ATP REQUIREMENT IN THE COURSE OF CALCIUM UPTAKE BY HUMAN MYOMETRIAL MITOCHONDRIA

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1. Introduction

The uptake of Ca by mitochondria can be energized by either ATP or respiration [1]. Liver mitochondria can accumulate Ca in the absence of added adenine nucleotides when a respiratory substrate, such as glutamate or succinate is present [2]. Recently it was shown that Ca uptake in cardiac mitochondria could also be supported either by succinate or by ATP with equal facility [3, 4]. In the experiments to be presented, Ca uptake in the presence of succinate or succinate and P_i, with or without ATP was studied. In contrast to liver or cardiac mitochondria, mitochondria from human myometrium were found to be unable to accumulate Ca in the absence of ATP. With succinate as substrate, ATP increased Ca uptake considerably. In the presence of oligomycin, there was still a marked stimulation of Ca uptake by ATP. Dinitrophenol inhibited Ca uptake whether it was supported by ATP alone or ATP and succinate.

2. Methods

Tissues were removed at operation, usually a cesarean section, and collected in Krebs-Ringer bicarbonate solution [5]. The endometrium was removed and the myometrium was cut into strips 2–3 mm wide which were blotted and placed in a weighed beaker containing ice-cold sucrose (0.25 M) and histidine (5 mM) solution. After weighing, the tissue was homogenized with a Polytron homogenizer as previously described [6]. The homogenate was centrifuged at 1000 *g* for 10 min and the pellet discarded. The supernatant was filtered through 3 layers of gauze and centrifuged at 15,000 *g* for 15 min. The sediment was suspended in sucrose–histidine medium and centrifuged at 10,000 *g* to obtain the mitochondrial pellet. The above procedure was carried out at 4°. The mitochondrial pellet was suspended in sucrose–histidine medium to give 0.5 to 1 mg of protein per ml and used for Ca uptake experiments immediately.

Unless otherwise stated standard Ca uptake medium consisted of 0.125 M sucrose, 0.1 M Tris buffer (pH 7.2), 5 mM $MgCl_2$, 0.1 mM $^{45}CaCl_2$ and 0.2–0.3 mg mitochondrial protein in a total volume of 1 ml. When used, the final concentration of ATP or succinate or inorganic phosphate was 5 mM. The reaction was carried out at 25° and stopped by removal of mitochondria with Millipore filters (0.45 μ diameter) as described previously [6]. Blanks which lacked only mitochondria were filtered simultaneously. Ca uptake was determined by measuring the radioactivity in the filtrate by a Packard Tri-Carb liquid scintillation counter. Protein concentration was determined by the method of Lowry et al. [7] using bovine serum albumin as a standard. Oligomycin was purchased from Sigma Chemical Company and 2,4-dinitrophenol (DNP) from British Drug Houses. Oligomycin was dissolved in absolute alcohol to prepare a stock solution. When the effect of oligomycin was studied, the control experiments contained an equal concentration (1%) of alcohol.

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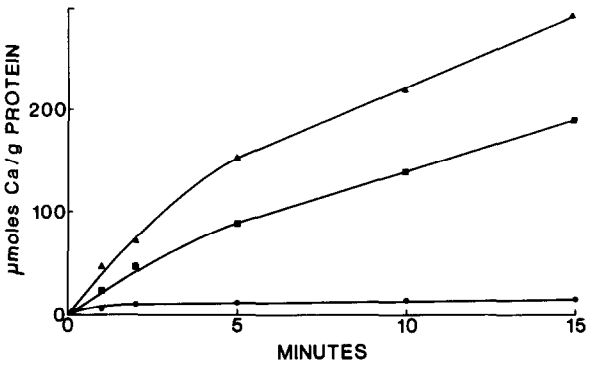

Fig. 1. Ca uptake by human myometrial mitochondria. Mitochondria were incubated at 25° in a medium containing 0.125 M sucrose, 0.1 M Tris (pH 7.2), 5 mM $MgCl_2$ and 0.1 mM $CaCl_2$. No additions or 5 mM succinate or 5 mM succinate + 5 mM Pi (•), 5 mM ATP (■), 5 mM ATP + 5 mM succinate (▲).

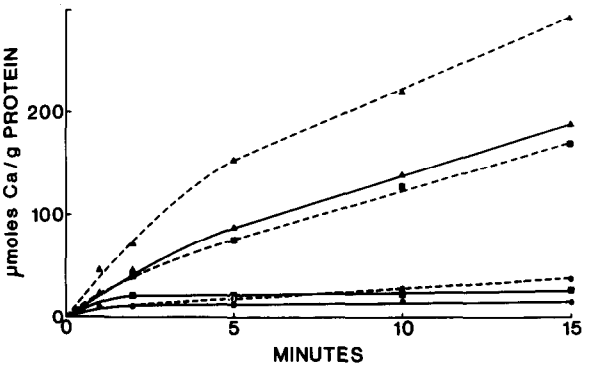

Fig. 2. Effect of inhibitors on Ca uptake by myometrial mitochondria. Mitochondria were incubated as in fig. 1, containing 5 mM ATP (solid lines) or 5 mM ATP + 5 mM succinate (broken lines). Without inhibitors (▲), with 4 µg per ml oligomycin (■), with 0.2 mM DNP (•).

3. Results and discussion

In the absence of ATP, myometrial mitochondria accumulated very little Ca (fig. 1), and addition of neither succinate nor succinate + P_i increased uptake. However, uptake of Ca was substantially increased by addition of ATP to the incubation medium. and still further increased by addition of ATP + succinate.

DNP completely abolished Ca accumulation in the presence of ATP and ATP + succinate (fig. 2) and in this respect the behaviour of myometrial mitochondria is similar to that of mitochondria from liver and cardiac muscle [1–3, 8]. Oligomycin completely abolished ATP supported Ca accumulation by the myometrial mitochondria. However, the uptake of Ca in the presence of ATP + succinate was only partially inhibited. That the inhibition was only partial was interesting since the same concentration of this inhibitor completely suppressed Ca uptake supported by energy from ATP.

The above results demonstrate that human myometrial mitochondria have the ability to accumulate large amounts of Ca and this process can be supported by energy from ATP. The process can also be supported by respiration but only when ATP is present. Thus somehow the ATP must allow energy from respiration to be utilized for mitochondrial Ca uptake. This was confirmed by using oligomycin, which is known to prevent the transfer of energy from ATP to mitochondrial metabolism. It inhibited ATP supported Ca uptake but seemed to exert little effect on respiration-dependent Ca uptake in the presence of ATP. From this is can be concluded that ATP itself cannot only support Ca uptake in myometrial mitochondria but also plays an essential role in mitochondrial Ca uptake supported by energy from respiration. The mechanism by which ATP plays the seond role is not clear at present. While this investigation was under way Tjioe et al. [9] demonstrated Ca uptake by brain mitochondria which had similar features as reported in the present study. In more recent experiments in this laboratory, ADP was found to be a poor substitute for ATP in supporting Ca uptake either alone or in the presence of succinate.

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