INTERACTION OF CALCIUM WITH MITOCHONDRIA ISOLATED FROM EHRLICH ASCITES TUMOUR CELLS

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SUMMARY

Calcium ions are accumulated by intact mitochondria isolated from Ehrlich ascites tumour cells in a buffered system supplemented with ATP or succinate. In the ATP-supplemented system, the tumour mitochondria, in contrast to rat liver mitochondria, retain the accumulated Ca^{2+}, do not exhibit a marked "irreversible" ATPase and do not swell. In the succinate-supplemented system, added Ca^{2+} stimulates respiration in either the absence or presence of added inorganic phosphate. Whereas respiration by rat liver mitochondria, measured in the presence of added phosphate, remains continuously activated after the addition of only a small amount of Ca^{2+}, that by the tumour mitochondria can be stimulated by several successive additions of 100 μM Ca^{2+} and at all times exhibit appreciable activation ratios.

INTRODUCTION

The ability of rat liver mitochondria to accumulate Ca^{2+} in preference to Mg^{2+} affords a means of altering the Mg^{2+}/Ca^{2+} ratio in the liver cell cytoplasm. Such an alteration can in turn lead to modification of enzyme activities, such as pyruvate kinase, sensitive to this ratio (1). These circumstances lend themselves to an interpretation in which movement of Ca^{2+} between mitochondria and the cytoplasm provides a metabolic link between mitochondrial and cytoplasmic reactions sensitive to Ca^{2+} (2).

The apparent "imbalance" between glycolysis and respiration in Ehrlich ascites tumour cells (reviewed in ref. 3) makes these cells a potentially suitable system for examining further the ionic control concept (2). This, together with an apparent abnormal calcium metabolism associated with some forms of cancer (see ref. 4) and the realisation that little attention has been

paid previously to the interaction of Ca^{2+} with energy-linked reactions in mitochondria from tumour cells in general, has prompted the present investigation. We show here that some of those properties such as Ca^{2+}-induced ATPase and swelling, which among others characterise the interaction of Ca^{2+} with rat liver mitochondria (5-7), are considerably modified in mitochondria isolated from Ehrlich ascites tumour cells.

MATERIALS AND METHODS

The Ehrlich ascites tumour used was a hyperdiploid-strain supplied by Dr. E.L. French, Animal Health Research Laboratories, Parkville, Victoria, Australia. Details of propagation are described elsewhere (8). The ascites cells were harvested from mice 8-11 days after inoculation. Cells from 6-8 mice were generally of sufficient quantity to allow a reasonable yield of mitochondria. The method described by Wu and Sauer (9) employing the Dounce homogeniser was used with slight modification to prepare the mitochondria. Rat liver mitochondria were prepared as described elsewhere (10). Oxygen uptake was measured using the polarographic technique (11). ATPase activity was measured by continuous recording of the release of protons into the incubation medium using a glass electrode in conjunction with a Townson Expansion pH meter connected to a Rikadenki recorder. Movement of Ca^{2+} was monitored using radioactive Ca^{2+}. The Millipore filtration technique was used to separate mitochondria from the suspending medium.

RESULTS

Mitochondria isolated from the ascites cells in this laboratory consistently exhibit acceptor control ratios of between 3 and 5 with succinate as substrate, undergo repeated respiratory state III-IV transitions retaining "controlled" rates of oxidation and possess a latent ATPase which is stimulated by DNP to a rate about ½ of that observed for rat liver mitochondria. Mg^{2+} has no effect while oligomycin and atractyloside both completely inhibit the ATPase. These criteria taken together suggest the tumour mitochondria prepared in this work possess a high degree of intactness. Examination of the preparations by electron microscopy confirms this supposition.

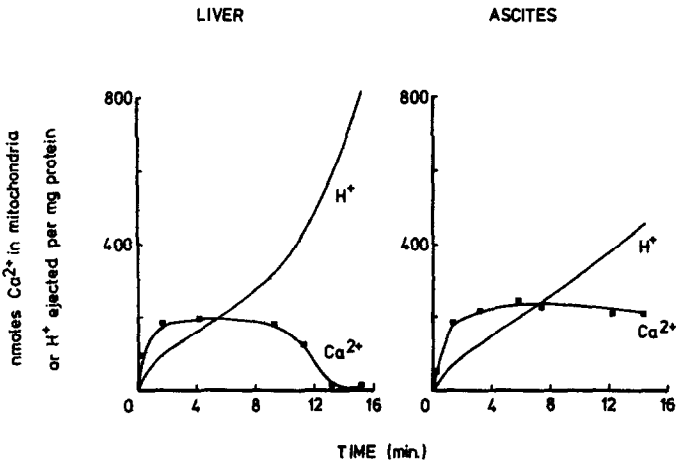

Fig. 1. pH changes and Ca^{2+} movements associated with ATP-supported Ca^{2+} accumulation in mitochondria from rat liver and from ascites tumour cells. The reaction system contained 80 mM sucrose, 16 mM Tris-HCl (pH 7.4), 3 mM ATP and 4 mg mitochondrial protein. The total volume was 3.0 ml and the temperature 25°. Ca^{2+} (600 nmoles for liver mitochondria and 900 nmoles for ascites mitochondria) containing $^{45}Ca^{2+}$ was added to start the reaction.

Considerable information about the interaction of Ca^{2+} with mitochondria was obtained in an incubation system containing ATP, buffer and Ca^{2+}, and by measuring both changes in pH and movement of $^{45}Ca^{2+}$ (see 5,6). Data in Fig. 1A show the pH and $^{45}Ca^{2+}$ uptake traces characteristic of rat liver mitochondria. The two phases of rapid H^+ ejection seen reflect, in the main, the initial accumulation of Ca^{2+} supported by energy derived from ATP hydrolysis (6,12) and secondly, the time-dependent uncoupler-like action of Ca^{2+}. This action induces irreversible ATPase activity and as well produces swelling and loss of Ca^{2+} from the mitochondria (5,7).

When the experiment is repeated using the ascites tumour mitochondria (Fig. 1B) and an equal or even greater concentration of Ca^{2+}, a different pattern of events is seen. There is an initial ejection of H^+ corresponding to accumulation of Ca^{2+} as observed with the rat liver mitochondria. However, there is no marked event corresponding to rapid H^+ ejection and loss of $^{45}Ca^{2+}$ from the mitochondria. Likewise no swelling of the tumour mitochondria as measured by changes in O.D.^{520}, could be

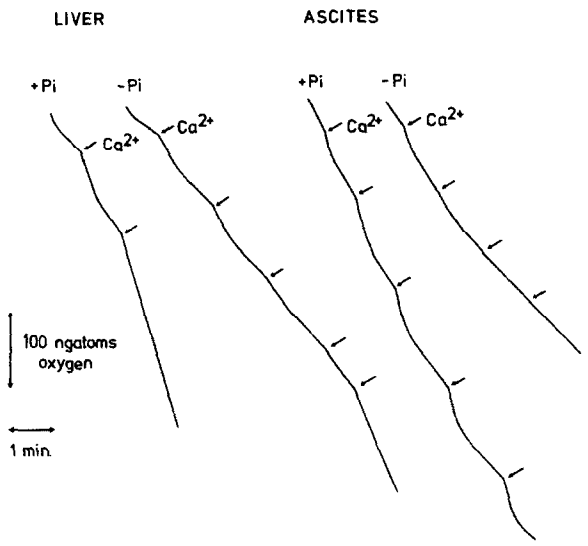

Fig. 2. Stimulation by Ca^{2+} of respiration in mitochondria from rat liver and from ascites tumour cells. The reaction system contained 250 mM sucrose, 2.5 mM Hepes buffer (pH 7.4) 6.5 mM succinate, 0.25 mM phosphate as indicated, 4 mg liver or ascites mitochondrial protein in a final volume of 2.0 ml. Temperature, 25°. At the points indicated by an arrow 200 nmoles of Ca^{2+} were added.

observed under conditions where rapid Ca^{2+}-induced swelling occurred in rat liver mitochondria.

In a second series of experiments, the ability of Ca^{2+} to stimulate respiration in the tumour mitochondria was examined. Fig. 2 compares data obtained with mitochondria from rat liver and from the ascites cells. Both types of mitochondria can undergo a "respiratory jump" as seen earlier with both liver and heart mitochondria (13,14). With tumour mitochondria the activation ratio for succinate (i.e. nmoles Ca^{2+} added to ngatoms extra oxygen taken up) was usually in the range 3.3-4.1 in the presence of added phosphate.

The addition of only 0.25 mM phosphate to rat liver mitochondria leads to rapid loss of Ca^{2+}-stimulated respiratory control, an observation previously described by Rossi and Lehninger (14).

Addition of an identical concentration of phosphate to tumour mitochondria on the other hand, allows Ca^{2+} to repetitively stimulate respiration; a similar activation ratio was obtained even after 5 successive additions of 200 nmoles Ca^{2+}.

DISCUSSION

It is clear from this broad survey that Ehrlich ascites tumour mitochondria and rat liver mitochondria differ in several fundamental respects with regard to their interaction with Ca^{2+}.

The usual uncoupling action of Ca^{2+} which elicits ATPase activity, swells mitochondria and causes the release of accumulated Ca^{2+}, is not evident in identical studies carried out with the tumour mitochondria which as far as can be judged, are functionally and morphologically intact. Furthermore, while our data clearly show that Ca^{2+} will induce a respiratory jump in ascites tumour mitochondria, its interaction with the respiratory assemblies in these mitochondria exhibits somewhat atypical properties, particularly with regard to the involvement of inorganic phosphate.

Current studies which will be described in detail elsewhere are aimed at further defining these interactions from the viewpoint of establishing whether the above-described differences (a) reflect a real difference in mitochondrial function and (b) how they might relate to the glycolytic/respiratory energy "imbalance" known to exist in these cells (3).

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