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THE ROLE OF MITOCHONDRIAL CALCIUM UPTAKE IN CONTRACTION AND RELAXATION OF THE HUMAN MYOMETRIUM

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SUMMARY

Ca^{2+} binding by the mitochondrial and microsomal fractions of human myometrium was studied in the presence of ATP. Mitochondria in contrast to microsomes were able to lower the Ca^{2+} concentration to $<10^{-7}$ M in a medium that originally contained 10^{-5} M Ca^{2+}. An estimate of the length of time required by the total amount of mitochondria present in the tissue to lower the cytoplasmic Ca^{2+} concentration from 10^{-5} to 10^{-7} M indicated that it was compatible with the observed relaxation in this smooth muscle.

INTRODUCTION

Ca^{2+} is intimately involved in excitation–contraction coupling and in relaxation of skeletal, smooth and cardiac muscles[1–3]. It is generally accepted that sarcoplasmic reticulum controls the level of intracellular Ca^{2+} in skeletal muscle below the threshold (10^{-7} M), needed for the activation of the contractile system[4,5]. This is achieved by an energy-dependent Ca^{2+} binding by the sarcoplasmic reticulum[4,5]. Mitochondria from muscle and other tissues have also been shown by many authors to be capable of binding considerable amounts of Ca^{2+} *in vitro*[6–8]. In cardiac and red skeletal muscle, due to a relatively small capacity of reticulum and the large abundance of mitochondria, Ca^{2+} uptake by mitochondria has been implicated as an additional mechanism controlling cytoplasmic Ca^{2+} in these tissues[8–10].

In smooth muscle it is not clear how the cytoplasmic Ca^{2+} is controlled, although some evidence in favour of the sarcoplasmic reticulum also regulating intracellular Ca^{2+} in this muscle, has been reported[11,12]. Previous studies from this laboratory demonstrated an ATP-dependent uptake of Ca^{2+} by the mitochondrial and microsomal fractions of the human myometrium[13,14]. The large Ca^{2+} binding capacity of the mitochondria and the selective inhibition of Ca^{2+} uptake by estrogens[15,16], led to the suggestion that these organelles may play a significant role in controlling the intracellular Ca^{2+} concentration and thereby contraction and relaxation of the myometrium. However, in order to judge whether the mitochondrial Ca^{2+} uptake system studied *in vitro* is able to control the Ca^{2+} activity and thus regulate relaxation in this muscle the following two important questions should be

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answered: (1) Can mitochondria lower the Ca^{2+} concentration below 10^{-7} M in the external medium? (2) Can the rate of Ca^{2+} uptake by the mitochondria account for the speed of relaxation? In the present investigation, an attempt was made to obtain this information.

MATERIALS AND METHODS

Pieces of myometrium were obtained usually at cesarean operations. A mitochondrial and a microsomal fraction were isolated as previously described[13,14]. Briefly, the tissue was homogenized in ice-cold sucrose (0.25 M) and histidine (5 mM) solution[13], centrifuged at $1000 \times g$ for 10 min and the pellet discarded. The supernatant was filtered through three layers of gauze and centrifuged at $15000 \times g$ for 15 min. The above supernatant was centrifuged at $90000 \times g$ for 1 h to obtain the microsomal pellet. The sediment obtained after centrifugation at $15000 \times g$ was suspended in the sucrose–histidine medium and again centrifuged at $10000 \times g$ to obtain the mitochondrial pellet. The above procedure was carried out at 0–4 °C. The mitochondrial and the microsomal pellets were suspended, in KCl (0.1 M) and imidazole (20 mM) solution to give a protein concentration of 1–2 mg/ml and used for the Ca^{2+} uptake experiments immediately.

Ca^{2+} uptake measurement was done by Millipore filtration technique using $^{45}Ca^{2+}$, essentially by the method reported previously[13,15] except the Ca^{2+} uptake medium was modified in composition to make it similar to the expected intracellular medium. It consisted of 100 mM KCl, 5 mM sodium succinate, 4 mM sodium ATP, 7 mM $MgCl_2$ and 20 mM imidazole. The Ca^{2+} concentration of the medium was 10^{-5} M except in some experiments where 10^{-4} M was used for comparison. The pH was 7.0, and the incubations were carried out at 35 °C.

RESULTS

Fig. 1 shows that from a medium containing 10^{-5} M Ca^{2+}, mitochondria removed 99% Ca^{2+} in 5 min whereas microsomes removed only 65% Ca^{2+} even after 10 min in spite of the fact that the concentration of microsomes (0.86 mg protein per

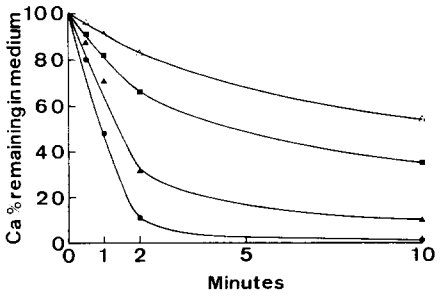

Fig. 1. Ca^{2+} removal by mitochondria (●) and microsomes (■) from a medium containing 10^{-5} M Ca^{2+}. The reaction mixture contained 100 mM KCl, 5 mM sodium succinate, 4 mM ATP, 7 mM Mg^{2+} and 20 mM imidazole. The mitochondrial and microsomal protein concentrations in these incubations were 0.36 and 0.86 mg/ml, respectively. The amount of Ca^{2+} remaining in the medium was measured at times shown. Ca^{2+} uptake from a medium with 10^{-4} M Ca^{2+} under identical conditions is shown for comparison, mitochondria (▲) and microsomes (△).

ml) was more than twice of mitochondria (0.36 mg protein per ml). Results of the experiment with a higher concentration of Ca^{2+} (10^{-4} M) in the medium (△, Fig. 1) indicated that microsomes were not filled to capacity but were actually not sensitive to low concentrations of Ca^{2+} in the medium. Here, the same concentration of microsomes accumulated at least 5 times more Ca^{2+}. Mitochondria also took up much more Ca^{2+} from the medium containing higher concentration of Ca^{2+}.

Experiments with very low Ca^{2+} concentrations in the medium with the use of Ca-EGTA buffers, confirmed that the microsomes were unable to take up Ca^{2+} from a medium containing approx. 10^{-7} M Ca^{2+} while mitochondria continued to take up until the Ca^{2+} concentration in the medium was lowered to approx. 10^{-8} M (Table I). The concentration of mitochondria in these experiments (0.34 mg protein per ml) was about 4 times less than their reported yield (1.3 mg protein) per g muscle[13]. Fig. 2 shows the relationship between the amount of Ca^{2+} removed from the medium both after 2 and 5 min and the amount of mitochondrial protein. As can be seen, there was a nearly linear relationship between the amount of Ca^{2+} removed from the medium and the amount of mitochondria present in the medium. A proportionately smaller amount of mitochondria protein was needed to remove the same amount of Ca^{2+} when the incubation time was increased from 2 to 5 min.

TABLE I

Ca^{2+} UPTAKE FROM MEDIA OF DIFFERENT Ca^{2+} CONCENTRATIONS

The reaction mixture for Ca^{2+} uptake was as in Fig. 1. Ca^{2+} concentration in the medium was varied by using appropriate EGTA concentration[13]. The amount of Ca^{2+} taken up was measured after 5 min.

Ca^{2+} concn (M)	*Ca^{2+} uptake (nmoles/mg protein)*	
	Mitochondria	*Microsomes*
$0.87 \cdot 10^{-7}$	87.53	3.78
$0.62 \cdot 10^{-7}$	33.88	0
$0.92 \cdot 10^{-8}$	5.65	0

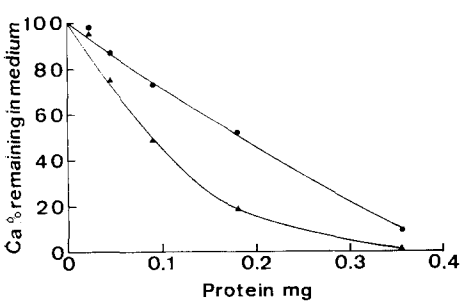

Fig. 2. Relation between mitochondrial protein and the amount of Ca^{2+} removed from the medium containing 10^{-5} M Ca^{2+}, measured after 2 min (●) and 5 min (▲). Other conditions as in Fig. 1.

DISCUSSION

The results of the present study clearly show that mitochondria in contrast to microsomes were able to lower the Ca^{2+} concentration below 10^{-7} M in a medium that originally contained 10^{-5} M Ca^{2+}. It should however be pointed out that this does not necessarily exclude the possibility that microsomes contribute to the regulation of cytoplasmic Ca^{2+} in this tissue under *in vivo* conditions. This uncertainty arises because of the fact that either the conditions for Ca^{2+} uptake measurements used in the present, and all other *in vitro* experiments, are not strictly comparable to those existing under *in vivo* situation, or the microsomes may have been damaged during the isolation procedure.

If the present yield represents 100% recovery of mitochondria (which is a gross overestimation), and if the cell water is about 410 ml/kg tissue[11], there would actually be 3.2 mg of mitochondrial protein per ml of cell water *in situ*. If the rate of Ca^{2+} uptake is linear with mitochondrial protein concentrations higher than that measured in the present experiments (Fig. 2), mitochondria would take about 30 s to lower the Ca^{2+} concentration in the cytoplasm from 10^{-5} to 10^{-7} M, that is to reverse the state of activation to complete relaxation.

The duration of the relaxation phase, as of the total contraction, of an isolated strip from the pregnant human myometrium varies considerably depending on the inherent activity of the preparation[17]. The relaxation phase during a noradrenaline induced contraction of the non-pregnant myometrium *in vitro* takes at least 1 min[18]. Similar duration for relaxation was observed when a contraction of a fully depolarized preparation was induced by acetylcholine (Aronson, S. and Batra, S., unpublished). These figures for the length of time for relaxation are much higher than the above calculated time (30 s) needed to lower the cytoplasmic Ca^{2+} concentration from 10^{-5} to 10^{-7} M by the mitochondria. However, if the concentration of cytoplasmic Ca^{2+} during contraction (which would depend on the nature and intensity of stimulus) is higher than 10^{-5} M[4,19] it would take correspondingly longer time for mitochondria to lower the Ca^{2+} concentration below 10^{-7} M. Furthermore, the Ca^{2+} uptake system if responsible for relaxation should remove the appropriate amount of Ca^{2+} in less than the time it takes for relaxation. It should also be mentioned that the Ca^{2+} which is to be removed by any system(s) to induce relaxation is not free in the cytoplasm but is presumably bound to the contractile proteins.

The present data show that mitochondria from human myometrium not only have the ability to lower the Ca^{2+} concentration in the medium below the threshold (10^{-7} M) needed for the activation of the contractile system[4–11] but can do so with the speed that is compatible with relaxation of this muscle. In view of the relatively sparse amount of sarcoplasmic reticulum in smooth muscle, an efficient Ca^{2+} accumulation by mitochondria would be in keeping with the needs of the myometrial cell for controlling the intracellular Ca^{2+}. Different smooth muscles, however, may vary in their amounts of sarcoplasmic reticulum[20] and mitochondria, and thereby, in the mechanisms for controlling myoplasmic Ca^{2+}. Finally, although not considered in the present discussion, the importance of membrane-bound and extracellular Ca^{2+} in the contraction of myometrium should not be disregarded[2,21].

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