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CALCIUM PERMEABILITY OF EHRLICH ASCITES TUMOUR CELL PLASMA MEMBRANE IN VIVO

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Passive Ca^{2+} entry into Ehrlich ascites tumour cells has been investigated. Passive equilibration of Ca^{2+} takes place in ascites tumour cells only under conditions of exhaustive energy depletion. The specific Ca^{2+} ionophore A23187 does not affect Ca^{2+} entry into ascites tumour cells under active metabolic conditions, but it increases the rate of Ca^{2+} equilibration in ascites tumour cells in the early stages of energy depletion. The results of the present experiments lead to the conclusion that in ascites tumour cell plasma membrane Ca^{2+} permeability is not a limiting step in the regulation of intracellular calcium content, while the energy-dependent Ca^{2+} extrusion is the main mechanism that prevents uncontrolled intracellular Ca^{2+} increase. The results taken together support the hypothesis that increased Ca^{2+} influx into the cell, caused by plasma membrane alteration, is responsible for permanently elevated mitotic activity and for deranged metabolic behaviour of these neoplastic cells.

Introduction

Cell Ca^{2+} metabolism is intimately involved in the regulation of many cell biological and biochemical features. Among these, cell division and proliferation processes, as well as glycolysis and respiration are examples of cell functions strongly affected by the level of cytosolic ionized calcium [1]. The problem of cell calcium metabolism becomes of outstanding importance if related to cancer cell characteristics. These cells show uncontrolled growth, increased aerobic glycolysis and decreased Pasteur effect, in addition to many other features (e.g. loss of contact inhibition, decreased adhesiveness and local as well as systemic invasiveness) that could depend on the altered content and movement of calcium in the cell [2,3].

Different models have been so far proposed in order to explain such a deranged cancer cell-calcium relationship, but up to now experimental results have not yet settled the subject. Increased tumour mitochondrial Ca^{2+} accumulation has been extensively documented [4] and cancer energy-dependent Ca^{2+} extrusion and uptake have been reported and characterized [5–11]; however no data are available on the neoplastic cell plasma membrane passive permeability to Ca^{2+} [6], except for a recent report by Cheney et al. [10] that produced evidence for a component of Ca^{2+} influx due to a Ca^{2+}/H^{+} electrogenic antiporter inhibited by membrane depolarization.

On the basis of recent results showing the inability of the Ca^{2+} ionophore A23187 to increase total calcium content in intact Ehrlich ascites and other tumour cells [12], we have now investigated the passive permeability to Ca^{2+} of the plasma membrane in whole Ehrlich ascites tumour cells.

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Abbreviation: TTFB, 4,5,6,7-tetrachloro-2-trifluoromethoxybenzimidazole.

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Much of the study has been performed on intact cells in condition of complete energy depletion. The results obtained lead to the conclusion that the Ehrlich ascites cell plasma membrane can be considered highly permeable to the external Ca^{2+} and that the main mechanism that prevents uncontrolled intracellular calcium increase is the effective energy-dependent extrusion of Ca^{2+} from the cell.

The results, taken together support the hypothesis that the uncontrolled influx of Ca^{2+} into the cell is an important pathogenetic step of malignant transformation.

Materials and Methods

Ehrlich ascites tumour cells (hyperdiploid strain) have been grown, harvested, washed and resuspended as described previously [5]. The cells have been washed and finally resuspended in Tris-buffered Ringer solution (146 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$ and 50 mM Tris-HCl, pH 7.4) devoid of calcium. Experimental incubations have been performed in shaking flasks which were gassed with pure O_2 when required (cell under active metabolic condition). The cells were usually equilibrated at 38°C for 15 min before Ca^{2+} ($CaCl_2$) addition. In the experiments reported in this work Ca^{2+} has been usually added at a concentration of 2.5 mM, but similar results were obtained with lower Ca^{2+} concentration. For the determination of $^{45}Ca^{2+}$ exchange, the tracer (0.05 μCi/ml) has been added after the period of equilibration necessary for the cells incubated with non-radioactive Ca^{2+} to obtain Ca^{2+} steady state in the different metabolic conditions. For the analysis of total cell calcium as well as of cell $^{45}Ca^{2+}$, determined by atomic absorption spectrophotometry and scintillation counting, respectively, the cells have been treated as previously described [5,13]. The method for the separation of the cells from the suspension medium allows accurate determination of intracellular Ca^{2+} with negligeable contamination from extracellular Ca^{2+} [14]. K^+ has been determined by flame emission photometry on the same calcium extracts with the appropriate standards. O_2 uptake and lactate production have been determined by the Warburg manometric method and enzymatic analysis, respectively, during and after 1 h incubation in Warburg vessels. ATP has been determined by hexokinase and glucose-6-phosphate dehydrogenase reactions on the acid cell extracts. Intracellular water has been measured gravimetrically as the difference between the wet and the dry weight of cell aliquots centrifuged 10 min at 5000 rev./min and dried overnight in oven at 95°C after discarding the supernatant and cleaning tube walls carefully with tissue paper. In the figures of this paper intracellular calcium has been expressed in mmol/kg intracellular H_2O. Modification of H_2O content of cells under inhibited metabolic conditions has been considered (see Fig. 7). Deionized water (> 20 MΩ) and analytical reagents used throughout.

Results

Fig. 1 shows the time course of total calcium content of intact Ehrlich ascites tumour cells, incubated under different metabolic conditions, upon addition of Ca^{2+} to the suspension medium. In the absence of added Ca^{2+}, ascites tumour cells retain an amount of cation of about 0.5 mmol/kg cell H_2O. The addition of 2.5 mM Ca^{2+} provokes a sudden increase of intracellular total calcium con-

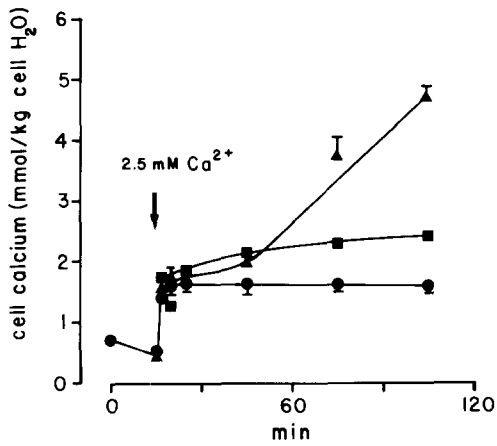

Fig. 1. Time course of Ca^{2+} accumulation in whole Ehrlich ascites tumour cells under different metabolic conditions. Ca^{2+} added to ascites tumour cells preincubated 15 min in order to allow temperature equilibration. ●——●, endogenous substrates; ■——■, rotenone (6.7 μM); ▲——▲, rotenone + iodoacetic acid (1.0 mM). About 3 mg protein/ml; 38°C. Vertical bars are S.E. of the media; where not present they fall inside symbols.

tent to about 1.5 mmol/kg cell H_2O irrespective of cell metabolic condition. Thereafter Ca^{2+} does not show further increase in the cells whose metabolism is supported by endogenous substrates, whereas it slowly increases to about 2.0 mmol/kg cell H_2O in the rotenone-treated cells. Rotenone inhibits ascites tumour cell respiration by more than 90% (see also Fig. 2). Ascites tumour cells treated with rotenone and iodoacetic acid, the well known inhibitor of glycolytic pathway, behave quite differently. Thus, after a lag period of about 30 min, their content of calcium progressively increases to about 4.5 mmol/kg cell H_2O. After 90 min of incubation, the accumulation of calcium reaches saturation and the content stabilizes (see also Fig. 3).

These experiments indicate that it is necessary to achieve complete metabolic inhibition in order to observe ascites tumour cells calcium accumulation and that, even in the presence of both metabolic inhibitors, energy depletion sufficient to allow Ca^{2+} entry requires about 30 min.

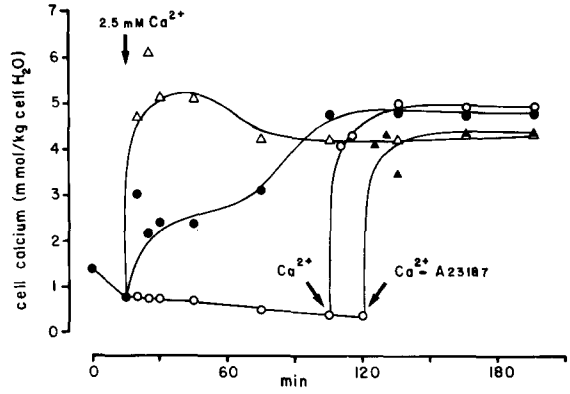

Fig. 3. Kinetics of Ca^{2+} accumulation in intact, energy-depleted ascites tumour cells. Cells treated with rotenone and iodoacetic acid. ●——●, control; △——△, A23187-treated cells; ○——○, cells preincubated 90 min in Ca^{2+}-free medium; ▲——▲, cells treated with A23187 after 90 min preincubation in Ca^{2+}-free medium. See the text for explanation. Cells about 3 mg protein/ml.

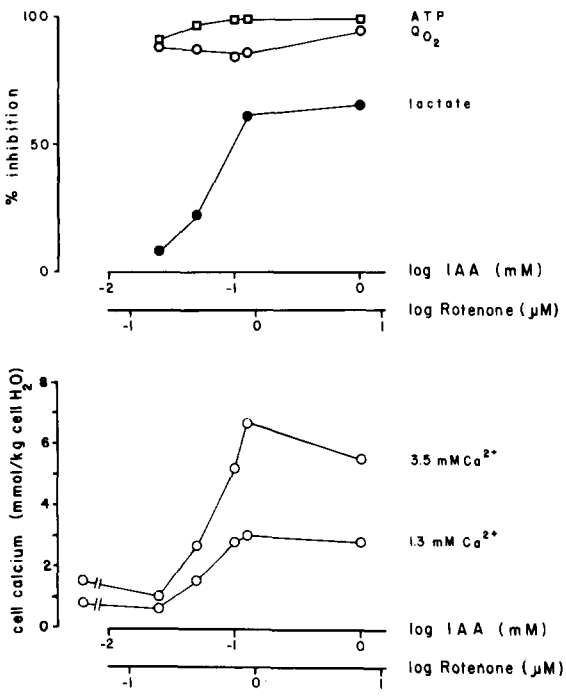

Fig. 2. Relation between metabolic activity (respiration and anaerobic lactate production), ATP and total calcium content in ascites tumour cells treated with increasing amount of rotenone and iodoacetic acid (IAA) in the presence of 1.3 and 3.5 mM external Ca^{2+}. 20 mM glucose as substrate. Total cell calcium content determined after 90 min incubation in the presence of inhibitors and Ca^{2+}.

Fig. 2 shows the correlation between cell metabolic parameteres and calcium level. In this case total cell calcium was measured 90 min after Ca^{2+} addition (1.3 and 3.5 mM) in the presence of increasing amounts of rotenone and iodoacetic acid. It can be seen that Ca^{2+} equilibration, whatever the external concentration, occurs when respiration and glycolysis are maximally inhibited. These experiments further show that glycolysis alone is sufficient to prevent Ca^{2+} entry since already at the lower rotenone concentrations, respiration is almost completely inhibited but a significant ATP content is still maintained, although only 10% of the original content. Calcium accumulation, however, is complete with the rotenone and iodoacetic acid concentrations usually added to cell suspension in the course of this work (6.7 μM rotenone and 1.0 mM iodoacetic acid).

Energy depletion is the only condition in which calcium entry can be observed. In fact, as already reported elsewhere [12] (see also Fig. 5), not even the addition of the specific Ca^{2+} ionophore A23187 [15] induces a similar effect.

To better characterize the passive equilibration of calcium, we performed the experiments of Fig. 3.

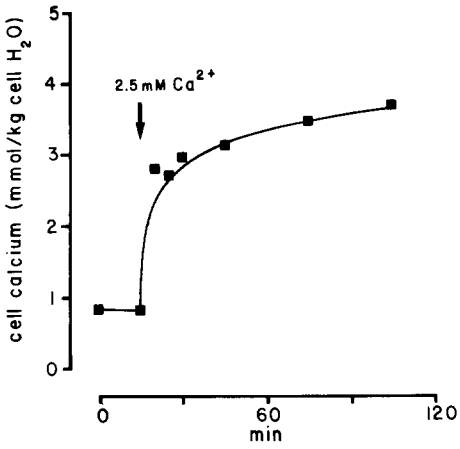

Fig. 4. Calcium accumulation at 0–4°C in energy-depleted ascites tumour cells. Cells preincubated 120 min at 38°C and than transferred to 0–4°C.

Ascites tumour cells were incubated with rotenone and iodoacetic acid up to about 3 h. Ca^{2+} was added to an aliquot of cell suspension after the usual 15 min of equilibration: in these conditions calcium accumulation followed the same pattern as that of Fig. 1, and equilibrium was achieved after 90 min. In the same conditions the addition of ionophore A23187 brought about an abrupt and strong accumulation of Ca^{2+} that required only 5 min to reach the same level as that reached after 90 min in the control cells. However it is interesting to note that if the cells are preincubated without Ca^{2+} for the extent of time necessary for a complete energy depletion (about 90 min), ascites tumour cells accumulate Ca^{2+} as quickly and promptly as the A23187-treated cells. Moreover if A23187 is added to cells preincubated in the presence of inhibitors, without Ca^{2+}, for the time required for energy depletion, it does not modify the kinetics of Ca^{2+} accumulation.

Depleted cells accumulate calcium with the same kinetic characteristic also at 0–4°C (Fig. 4) indicating that this is a truly passive equilibration that takes place, likely, through aqueous channels.

The lack of effect of A23187 upon ascites tumour cells both under energy-supplemented and energy-depleted conditions suggests that these cancer cells possess plasma membranes physiologically highly permeable to external Ca^{2+}. The experiments of Fig. 5 have been performed in order

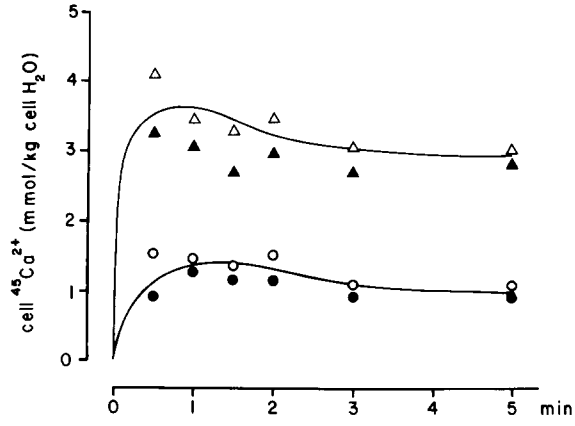

Fig. 5. $^{45}Ca^{2+}$ exchange in intact ascites tumour cells incubated under condition of active metabolism (circles) and energy-depletion (triangles) with (solid symbols) and without added A23187 (open symbols). $^{45}Ca^{2+}$ added in tracer amount to cells preincubated with 2.5 mM Ca^{2+} 60 min (metabolizing cells) and 120 min (depleted cells) in order to achieve steady-state and equilibrium condition, respectively. Upon addition of $^{45}Ca^{2+}$ the exchange takes place with similar kinetic characteristic irrespective of ionophore treatment. Note the time scale in the minute range.

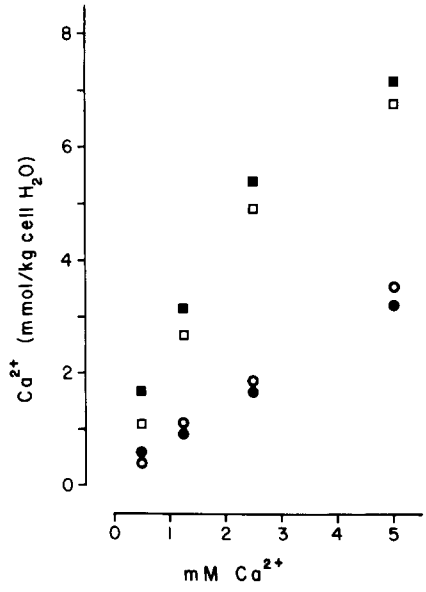

Fig. 6. Plot of apparent ascites tumour cells intracellular Ca^{2+} concentration at increasing extracellular Ca^{2+} concentration. Circles refer to cells with active metabolism and squares to depleted cells (rotenone + iodoacetic acid). Closed symbols: control; open symbols: cells supplemented with 10 μM A23187. Cell calcium content determined after incubations of 120 and 60 min for energy depleted and metabolically active cells, respectively. See text for further explanations.

to study the effect of the ionophore upon $^{45}Ca^{2+}$ exchange properties in ascites tumour cells in the condition of the previous experiments. $^{45}Ca^{2+}$ has been added in tracer amount to cells preincubated in the presence of non-radioactive Ca^{2+} for the time required to reach steady state (60 min for the cells under metabolically favourable conditions (see also Figs. 1 and 2) and 120 min for the energy-depleted ascites tumour cells (see Fig. 3). Our experimental procedure allowed cell samples to be taken, at the earliest, 30 s after tracer addition. The results of these experiments (Fig. 5) show that $^{45}Ca^{2+}$ exchange is complete within 30 s from the addition, irrespective of the A23187 treatment.

These data give an additional indication that intact ascites tumour cell plasma membrane Ca^{2+} permeability is not affected by ionophore treatment and, thus, it can be considered already high under physiological condition. This conclusion is strenghtened by the results of Fig. 6. This plot reports the apparent Ca^{2+} intracellular concentration attained at increasing external Ca^{2+} concentration both in energy-supplemented and energy-depleted ascites tumour cells, with and without A23187. The plot of energy depleted cells gives a straight line, except for the last point which slightly declines probably because of the exiguous cell loss occurring at this concentration of Ca^{2+}, and shows that equilibrium between extra- and intracellular Ca^{2+} concentration has been achieved. The apparent intracellular Ca^{2+} concentration exceeds by a factor of about two the medium Ca^{2+} concentration indicating a strong Ca^{2+} buffering capacity of intracellular components [16]. The line obtained in the presence of A23187 virtually coincides with the control. The results pertaining to metabolically active cells show that in these conditions intracellular Ca^{2+} concentration is far from equilibrium, although a slight increase is also evident, and that A23187 is ineffective.

The last aspect of the problem we have investigated is that regarding the early effect of A23187 upon inhibited cells before complete energy depletion (see Fig. 3). All evidence indicates the failure of A23187 to induce Ca^{2+} permeabilization in ascites tumour cells so that this effect could be related to a nonspecific ionophore effect, such as a shortening of the energy depletion time.

Since attempts to determine differences of cell energy level by measuring ATP did not give significant differences, due to the low amount of endogenous ATP in the depleted cells, we measured the entry of H_2O into ascites tumour cells, as an alternative parameter of cell energy loss. This process, in fact, reflects the cell energy state [17]. Fig. 7 shows that, indeed, A23187 brings about ascites tumour cells H_2O increase earlier than that from spontaneous energy depletion. In both cases H_2O gain follows the same pattern of Ca^{2+} passive equilibration (compare to Fig. 3), showing direct connection between these two processess.

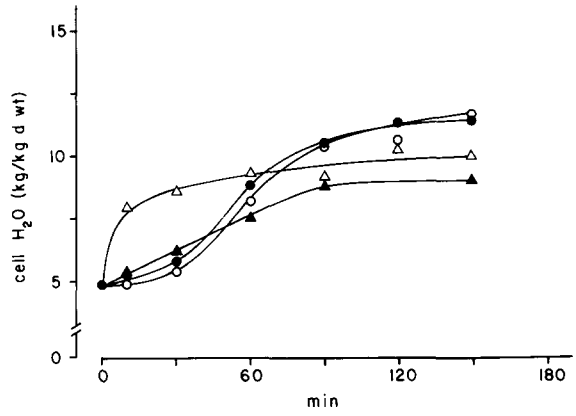

Fig. 7. Effect of metabolic inhibition and A23187 upon H_2O content in ascites tumour cells. Cells treated with rotenone and iodoacetic acid: ●——●, no added Ca^{2+}; ○——○, 2.5 mM Ca^{2+}; ▲——▲, 10 μM A23187; △——△, A23187 +2.5 mM Ca^{2+}. Energy depletion, with or without Ca^{2+}, provokes slow increase of intracellular H_2O which proceeds in parallel with cell calcium accumulation (compare to Fig. 4). Intracellular water determined gravimetrically as under Materials and Methods.

Following the same rationale we determined also the time course of K^+ loss from ascites tumour cells treated as in the previous experiments. The maintenance of a K^+ gradient across plasma membrane is an energy requiring process that could give additional indication about the nature of the phenomenon under study. Fig. 8 reports such results. It is evident that the loss of K^+ takes place at the same time as Ca^{2+} gain occurs. It is, however, independent from it since it takes place even in the cells without calcium. The addition of A23187 accelerates cell K^+ loss but only when Ca^{2+} is present. The effect of A23187 in the early stages of cell energy depletion can be, then, con-

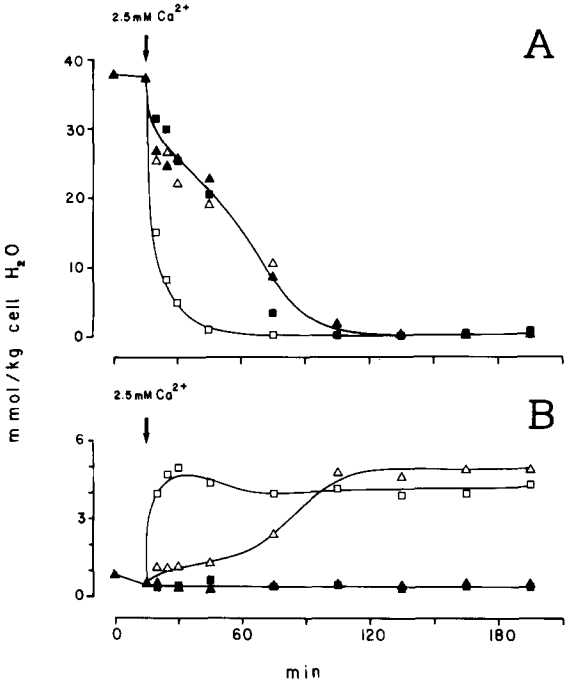

Fig. 8. Time course of K^+ loss from energy-depleted intact ascites tumour cells (part A) compared to Ca^{2+} accumulation (part B). Conditions similar to those of Fig. 3. 6.7 μM rotenone and 1.0 mM iodoacetic acid throughout. ▲——▲, no Ca^{2+} added; △——△, 2.5 mM Ca^{2+}; ■——■, 10 μM A23187; □——□, A23187+2.5 mM Ca^{2+}. Ca^{2+}-A23187 accelerates cell K^+ loss as well as Ca^{2+} accumulation (see also Fig. 3). Results discussed in the text. Cells about 3.0 mg protein/ml.

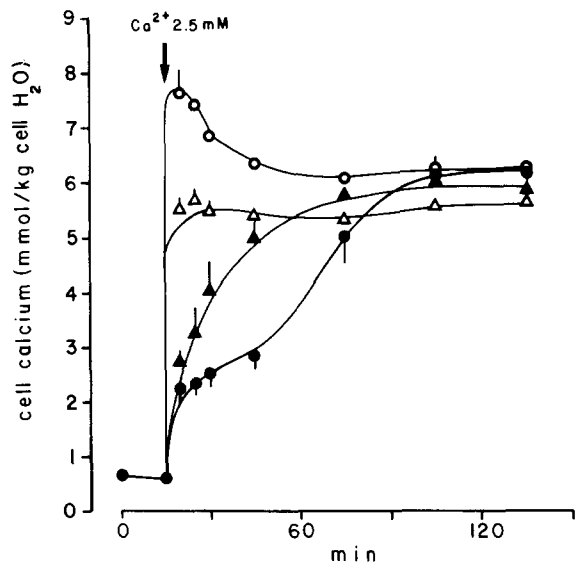

Fig. 9. Effect of oligomycin (10 μg/ml) and TTFB (2.0 μM) on Ca^{2+} accumulation in energy-depleted ascites tumour cells. Ascites tumour cells incubated with rotenone and iodoacetic acid (●——●) take up Ca^{2+} as described in Fig. 3. The addition to the inhibited cells of oligomycin and TTFB (▲——▲) eliminates the lag phase of Ca^{2+} accumulation. Oligomycin and TTFB (△——△) strongly reduce Ca^{2+} accumulation in the inhibited ascites tumour cells treated with A23187 (○——○). For further explanation see the text.

sidered as due to shortening of the time required to achieve energy depletion.

Following this line of evidences we looked for other means to accelerate energy depletion of rotenone and iodoacetic acid-treated ascites tumour cells. Fig. 9 reports the results of such experiments.

The addition of TTFB and oligomycin, uncoupler and inhibitor of oxidative phosphorylation respectively, to ascites tumour cells pretreated with rotenone and iodoacetic acid (solid triangles), indeed, increases the initial rate of Ca^{2+} entry in comparison with the control (solid circles). Under the above mentioned conditions, the addition of A23187 slightly increases Ca^{2+} equilibration rate (open triangles), which is, however, slower than that occurring in ascites tumour cells without TTFB and oligomycin (open circles). The results of these experiments support the hypothesis that the lag phase of Ca^{2+} accumulation in inhibited ascites tumour cells is due to the achievement of a complete cell energy depletion, while they clearly show that a certain degree of Ca^{2+} permeabilization by A23187 occurs in the inhibited cells. This is further supported by the different kinetics of Ca^{2+} equilibration in A23187-treated ascites tumour cells with and without TTFB and oligomycin. The latter compounds inhibit the initial overshoot of Ca^{2+} entry into ascites tumour cells suggesting the involvement of the mitochondrial compartment in the initial accumulation of Ca^{2+} that follows Ca^{2+} permeabilization. Thus, complete energy depletion is also achieved because of Ca^{2+} cycling at mitochondrial level. This has been further prooved by experiments carried out at temperature lower than 38°C, in which the kinetics of this effect was better resolved (not shown).

Discussion

In the course of our studies on cancer cell calcium metabolism, we had indications, strengthened by the results of other authors [7–10], that the plasma membrane of these cells does not constitute an efficient barrier against calcium entry into intracellular space [6]. The addition of Ca^{2+} to ascites tumour cells usually gives rise to cell uptake of this cation which is mostly accumulated by the mitochondrial compartment in vivo. On the other hand, also in these cells other mechanisms operate in order to keep low cytosolic calcium. These are the energy-dependent plasma membrane extrusion [5] as well as the energy-dependent uptake by intracellular compartments (e.g. mitochondria and microsomes) [4,18,19]. It is, however widely acknowledged that the main mechanism for 'long term' intracellular Ca^{2+} regulation is that found in the plasma membrane. Plasma membranes usually possess Ca^{2+} extrusion mechanisms either energy or electrochemically driven [20,21], however it is the intrinsic Ca^{2+} permeability of plasma membrane that, first of all, opposes calcium entry into the cell. Recent results by Cheney et al. [10] have given evidence for other components of Ca^{2+} influx, which depend on the electrochemical gradient at the plasma membrane and on mitochondrial activity. Membrane has been extensively investigated in red blood cells and it has been concluded that these cells are virtually impermeable to external Ca^{2+} although exhibiting a powerful outwardly directed ATP-supported Ca^{2+} pump, whose importance is, thus, questioned (for review, see Ref. 22).

The problem of the intrinsic passive plasma membrane permeability is of outstanding importance in many other cells. For example, it is postulated that plasma membrane permeability modulation, either spontaneous or provoked, represents the trigger for the cell 'decision to divide' (viz. cell division by mitogen-induced Ca^{2+} influx) [23]. Ascites tumour cells possess an efficient energy-dependent Ca^{2+} extrusion [5] so that, in order to observe the passive calcium entry, that leads to equilibrium between extra- and intracellular compartments, an exhaustive depletion of cell energy stores is required. Energy depletion is obtained by complete metabolic inhibition. Respiration inhibition by rotenone, that reduces cell ATP by 90% (Fig. 2), is not sufficient to allow entry of Ca^{2+}; it is necessary to inhibit also glycolysis by iodoacetic acid. This compound is a powerful sulphydryl group reagent, so that, at present, an effect on membrane proteins cannot be excluded. However, experiments carried out with other normal cell systems (intact lymphocytes) in which iodoacetic acid does not affect plasma membrane responsiveness to A23187, rule out this possibility (unpublished observation).

Our results indicate that in intact ascites tumour cells energy from endogenous metabolism strongly opposes intracellular calcium increase. Even the addition of A23187 is without effect (Figs. 3 and 5).

It is known that the Ca^{2+} ionophore A23187 causes uncontrolled calcium rise by increasing passive Ca^{2+} leak through hydrophilic channels open in the membrane. It could be argued that in the above mentioned condition the failure to increase permeability of the plasma membrane is only apparent because the level of total calcium is kept low by increase of extrusion activity. The experiments with $^{45}Ca^{2+}$ (Fig. 5) exclude this possibility, since also $^{45}Ca^{2+}$ exchange is not affected by the ionophore. Furthermore a rough estimate of intracellular $^{45}Ca^{2+}$ specific activity, indicates that also this parameter remains unchanged. Thus it clearly appears that even under physiological conditions the inward leak proceeds at its maximal rate. This rate can be increased only by profound modification of the membrane structure as those elicited by dextran sulphate [9] or digitonin treatment. In order to increase ascites tumour cell total calcium it is necessary to achieve ATP depletion of more than 90%. Though not directly demonstrated and according to the results of previous authors on different cell models [24], this observation suggests high affinity for ATP of the extrusion mechanism.

It has been recently reported that ascites tumour cells incubated at 0–4°C, do not take up Ca^{2+} and this has been ascribed to unusual low Ca^{2+} plasma membrane permeability [8]. Our experiments do not agree with such a conclusion since energy depleted ascites tumour cells take up Ca^{2+} at 0–4°C to the same extent and at the same apparent rate as cells at 38°C (Fig. 4). The apparent discrepancy may be linked to the fact that the cells

require a wide extent of time in order to undergo a sufficient reduction of their energy charge and probably still extrude calcium. This would not be an unusual occurrence since other Ca^{2+}-related energy-dependent mechanisms have been shown to operate even at sub-zero temperature [25].

Massive calcium entry into ascites tumour cells leads to equilibrium of concentration across plasma membrane, as seen by the experiments at different external Ca^{2+} concentrations, when the functional distinction between all relevant intracellular compartments is lost because of energy depletion (Fig. 6). In these conditions Ca^{2+} entry is maximal without the addition of A23187 (Figs. 3 and 5). The results of these experiments further indicate that the fast initial Ca^{2+} increase in the active cells, is concentration dependent (see also Ref. 10). Apparent cell calcium concentration increases proportionally to the extracellular Ca^{2+} but it lays below the theoretical equilibrium. This suggests that activation of the extrusion mechanism occurs probably at high concentration of intracellular Ca^{2+}.

It must be mentioned that permeabilization to Ca^{2+} by A23187 has been observed in all the several cell types up to now investigated; in addition in red blood cells A23187 has been described to increase Ca^{2+} content both in energy-supplemented and energy-depleted cells [22]. The only effect of permeabilization we have observed is that in the early stages of ascites tumour cells energy depletion (Figs. 3 and 8). Nevertheless, for a number of reasons, this effect has to be interpreted partly as a nonspecific one. First of all, the above mentioned effect disappears after the cells are thoroughly de-energized (Fig. 3). Moreover, evidence has been presented which shows how the ionophore affects well known energy-dependent parameters, such as cell H_2O and K^+ content. Cell water content increases with the same characteristics of Ca^{2+} accumulation and K^+ loss follows an inverse pattern. A23187 accelerates these processess. The results of Fig. 3 and 9, however, indicate that A23187 increases Ca^{2+} permeability in the plasma membrane of ascites tumour cells during the early stages of energy depletion. This effect proposes interesting consideration for the explanation of the whole problem concerning the observed failure of A23187 to increase Ca^{2+} permeability in ascites tumour cells under active metabolic condition. First, it could be argued that the level of endogenous energy, probably ATP, provokes membrane modifications such to inhibit the plasma membrane-ionophore interaction required in order to observe Ca^{2+} permeabilization. A second, more likely, probability is that under active metabolic condition, intracellular proton concentration, sustained by glycolytic activity, makes ineffective the A23187 molecule bound to the membrane. This occurrence would be supported by the evidence that only when glycolytic pathway is exhaustively inhibited by iodoacetic acid, ionophore-induced Ca^{2+} entry occurs.

The overall results indicate that passive Ca^{2+} permeability of in vivo ascites tumour cells is physiologically high (several fold higher than that found in red blood cells) and is not further increased by the treatment with the specific Ca^{2+} ionophore A23187. This occurrence could well explain the permanent mitogenic stimulation under which these cells physiologically are, and that is normally induced by variation of Ca^{2+} influx into the cell. This conclusion fits with the model that focuses as the main cancer cell alteration a plasma membrane modification such that the cell is under a permanent 'ionophore effect' of unknown origin. Much importance to this regard, is also attributed to Mg^{2+} movement [26].

Preliminary experiments performed with a new method for the direct monitoring of intracellular Ca^{2+} concentration, based on the incorporation of fluorescent dyes sensitive to free Ca^{2+} concentration [27,28], support the results shown in this paper (Pozzan, T. and Cittadini, A., unpublished data).

On the basis of the above considerations many other ascites tumour cell features could find an explanation in the increased Ca^{2+} influx (high aerobic glycolysis and low Pasteur effect) [13]. It would be of great interest to investigate Ca^{2+} extrusion from ascites tumour cells by this experimental model, namely in vivo after massive loading, but, up to now, the extensive cell damage induced by uncontrolled Ca^{2+} increase made useless every efforts. Further work is in progress in our laboratory in order to better clarify this subject, to extend these observations to other tumour systems and especially to compare normal and neoplastic cells of the same origin.

Conclusion

The results presented in this paper lead to the conclusion that in vivo Ehrlich ascites tumour cells are physiologically highly permeable to extracellular Ca^{2+}. Passive Ca^{2+} entry, in these cells, proceeds physiologically at high rate that is not further increased by the specific Ca^{2+} ionophore A23187. The main mechanism that prevents uncontrolled Ca^{2+} increase is the energy-dependent extrusion. Ca^{2+} can enter intact ascites tumour cells only when their metabolism, and especially their glycolytic activity, is exhaustively inhibited. Our results fit with the model that explains neoplastic cell biological as well as biochemical behaviour as dependent on increased Ca^{2+} influx, and probably content, in tumour cells.

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