THE ROLE OF MITOCHONDRIA IN UTERINE CONTRACTIONS

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1. Introduction

The contractile activity of smooth muscle, like that of all muscle types, is controlled by changes in the myoplasmic free calcium ion concentration [1–3]. Muscle concentration is initiated by a rise in the calcium concentration and relaxation occurs when the calcium level has been lowered to the initial level. It is known that extra-cellular calcium is necessary for normal contractile activity of the isolated uterus, but some activity has been demonstrated in a calcium-free medium indicating the presence of an intracellular calcium pool [4,5]. Carsten [6] has proposed that the control of smooth muscle contraction is the same in principle as in skeletal muscle. According to this model, which seems well established for the case of skeletal muscle, the sarcoplasmic reticulum (SR) releases calcium upon stimulation and subsequently accumulates the released ions. However, the poorly developed SR and the absence of the triad system as seen in electron micrographs [7], as well as the low calcium capacity of isolated myometrial SR has lead some authors to doubt this type of mechanism. Consequently, the possibility that other cell organelles such as the plasma membrane and the mitochondria might be involved in the release and reaccumulation of calcium ions in smooth muscle has been proposed [8–10].

In the present work we have attempted to elucidate the role of mitochondria in regulation of intracellular calcium in smooth muscle by studying the kinetics and general properties of calcium transport in isolated myometrial mitochondria. In addition we report on the activity and properties of α-glycerophosphate (αGP) oxidation by these mitochondria.

Mitochondria isolated from various tissues are known to take up calcium ions either in a respiration-dependent fashion or driven by ATP hydrolysis [11,12]. This process has been studied most thoroughly in liver and heart mitochondria [13]. The uptake is thought to be mediated by a carrier mechanism because it shows saturation kinetics [14] and sensitivity to specific inhibitors [15,16]. The kinetics of mitochondrial calcium uptake is apparently co-operative, showing a sigmoidal relation between uptake velocity and calcium concentration [14,17]. Since the free intracellular calcium ion concentration is probably very low, in the order of 10^{-7} M in relaxed and 10^{-6} M in maximally contracted muscle respectively [18], and the rate of mitochondrial calcium uptake at these concentrations is very low in the tissues so far studied, a major contribution of mitochondria to the regulation of the cytosolic calcium level and hence of the contractile activity has been seriously questioned for the case of heart muscle [19].

2. Materials and methods

Mitochondria were isolated by the following procedure. A fresh bovine uterus was freed of endometrium and connective tissue, ground in a hand-driven meat grinder, and suspended at 1:6 (w:v) in a medium containing 100 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA and 50 mM Tris–HCl pH 7.2. The suspension was homogenized in 250 ml aliquots for 1 min with an Ultra-Turrax homogenizer and centrifuged for 10 min at 1000 *g*. The supernatant fraction was filtered through a triple cheesecloth and the filtrate centrifuged for 10 min at 10 000 *g*. The pellet

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was suspended in basically the same medium except that EDTA was omitted and $MgCl_2$ reduced to 3 mM. The suspension was centrifuged for 10 min at 1000 *g*, and the supernatant fraction was centrifuged 10 min at 10 000 *g*. The resulting pellet was washed twice in the same medium. The washed mitochondria were resuspended in a small volume of 0.25 M sucrose, pH 7.2. The entire procedure was carried out at 0–4°C.

3. Results and discussion

In order to get information about the functional integrity of the mitochondrial preparation we measured oxygen consumption polarographically. In intact mitochondria respiration is tightly coupled to energy conservation [20]. Experiments with different substrates showed that respiration was greatly stimulated upon the addition of calcium ions in the presence of inorganic phosphate. When the added calcium had been taken up (see below), respiration reverted to the initial rate (see fig.1A). The mitochondria were thus tightly coupled.

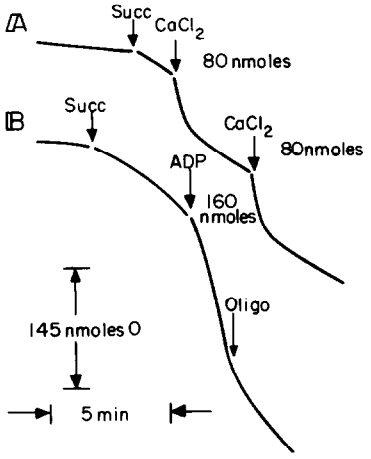

Fig.1. Initial velocities of calcium uptake at different calcium concentrations. Calcium uptake was measured spectrophotometrically with the murexide technique employing an Amino DW-2 spectrophotometer at the wavelength couple 540 minus 507 nm. To the basic medium consisting of 0.2 M sucrose, 0.02 M KCl, 0.02 M *N*-2-hydroxypethylpiperazine-*N'*-2-ethane-sulphonate (HEPES) buffer pH 7.2, 12 μM rotenone, 4 mM KH_2PO_4, 2 mM $MgCl_2$ the following additions were made: succinate 4 mM, murexide 60 μM, 0.4 mg/ml BSA (essentially lipid free bovine serum albumin) and approx. 1 mg mitochondrial protein. The final vol was 2.5 ml and the light path 1.0 cm. $CaCl_2$ was added manually and the initial rate was recorded at 1–2 sec after the addition. Temperature 22–23°C.

In coupled mitochondria the rate of respiration is controlled by the phosphate potential, a function of the [ATP]/[ADP] [P_i] ratio [21–23]. When the phosphate potential is high the respiratory rate is low and vice versa. Addition of ADP to the mitochondrial preparation in the presence of substrate and inorganic phosphate (P_i) stimulated respiration due to lowering of the phosphate potential (fig.1B). However, the rate of respiration did not revert to the initial level but remained high. This is due to ATPases of other than mitochondrial origin in our preparation, hydrolyzing the ATP generated by oxidative phosphorylation whereby the phosphate potential is kept constantly low. This is demonstrated by the finding that the ADP-stimulated respiration was inhibited by oligomycin (fig.1B), the specific inhibitor of oxidative phosphorylation [24,25], again indicating functional integrity of the isolated mitochondria. It may be noted that the rate of respiration was always much faster during active calcium accumulation than during oxidative phosphorylation, a phenomenon that was very pronounced in some of the mitochondrial preparations (fig.1 and [10]). The reason for the relatively slow rate of oxygen consumption during ATP synthesis is not yet understood [10]. A related interesting facet of the myometrial mitochondria is the consistently much higher respiratory control of calcium accumulation as compared with that of ATP synthesis (fig.1).

Similar experiments revealed a very high activity of α-glycerophosphate (αGP) oxidation in the myometrial mitochondria. In the uncoupled state the respiratory rate with αGP as substrate was about one third of the rate with succinate, approx. 70 nmol/min × mg of protein. The apparent K_M for αGP, calculated from the respiratory rate at different substrate concentrations was found to be low (0.25 mM, see fig.2), about one order of magnitude lower than the value reported for blowfly flight muscle mitochondria [26]. The apparent K_M for αGP was unaffected by calcium, in contrast to the case in flight muscle mitochondria. αGP oxidation by oxygen was fully inhibited by antimycin in the myometrial mitochondria, but the activity with ferricyanide as electron acceptor was

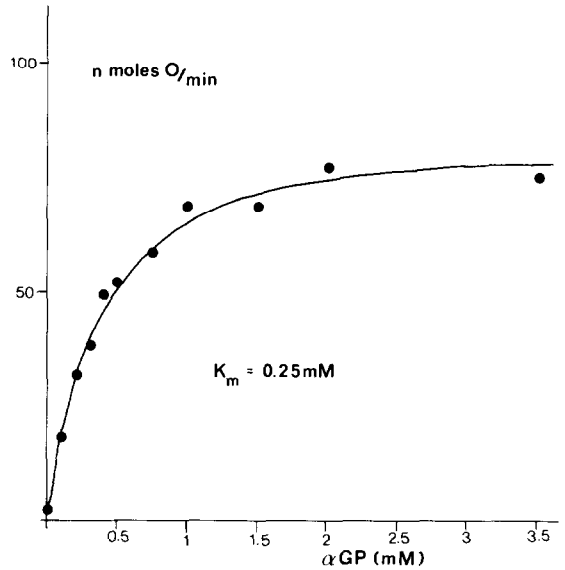

Fig.2. Respiratory stimulation by calcium (trace A) and ADP (trace B). The reaction was recorded polarographically with a Clark electrode in a closed 1.2 ml vessel thermostated at 28°C. The basic medium described under fig.1 was supplemented with: mitochondria (1 mg protein/ml) and BSA 1 mg/ml. Succinate (succ, 4 mM), $CaCl_2$, ADP and oligomycin (8 μg/ml) were added as indicated.

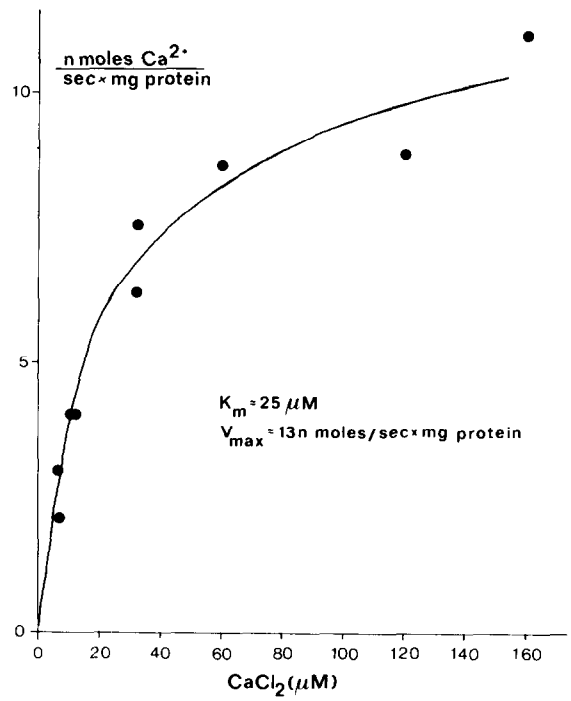

Fig.3. Oxygen consumption as a function of α-glycerophosphate concentration. Experimental conditions as in fig.2. Additions: 1.1 mg mitochondrial protein, 1 mg/ml BSA and 4 μM FCCP (carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone). α-Glycerophosphate was added as indicated.

insensitive to this inhibitor. This suggests that αGP dehydrogenase is located externally at the inner membrane of the myometrial mitochondria, similar to flight muscle mitochondria, where it is accessible to the non-penetrant ferricyanide. The high activity and low apparent K_M of αGP oxidation by bovine myometrial mitochondria suggest that the αGP shuttle [27] may be the main pathway for oxidation of cytoplasmic NADH generated by glycolysis in this tissue. This proposal is consistent with experiments with spontaneously contracting rat-uterus horns [28], which have shown that cycloserine, an inhibitor of the transamination reactions involved in the alternative mechanism for oxidation of cytoplasmic NADH, the malate shuttle [29], had no effect on the spontaneous contractile activity.

Calcium uptake of the isolated myometrial mitochondria was measured spectrophotometrically using the murexide technique [30,31]. The initial rates of calcium uptake at different calcium concentrations are plotted in fig.3. As shown in the figure, calcium transport in bovine myometrial mitochondria is hyperbolic with an apparent K_M of approx. 25 μM. This contrasts our preliminary findings with a more crude mitochondrial preparation suggesting sigmoidal kinetics [10]. With the refined isolation procedure reported here we have, however, consistently observed hyperbolic calcium uptake kinetics. The suggests that the uptake velocity at the low calcium concentrations assumed to prevail in the muscle cell during contraction may be much faster in the myometrium than in heart muscle where calcium uptake kinetics are strongly sigmoidal [14,17,19]. Thus a central role of mitochondrial calcium uptake during the relaxation phase must seriously be taken into consideration for the case of the uterus. This possibility is further supported by our finding [10] that myometrial mitochondria have a high capacity for calcium, in the order of 1500 nmol calcium per mg of protein. This capacity may be compared to that of isolated myometrial SR

which has been reported to be lower by three orders of magnitude [32,33].

This study shows for the first time a mitochondrial preparation from mammalian tissue with hyperbolic calcium transport kinetics at least down to about 5 μM of Ca^{2+}, the lower limit of the murexide technique. It is of interest that Vallieres, Somlyo and Scarpa [34] have very recently observed similar properties of mitochondria isolated from vascular smooth muscle, and have shown by using a more sensitive technique that calcium uptake is essentially hyperbolic down to about 1 μM concentrations of the cation. This property thus seems to be general for smooth muscle mitochondria and suggests a central role of these organelles in the control of myoplasmic calcium concentration in smooth muscle and thus in smooth muscle contractions.

Acknowledgement

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