nount of ^{45}Ca in the cells was followed for a period of 12 in, taking 200-μl aliquots for assay as above. Samples of the pernatant and of the pellets, extracted using Trion X-100 and ecipitated with trichloroacetic acid as described elsewhere 4], were counted by liquid scintillation spectrometry. Any extracellular ^{45}Ca associated with the pellet was corrected for by using the [^{3}H]inulin counts. The intracellular Ca content is expressed as a percentage of that found in the initial sample taken before additions had been made.

hepatocytes [5]. In the latter case, however, there was an initial transient rise in cellular ^{45}Ca and this was presumably related to a ^{45}Ca influx through the hepatocyte plasma membrane taking place before the ionophore affects intracellular membranes. That this rise was not seen with enterocytes may have been because the increase was below the resolution of the method, or because both the mobilisation of the internal stores and the increase in membrane permeability occurred simultaneously. Addition of solvent alone failed to provoke any change in amount of ^{45}Ca associated with the cells (curve b in Fig. 2), a finding which confirms that isotope exchange had been completed during the preincubation period. There did not appear to be any replinishment of intracellular ^{45}Ca in the presence of the calcium ionophore.

Potassium efflux was measured from cells bathed in nominally calcium-free buffers in an attempt to assess the dependence of the amino acid or sugar induced increase in potassium efflux upon external calcium. Under these conditions the cells became more permeable to potassium (Table I) and potassium efflux remained unaffected by the presence of alanine. These experiments do not prove the importance of external calcium, however, because even if alanine did have an affect this would probably have been masked by the high basal rate of potassium efflux. The removal of calcium from buffers bathing isolated rat pancreatic acinar cells is known to increase the permeability of their membranes [10] and the same seems to occur with enterocytes [7]. Attempts to use lanthanum as a calcium substitue to maintain normal basal potassium permeability, as suggested for smooth muscle cells [10], proved unsuccessful with enterocytes (results not shown).

If it is assumed that after preloading ^{45}Ca is uniformly distributed in the intracellular water [2], the calculated intracellular calcium concentration would be of the order of 1 mM. This is inconsistent with generally accepted calcium levels in other epithelial cells, which are around 100 nM [12,13], strongly suggesting intracellular binding or

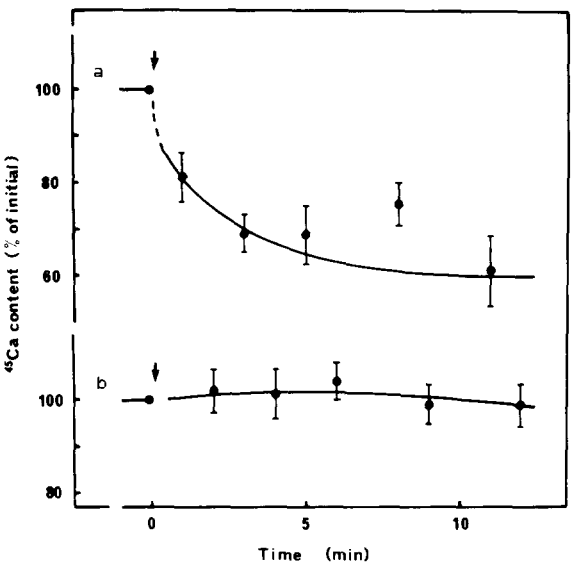

Fig. 2. Calcium-ionophore effect on ^{45}Ca content of pre-loaded isolated enterocytes. A234187 (10 μM) was added, at the time shown by the arrow, from a 10 mM stock, to cells preloaded in ^{45}Ca (curve a). Points are expressed as a percentage of the initial content and are means $\pm$ S.E. of four experiments. Curve b shows that the addition of solvent (dimethylsulphoxide) alone does not affect ^{45}Ca content of the cells ($n = 4$). Other details as in legend to Fig. 1.

sequestration. Experiments with enterocytes whose plasma membrane had been rendered highly permeable show that intracellular stores are capable of accumulating large amounts of calcium in a nonmitochondrial compartment that has been proposed to be the endoplasmic reticulum [7]. It is possible that efflux of ^{45}Ca from preloaded enterocytes seen in the present work might represent mobilisation from this kind of intracellular store followed by pumping out of the cells via the calcium pump. The time-course of this efflux is similar to that seen with α-adrenergic agonists in isolated hepatocytes [5]. The apparent ability of the ionophore A23187 to mobilise intracellular calcium is in keeping with previous studies [5,7] and supports the view that calcium is in a membrane enclosed space rather than simply bound to membranes or soluble proteins.

In summary, we have demonstrated that the increase in potassium efflux caused by sugars or amino acids, is accompanied by release of calcium from intracellular stores. Further work will now have to be undertaken to ascertain how this release

TABLE I

EFFECT OF EXTERNAL CALCIUM REMOVAL ON ALANINE-PROVOKED INCREASE IN POTASSIUM EFFLUX

Potassium efflux was measured after preloading enterocytes with ^{86}Rb as described previously [14]. Values are rate constants for single-exponential decrease in intracellular ^{86}Rb. The alanine concentration was 20 mM while control incubations contained 20 mM mannitol. Results are the means $\pm$ S.E. of four experiments.

Rate constant (min^{-1})			
Control	+ Alanine	− Calcium	− Calcium + Alanine
0.043 ± 0.008	0.078 ± 0.012	0.092 ± 0.004	0.085 ± 0.008

is brought about and to test whether it is causally related to changes in potassium permeability. The involvement of secondary messengers, possibly produced in response to changes in membrane potential or cell volume cannot be discounted.

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