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CALCIUM METABOLISM IN EHRLICH ASCITES TUMOUR CELLS

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Summary

Ehrlich ascites tumour cells are able, under the proper experimental conditions, to extrude a substantial amount of Ca^{2+} from the intracellular space. The Ca^{2+} extrusion mechanism, probably located at the plasma membrane level, appears to be similar to that found in red blood cells. It is energy-dependent and both respiration and glycolysis are able to drive it. The use of some inhibitors and uncouplers, besides showing that this activity is different from that linked to the mitochondrial Ca^{2+} pump which acts in the opposite direction, proposes some speculations on the energy compartmentation in the Ehrlich ascites tumour cells.

The regulation of intracellular calcium metabolism and concentration is one of the most interesting problems in cell biology [1]. Much evidence is accumulating which shows the importance of this cation in the regulation of different cellular metabolic pathways, such as glycolysis, respiration, guanylcyclase and protein phosphokinases activation, and cell properties, such as intercellular communication, permeability, deformability, adhesiveness and cell growth [2—14]. Furthermore calcium is thought to play a primary role in the determination of some peculiar tumour cells characteristics (e.g. glycolytic rate, Crabtree effect and uncontrolled growth), which would depend on an impairement of tumour cell calcium metabolism [7,15,16]. In contrast with the findings of previous authors [6,7,17,18] we have already shown that Ehrlich ascites tumour cell cytoplasmic membranes are permeable to calcium and that these cells are able, under certain circumstances, to take up calcium from the external medium by a metabolism-driven pump related exclusively to the mitochondrial activity [19,20].

In this communication we report the existence in the Ehrlich ascites tumour cell of a Ca^{2+}-extruding mechanism, supported by metabolic energy, most probably located at the cell membrane level and closely resembling the Ca^{2+}-extruding pump found in red blood cells [21].

The results of our experiments show that Ehrlich ascites tumour cells are able to bring about the extrusion of about 45 mmol Ca^{2+}/kg cell protein and

that this extrusion mechanism requires energy derived either from respiration or glycolysis. The use of some well known inhibitors of mitochondrial Ca^{2+} uptake clearly leads to the conclusion that this activity is different from that present in the mitochondria and procedes in the opposite direction.

Ehrlich ascites tumour cells (ATC) (hyperdiploid strain) were grown, as usual, by weekly intraperitoneal transplantation in Swiss albino mice. The cells harvested after 7—9 days were washed twice (see medium below) and resuspended at a concentration of about 4—6 mg dry wt./ml. The medium used throughout the present investigation had the following composition: 0.225 M mannitol, 0.075 M sucrose, 0.001 M $MgCl_2$, 0.0013 M $CaCl_2$ and 0.010 M Tris·HCl, pH 7.4. This medium has been utilized in order to avoid possible interference of other ions on calcium movements [19]. The cell suspension was incubated for different lengths of time in an ice-bath (0—1°C) and, after gassing with pure O_2, warmed to 38°C in the presence of the appropriate substrates and inhibitors in Warburg vessels for the contemporary measurement of oxygen uptake. Aliquots (usually 1 ml) of cell suspension were withdrawn after the incubation (both at 0—1 and 38°C) and stratified over a layer of 0.3 M sucrose (salt-free) in conical centrifuge tubes. The samples were rapidly centrifuged at 3200 rev./min in a bench centrifuge for 75 s. The clear supernatant was discarded by a suction pump and the tube walls carefully dried with the aid of tissue paper avoiding any contact with the cell pellet. This procedure has been shown to be essential to avoid contamination of the cells by the Ca^{2+} of the incubation medium and gives highly reproducible and reliable estimates of intracellular calcium. The cell sediment was subsequently dried in an oven at 100—120°C for at least 4 h and the Ca^{2+} quantitatively extracted by 3 ml of 0.1 N HNO_3 [22]. Samples of the extract were analyzed for calcium by atomic absorption spectrophotometry in the presence of 1% $LaCl_3$. The protein concentration was determined by the biuret reaction modified for turbid solutions [23].

Ascites tumour cells incubated in a medium containing 1.3 mM Ca^{2+} at 0—1°C, hence in conditions of inhibited endogenous metabolism, take up a substantial amount of Ca^{2+} from the external medium. The accumulation is linear during the first 60 min and declines thereafter, reaching the equilibrium in the following 30 min at a level of about 70 mmol/kg cell protein (Fig. 1, solid circles). When the cells are incubated under favourable metabolic conditions, namely at 38°C in the presence of an oxidizable substrate, a rapid and substantial extrusion of Ca^{2+} takes place. Fig. 1, in fact, shows that the level of ATC intracellular Ca^{2+} quickly falls to about 25—30 mmol/kg protein, both after 30 and 90 min of cold incubation (open circles in Fig. 1). The relationship between Ca^{2+} extrusion and ascites tumour cell metabolism is further shown by the results of Table I. It can be seen that the lower the rate of O_2 uptake, the higher the level of cellular Ca^{2+}. In fact, ascites tumour cells respiring with a Q_{O_2} of 4.7 (endogenous substrates or 10 mM glucose) extrude Ca^{2+} in an amount of about 30—35 mmol/kg protein, while respiring with a Q_{O_2} of 7.3 (10 mM pyruvate)(+55% of the endogenous), they extrude about 45 mmol Ca^{2+}/kg protein. In the experiments shown, ascites tumour cells fail to exhibit the classical Crabtree effect (inhibition of the respiration by glucose) and we suppose that this can be due to the special condition in which the cells are in-

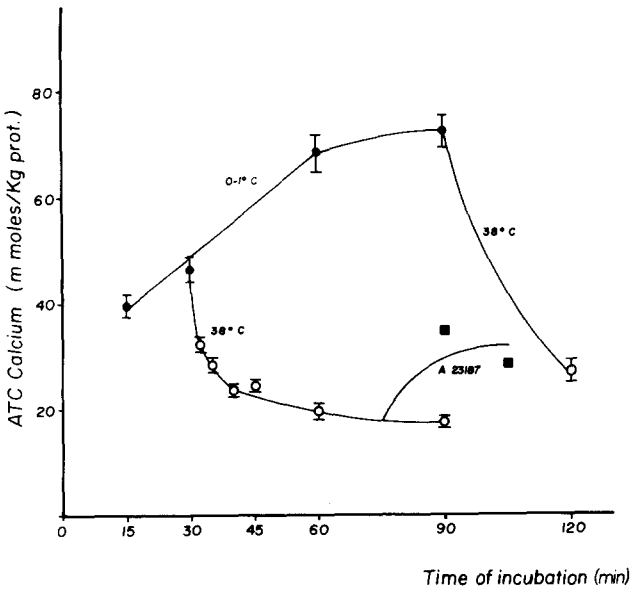

Fig. 1. Time course of Ca^{2+} accumulation at 0—1°C (•) and extrusion at 38°C (○) by Ehrlich ascites tumour cells (ATC) supplemented with 10 mM pyruvate, incubated in: 225 mM mannitol, 75 mM sucrose, 1.0 mM $MgCl_2$, 1.3 mM $CaCl_2$, and 10 mM Tris • HCl, pH 7.4. The inhibiting effect of the antibiotic ionophore A23187 (■) on cell Ca^{2+} extrusion is also reported.

TABLE I

TOTAL CALCIUM CONTENT AND OXYGEN UPTAKE OF INTACT EHRLICH ASCITES TUMOUR CELLS INCUBATED IN MST MEDIUM AT 0—1°C AND 38°C IN ENDOGENOUS AND SUBSTRATES SUPPLEMENTED CONDITIONS

Incubation was conducted for 45—50 min at 0—1°C in an ice bath, the same cell suspension was, then, transferred at 38°C in Warburg vessels for additional 30 min in aerobic conditions. Lactate production is also reported for the glucose-supplemented cells. For further technical details see the text.

Incubation	Additions	Calcium (mmol/kg protein)	O_2 uptake (Q_{O_2})**	Lactate production (μmol/100 mg dry wt./30 min)
45—50 min at 0—1°C	None	73.1 ± 3.8(56)*		
45—50 min at 0—1°C and 30 min at 38°C	None	43.2 ± 1.9(55)	−4.7 ± 0.3(21)	
	10 mM glucose	37.1 ± 2.6(29)	−4.7 ± 0.3(14)	5.0 ± 0.9(12)
	10 mM pyruvate	26.7 ± 1.1(48)	−7.3 ± 0.2(27)	

*Mean ± S.E.M. (number of observations).
**μl/h/mg dry wt.

cubated (Na^+, K^+ and P_i-free medium). However the phenomenon requires further investigation. Glycolysis is one of the most impressive features of cancer cell metabolism [24] and its role in the biology of such cells is still an intriguing problem. Indeed, the energy derived from the glycolytic pathway, in cancer cells, has been shown to be as effective as the mitochondrial one in many respects and in particular in the maintenance of cell ion gradients [25]. In preceding reports, we underlined that glucose fails to support Ca^{2+} uptake by ascites tumour cells [19,20]. On the contrary, the energy from glycolysis is as effective as that from respiration in supporting the extrusion of Ca^{2+} from the cell. Table II shows such results. Rotenone blocks the Ca^{2+} extrusion in

TABLE II

EFFECT OF ROTENONE AND TTFB IN THE PRESENCE OR ABSENCE OF GLUCOSE ON THE CALCIUM EXTRUSION BY EHRLICH ASCITES TUMOUR CELLS INCUBATED FOR 30 min AT 38°C AFTER PREINCUBATION AT 0–1°C FOR 45–50 min

For more explanations, see the text.

Additions	Glucose (10 mM)	Calcium (mmol/kg protein)	Lactate production (μmol/100 mg dry wt./30 min)
Rotenone 6.7 μM	–	76.9 ± 7.6(19)*	
	+	41.6 ± 2.9(36)	38.5 ± 3.5(7)
TTFB 2.6 μM	–	65.8 ± 4.0(14)	
	+	35.0 ± 3.5(9)	54.5 ± 2.5(6)

*Mean ± S.E.M. (number of observations).

endogenous conditions, but the activity is restored by the addition of glucose, condition in which the level of Ca^{2+} is equal to that found in the uninhibited endogenous conditions (Table I). Similar results are obtained using the uncoupler 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TTFB) (Table II). The uncoupling inhibits calcium extrusion, which is, however, restored by the addition of glucose to the cells. The amount extruded is still the same as in endogenous conditions. The above-mentioned TTFB experiments, besides stressing the energy-dependence of the ascites tumour cells Ca^{2+}-extruding mechanism, show that the mitochondrial Ca^{2+} pump is not involved in the phenomenon since, in these conditions, mitochondria loose calcium while the cells gain it. Furthermore, the same results propose interesting speculations on the competition among different energy-utilizing systems in tumour cells ("energy compartmentation") [26,27]. In fact, the ability of glucose to support cell Ca^{2+} extrusion in the presence of the uncoupler, together with its inability to support the Ca^{2+} uptake by the ascites tumour cell mitochondria in vivo [19,20] suggests that in these cells the plasma membrane Ca^{2+}-extruding mechanism competes favourably with mitochondrial ATPase for cytosolic ATP. This could be due to: (a) higher affinity of the cell membrane Ca^{2+} pump for cytosolic ATP, and/or (b) alteration of tumour mitochondrial ATPase, the ability of which to hydrolyze exogenous (cytosolic) ATP could be deeply impaired, as shown by Pedersen et al. [28]. These considerations which of course require further proof, are in contrast with the results obtained by others on different normal cell preparations, which show that cytosolic ATP freely reacts with mitochondrial ATPase [29]. Finally the effect of the antibiotic ionophore A23187 on Ca^{2+} extrusion by ascites tumour cells is reported in Fig. 1. This compound, which equilibrates the concentration of divalent cations (especially Mg^{2+} and Ca^{2+}) across many biological membranes [30], exhibits an inhibiting effect also on Ca^{2+} extrusion by ascites tumour cells.

On the basis of the above experiments, we draw the following conclusions: ascites tumour cells are capable of extruding from the intracellular space substantial amounts of Ca^{2+} and the mechanism is metabolism- and energy-dependent. The extrusion is proportional to the rate of O_2 consumption. Ascites tumour cell glycolysis is capable of supporting cell Ca^{2+} extrusion differently from that found in other perenchymal cell species; in such respect, the mechanism resembles the one acting in red blood cells. Furthermore, the net extrusion is not dependent on the exchange diffusion of Na^+, which is

absent from the incubation medium, and seems to be in good agreement with the recent report about Ca^{2+}-ATPases found in ascites tumour cells and other tumour cell plasma membranes [33,34]. All these features make the Ehrlich ascites tumour cells the ideal experimental model to study the regulation of cell Ca^{2+} content and the effect of Ca^{2+} on cell metabolism. Experiments are in progress in both directions.

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