Biochimica et Biophysica Acta 896 (1987) 113–116
Elsevier

BBA 70267

BBA Report

Inositol trisphosphate and calcium mobilisation in permeabilised enterocytes

A. Ilundain *, J.A. O'Brien, K.A. Burton and F.V. Sepúlveda

AFRC Institute of Animal Physiology, Babraham, Cambridge (U.K.)

(Received 30 June 1986)

Key words: Intestinal transport; Ca^{2+} transport; Inositol trisphosphate; (Chicken intestine)

Saponin-permeabilised epithelial cells isolated by hyalurodinase incubation from chicken small intestine were used to study ^{45}Ca uptake into intracellular stores. At low ($6.7 \cdot 10^{-7}$ M) free Ca^{2+} concentration most of the Ca^{2+} appears to be taken up into non-mitochondrial stores, whilst the mitochondria seem to play a major role at high ($2 \cdot 10^{-5}$ M) Ca^{2+} concentration. Addition of inositol trisphosphate (IP_3) causes a rapid and reversible release of ^{45}Ca from non-mitochondrial stores, with a half-maximal effect of approximately 1 μM.

The importance of cytoplasmic Ca^{2+} in the regulation of intestinal transport has been well established. Studies with both small and large intestine have demonstrated that manoeuvres supposedly affecting cytoplasmic Ca^{2+} concentration alter transepithelial Na^+ and Cl^- transport [1,2]. Work with isolated intestinal cells or perfused intestine has also demonstrated a dependence of K^+ fluxes upon intracellular Ca^{2+} [3–5]. The free Ca^{2+} concentration of the cytoplasm is thought to be determined by the interplay between the rate of Ca^{2+} entry from the extracellular space, the pumping of the Ca^{2+} out of the cell through the plasma membrane and the action of intracellular buffering systems. The role of the plasma membrane systems has been studied using isolated plasma membrane vesicles [6]. The ability of intracellular organelles of intestinal cells to act as stores or buffer systems for Ca^{2+} has been explored using cells whose plasma membrane had been previously rendered highly permeable [7,8] or in subcellular membrane fractions [9,10]. No information is available, however, on the possible ways by which external stimuli could modify intracellular Ca^{2+} in transporting enterocytes. It has recently become apparent that inositol 1,4,5-trisphosphate (IP_3) is produced as a response to external stimuli in various cell types and that its liberation into the cytoplasm is followed by Ca^{2+} mobilization from intracellular stores [11,12] but no information on this is available for intestinal epithelial cells. In the present report we demonstrate that inositol 1,4,5-trisphosphate is capable of releasing Ca^2, probably accumulated in a non-mitochondrial store, in transporting intestinal epithelial cells with highly permeable plasma membrane.

White Leghorn chickens 5–10-weeks old were used throughout. Intestinal cells were isolated as described in Ref. 13 with minor modifications. After isolation cells were washed twice in a modified Hank's buffer and then immediately suspended in a high K^+ medium of the following composition (mM): 120 KCl, 10 $MgCl_2$, 1.2 KH_2PO_4, 1 EGTA, 0.5 mg/ml bovine serum albumin (BSA) and 25 Tris-Hepes (pH 7.2), that

* Permanent address: Departamento de Fisiologia Animal, Facultad de Farmacia, Seville, Spain.

Correspondence: Dr. F.V. Sepúlveda, AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

contained 50 μg/ml saponin and incubated for 5 min at 37°C. This incubation was terminated by washing in saponin-free, high potassium medium. The cells obtained in this way are defined as permeabilised cells.

Ca^{2+} transport experiments were performed in cells previously exposed to saponin, a detergent that is thought to render the plasma membrane permeable by complexing with cholesterol [14]. The membranes which contain low amounts of cholesterol, such as those of the mitochondria and endoplasmic reticulum, do not seem to be affected by this treatment. The cell suspension was diluted, generally 1 ml of cells plus 4 ml of buffer of the following composition (mM): 120 KCl, 10 $MgCl_2$, 1.2 KH_2PO_4, 5 Tris ATP, 5 sodium phosphocreatine, 9.5 units/ml creatine phosphokinase, 0.5 mg/ml bovine serum albumin and 25 Tris-Hepes (pH 7.2) which contained in addition ^{45}Ca (5 μCi/ml). Samples of the cell suspension (200 μl) were taken at timed intervals to measure ^{45}Ca content of the cells. These were added to ice-cold microcentrifuge tubes containing an upper layer of radioactivity-free high potassium solution and a lower layer of dense immiscible oil [3] and immediately centrifuged (20 s, 12000 × *g*). ^{45}Ca in the cells was measured by liquid scintillation counting. In some experiments Ca^{2+} uptake was measured at fixed external free Ca^{2+} concentrations of $2 \cdot 10^{-5}$ M and $6.7 \cdot 10^{-7}$ M obtained with the chelators *N*-hydroxyethylethylenediamine triacetic acid (HEDTA) and ethyleneglycol bis(β-aminoethyl-*N*,*N'*-tetraacetic acid (EGTA), respectively. The necessary amounts of $CaCl_2$ and ligand were calculated by iteration using a microcomputer programme [15].

As demonstrated previously for rabbit enterocytes [7], leaky chicken intestinal epithelial cells take up ^{45}Ca from a medium mimicking the intracellular composition (Fig. 1). The upper panel shows that rapid uptake occurred at $2 \cdot 10^{-5}$ M Ca^{2+}, and that this was sensitive to a mixture of the mitochondrial blockers oligomycin and rotenone. The reduction in uptake was of the order of 65%. Addition of the ATPase inhibitor vanadate, caused only a very small further decrease in Ca^{2+} uptake. When the free Ca^{2+} concentration was reduced to $6.7 \cdot 10^{-7}$ M the inhibition by mitochondrial inhibitors was only about

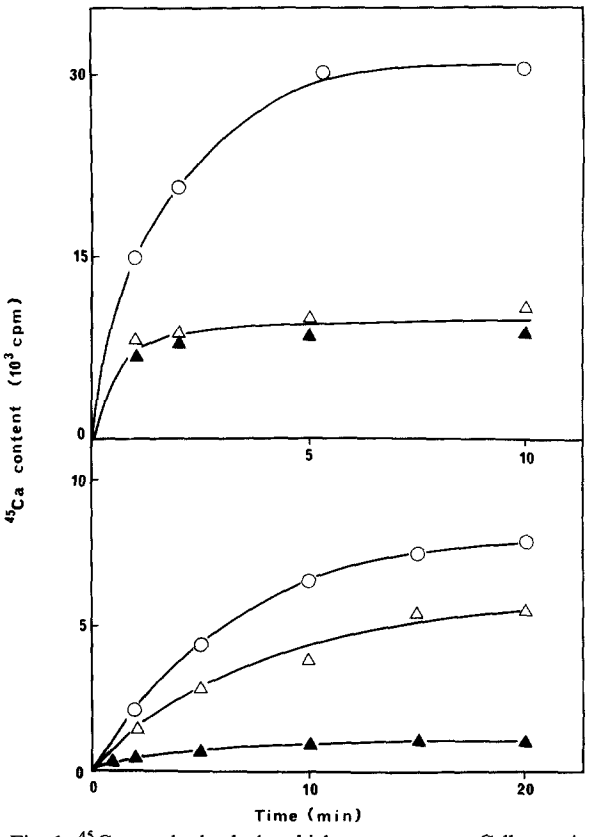

Fig. 1. ^{45}Ca uptake by leaky chicken-enterocytes. Cells previously permeabilised with saponin were incubated at 37°C and pH 7.2 in a buffer mimicking the intracellular composition and containing 5 mM ATP and an ATP-regenerating system. Initial free Ca^{2+} concentration was either $2 \cdot 10^{-5}$ M (upper panel) or $6.7 \cdot 10^{-7}$ M (lower panel) obtained by HEDTA and EGTA buffering, respectively. Incubation solutions contained either 3 μg/ml oligomycin and 0.5 μg/ml rotenone (△); 3 μg/ml oligomycin, 0.5 μg/ml rotenone and 5 mM vanadate (▲). Control solution (○).

30%. Addition of vanadate, however, caused a further inhibition that lowered uptake to 10% of that under control conditions. These results suggest that, as reported before for rabbit enterocytes [8], permeable chicken enterocytes transport Ca^{2+} into mitochondrial and non-mitochondrial intracellular stores. No uptake was observed when ATP in the solution was decreased by addition of hexokinase and glucose (not shown). Uptake into mitochondrial stores was predominant at high ($2 \cdot 10^{-5}$ M) free Ca^{2+} concentration, while the non-mitochondrial stores became dominant at lower ($6.7 \cdot 10^{-7}$ M) free Ca^{2+}.

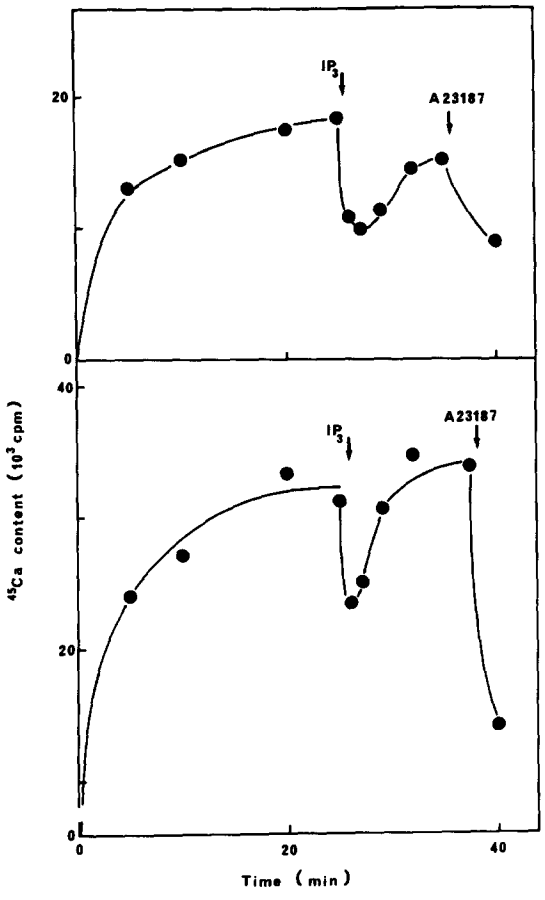

Fig. 2. Effect of IP_3 on the ^{45}Ca content of leaky chicken-enterocytes. Cells were incubated in the same buffer as that used in Fig. 1, but with no added Ca^{2+} or chelators. The initial Ca^{2+} concentration was 500 nM as measured with a Ca^{2+}-selective electrode. Incubations were performed in the absence (lower panel) or presence (upper panel) of oligomycin (3 μg/ml) and rotenone (0.5 μg/ml). Additions: 5 μM IP_3; 10^{-5} M Ca^{2+}-ionophore A23187.

To test the effect of the intracellular messenger IP_3, leaky chicken enterocytes were allowed to take up ^{45}Ca from a solution similar to that in the experiments described above but containing no added Ca^{2+} or chelators. Fig. 2 shows the time course of ^{45}Ca uptake, either in the absence of inhibitors (lower panel) or when the mitochondrial inhibitors rotenone and oligomycin were present in the medium (upper panel). Under both conditions addition of IP_3 induced a rapid and transient release of accumulated ^{45}Ca. The decrease in ^{45}Ca associated with the cells was from 19.5 ± 1.5 to 10 ± 1.4 10^3 cpm ($P < 0.01$ by paired t-test) and from 310 ± 30 to 232 ± 28 10^3 cpm ($P < 0.05$ by paired t-test) in the presence and absence of inhibitors, respectively (means $\pm$ S.E., $n = 4$). Most of this Ca^{2+} was lost within the first minute of stimulation with IP_3. The magnitude of the release was similar in both situations but the re-uptake process was slower in the inhibited cells. Release of accumulated ^{45}Ca could also be induced by addition of the calcium-ionophore A23187, suggesting that ^{45}Ca sequestration was in a membrane-enclosed compartment. Substances with no effect included Inositol 1,4-bisphosphate (IP_2, 10 μM) and *myo*-inositol (20 μM). Similar results were obtained with rabbit enterocytes with highly permeable plasma membranes (not shown). The concentration-dependence of IP_3-induced ^{45}Ca release from leaky chicken enterocytes in the presence of mitochondrial inhibitors is shown in Fig. 3. A maximal release of 51 ± 7 percent of the accumulated ^{45}Ca was extrapolated assuming that the relationship between release and IP_3 concentration was a rectangular hyperbola. The concentration of IP_3 giving half maximal release was 1.0 ± 0.4 μM (mean $\pm$ S.D. of four separate experiments). This value is similar to the half-maximal concentrations previously described for other cell types [12].

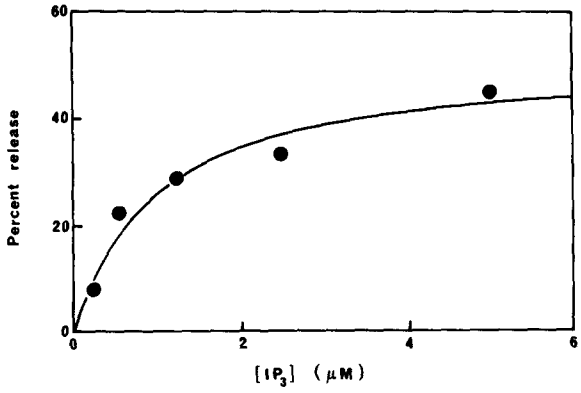

Fig. 3. Concentration-dependence of IP_3-induced ^{45}Ca release from leaky epithelial cells isolated from chicken intestine. Saponin-treated cells were incubated in the presence of ^{45}Ca for 20 min as described for the upper panel of Fig. 2. Percent release indicates the percent of total accumulated ^{45}Ca lost by the cells upon addition of the appropriate concentration of IP_3 and is based on the maximal loss of ^{45}Ca occurring after addition; this was usually one minute after IP_3 addition. Results are means of four separate experiments.

Release of Ca^{2+} under the effect of IP_3 could also be shown by monitoring ambient free Ca^{2+} concentration in the presence of leaky cells, with a Ca^{2+}-selective electrode [8]. In these experiments release is assessed by comparison between elevation in Ca^{2+} concentration induced by IP_3 with that observed upon direct addition of known amounts of $CaCl_2$. These measurements (not shown) revealed that 5 μM IP_3 evoked the release of about 2.4 nmol Ca per mg of cell protein.

Intracellular Ca^{2+} has been proposed to play a crucial role in regulating intestinal transport, and there are numerous recent reports showing that in many cells IP_3 is an important link between receptor-agonists binding and Ca^{2+} mobilisation from intracellular stores [11,12]. The fact that IP_3 produces Ca^{2+} mobilisation in leaky enterocytes suggests that this compound might act in these cells as an intracellular signal mediating the response of the intestine to external stimuli, such as secretagogues, which are known to be Ca^{2+}-dependent [1,2]. In addition this effector might be the signal involved in intestinal transport regulation events which appear to be accompanied by Ca^{2+} mobilisation [16].

Part of this work was presented at the April 1986 meeting of the Physiological Society [17].

We thank Dr. Robin Irvine for the supply of IP_2 and IP_3 and to Dr. C.J.M. Nicol for making his computer programme available to us. A.I. acknowledges the support of the Junta de Andalucia and the British Council Acciones Integradas travel grant.

References

1 Fondacaro, J.D. (1986) Am. J. Physiol. 250, G1–G8
2 Donowitz, M. and Welsh, M.J. (1986) Annu. Rev. Physiol. 48, 135–150
3 Brown, P.D. and Sepúlveda, F.V. (1985) J. Physiol. 365, 271–285
4 Sepúlveda, F.V. and Mason, W.T. (1985) FEBS Lett. 191, 87–91
5 Boyd, C.A.R. and Walley, G.J. (1985) J. Physiol. 358, 77P
6 Van Corven, E.J.J.M., Roche, C. and Van Os, C.H. (1985) Biochim. Biophys. Acta. 820, 274–282
7 Sepúlveda, F.V. (1985) J. Physiol. 367, 58P
8 Sepúlveda, F.V. and Smith, S.M. (1986) Pflügers Arch. in the press
9 Rubinoff, M.J. and Nellans, H.N. (1985) J. Biol. Chem. 260, 7824–7828
10 Bikle, D.D., Askew, E.W., Zolock, D.T., Morrissey, R.L. and Herman, R.H. (1980) Biochim. Biophys. Acta 598, 561–574
11 Berridge, M.J. and Irvine, R.F. (1984) Nature, 312, 315–321
12 Sekar, M.C. and Hokin, L.E. (1986) J. Membrane Biol. 89, 193–210
13 Kimmich, G.A. (1970) Biochemistry 9, 3659–3668
14 Burgess, G.H., McKinney, J.S., Fabiato, A., Leslie, B.A. and Putney, J.W. (1983) J. Biol. Chem. 258, 15336–15345
15 Nicol, C.J.M. (1985) J. Physiol 367, 10P
16 Sepúlveda, F.V., Burton, K.A. and Brown, P.D. (1986) Biochim. Biophys. Acta. 856, 185–187
17 Burton, K.A., Ilundain, A., O'Brien, J.A. and Sepúlveda, F.V. (1986) J. Physiol. 378, 77P