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The Role of Ca^{+2}– mg^{+2} ATPase Activity in the Sub-cellular Fractions in calcium sequestration and the effect of Quinine Sulphate on the Mechanical Activity of the Golden Hamster Vas Deferens Smooth Muscle

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ABSTRACT

The effect of Ca^{2+} and Mg^{2+} on total ATPase activity of vas deferens microsomal and mitochondria fractions was investigated. The results suggest the existence of Ca^{2+}-Mg^{2+} activated ATPase in this smooth muscle. However in the presence of moderate concentrations of Mg^{2+} (2-4Mm), the absence of calcium did not affect total ATPase activity. Microsomes and mitochondria from vas deferens are capable of accumulating calcium in the presence of Mg^{2+} and ATP. Quinine, while causing inhibitory effect on contractile activity, enhanced the calcium binding capacity of microsomes and mitochondria isolated from vas deferens muscle. These results may suggest that quinine may inhibit the contractile activity of this kind of smooth muscle through its ability to enhance Ca^{2+} accumulation by the sub cellular agencies which lead to a decrease of the free Ca^{2+} required for contraction.
KEYWORDS: Mitochondria; Microsomes; Calcium; ATPase, smooth muscle.

INTRODUCTION

As in skeletal and cardiac, the smooth muscle contractive activity and relaxation ultimately are dependent on an increase and decrease, respectively, of concentration of free calcium [2, 16, 17]. In skeletal muscle, there are conclusive evidences that calcium involved in the contraction relaxation cycle is of intracellular origin [3, 16, 18, 19] and that the sarcoplasmic reticulum is the major site for the sub cellular release and binding of this calcium [10, 20]. However, unlike skeletal muscle, the source of activator calcium is less clear in many smooth muscles, since many smooth muscles lack well defined sarcoplasmic reticulum [20, 22, 23, 37]. Thus, this has been led to a proposition that the sub cellular structures such as the membrane and sub-membrane structures and mitochondria may possess the ability of calcium regulation during the excitation – contraction coupling [5, 8, 24, 25, 37]. It has been well known that quinine cause enhance contraction at low concentration and cause contracture in skeletal muscle at higher concentration [16, 21, 22].This action of the drug is believed to be due to an increase in free Ca^{2+} released from sarcoplasmic reticulum [28,29].

On other hand, quinine at low and moderate concentrations caused a strong inhibition of the contractile activity of rat vas deferens smooth muscle [24, 25] and ileal smooth muscle of the Goden hamster [30]. In addition, parenteral administration of therapeutic doses of quinidine and quinine caused forearm vasodilatation and decreased mean arterial pressure in humans [49]. This study is an attempt to find out the role of Ca^{2+} –Mg^{2+} ATPase activity in the membrane vesicles and mitochondria and their role of Ca^{+2} binding which may play a role in the contraction relaxation cycle in this kind of smooth muscle. Also the effect quinine was tested on the Ca^{+2} up take by the fraction to correlate the effect of the drug on the mechanical activity with its effect on Ca^{+2} sequestration by the fractions.

MATERIALS AND METHODS

Smooth muscle strips from the vas deferens of Golden hamster were used throughout this study. The preparations were maintained in Krebs buffer solution containing (in mM) NaCl 120.7 , KCl 5.9 , $CaCl_2$ 2.5 , $MgCl_2$ 1.2, NaH_2PO_4 1.2 , $NaHCO_3$ 15.5 and glucose,11.5 at PH 7.3 , continuously aerated and kept at 37°C . Quinine sulphate stock solution was made up in normal krebK's solution.

For tension recording the preparation was mounted in a 50ml organ bath which continuously aerated with 95% O_2 and 5% CO_2 and kept at 37°C. The preparation connected via a conventional strain gauge directly into a Washington 400 MD / 1 ink- writing pen recorder.

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For the study of Ca^{45} up take and the enzyme activity by the sub cellular fractions, microsomes and mitochondria, were prepared by homogenization and differential centrifugation. The methods used have already been described in detail elsewhere [24; 25]. The up take reaction was started by mixing 1 ml of the suspended fraction (0.5-1.0 mg protein / ml) with 1ml of incubation medium containing 2mM ATP, 4mM $MgCl_2$ and 2.5mM $CaCl_2$ in 40 mM Tris-Maleate buffer at PH 7.0 with 0.1 µCi / ml Ca^{45}.The reaction was stopped by passage through a membrane filter of pore size 0.45 µm and after washing to remove non-specifically bound Ca^{45} label the filters were dried and counted in a Packard Trycarb Liquid Scintillation counter.

RESULTS AND DISCUSSION

Primary test of the total activity of Ca^{+2}- Mg^{+2} ATPase:

The implication of the sub cellular fractions in calcium regulation in smooth muscle, suggesting that calcium pump activity is essential function of these membrane systems, therefore, to test this hypothesis a primary investigation carried out to define the existence of of Ca^{2+} - Mg^{2+} ATPase activity in the microsomal and mitochondrial fractions in a known standard reaction medium.

As can be seen in figure (1), the total ATPase activity increased linearly over the 15 minutes duration of the measurement in both microsomal and mitochondrial fractions. It is found that the rate of ATP hydrolysis was considerably higher in the microsomal fraction than in the mitochondria.

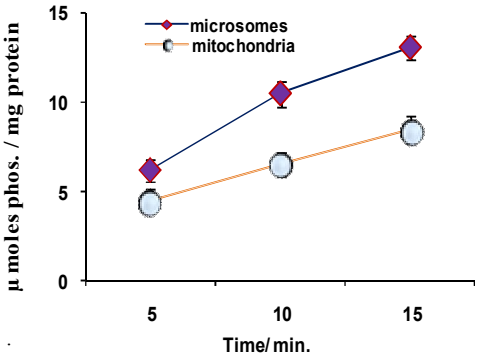

Figure (1): The enzyme activity of Ca^{2+}andMg^{2+} ATPase of typical preparations of vas deferens smooth muscle micromes and mitochondria in a standard incubation medium. Each point is the mean ± S.E. of the mean of ten replicates.

The effect of Ca^{2+} and Mg^{2+}ions on the ATPase activity:

The effect of calcium on microsomal and mitochondria ATPase activity was investigated over the range 0-2mM calcium in standard reaction medium over a period of 15 minutes. The results of such experiments are shown in (fig. 2). The difference in enzyme activity with and without calcium in the incubation medium was not significant. Therefore, under these experimental conditions calcium was without effect on ATPase activity.

The insensitivity of the ATPase to calcium may be due to its activity already being maximal in these preparations because of the high level of intrinsic membrane calcium. Therefore, another two sets of experiments were undertaken. In the first, intrinsic calcium was reduced by including 1mM EGTA in the reaction medium in the absence of both Mg^{2+} and calcium. In the second, EGTA was omitted. The results are shown in (fig 3). As can be seen, the enzyme activity was almost completely abolished in the presence of 1mM EGTA, whereas in those in similar experimental conditions but without EGTA treatment, the ATPase activity was unmasked. These results may suggest the existence of Ca^{2+} , Mg^{2+} activated ATPase in this smooth muscle. However, in the presence of moderate concentration of Mg^{2+}(2-4mM), the absence of calcium did not affect total ATPase activity (fig 4) It would appear therefore, that microsomal and mitochondrial ATPase is largely Mg^{2+}dependent. This finding is consistent with the possibility that the very high Mg^{2+}-ATPase activity would tend to mask any reasonable amount of Ca^{2+}-ATPase [32]. However, since both magnesium and calcium will stimulate the enzyme, they may compete for the same site to stimulate the activity. This suggestion is supported by the finding of [50] that ATPase activity of aortic muscle microsomal and mitochondrial fractions showed a greater affinity for Mg^{2+} than for calcium at equimolar

concentrations. Also it has been showed that enzyme activity was stimulated to differing extents by increasing calcium in the absence of Mg^{2+}. However, when calcium added to the reaction medium in the presence of 2mM Mg^{2+} inhibited microsomal and mitochondria ATPase activity.

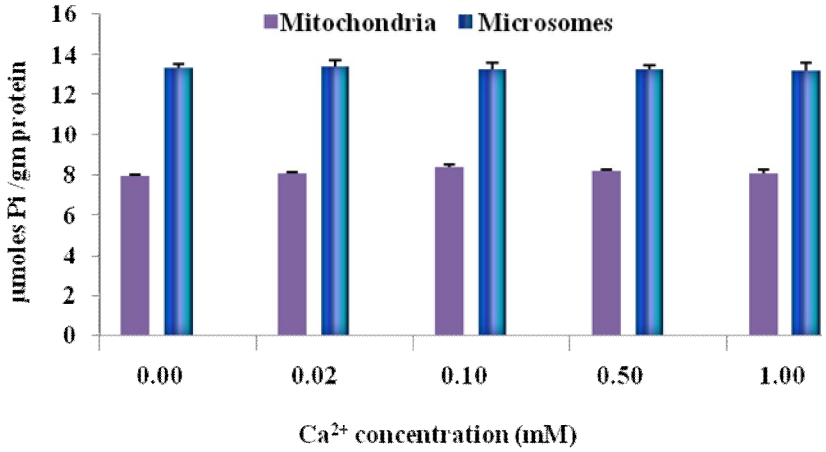

Figure (**2**): The effect of different concentrations of Ca^{2+} on the ATPase activity of microsomal and mitochondria fractions of vas deferens smooth muscle. Each point is the mean ± S.E. of six replicates.

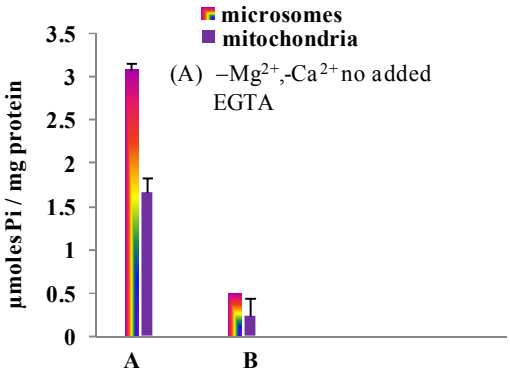

Figure (**3**) : show the mean ± S. E. of ten replicates of the effect of the omission of Ca^{2+} and Mg^{+2} on the ATPase activity in the presence and absence of EGTA .

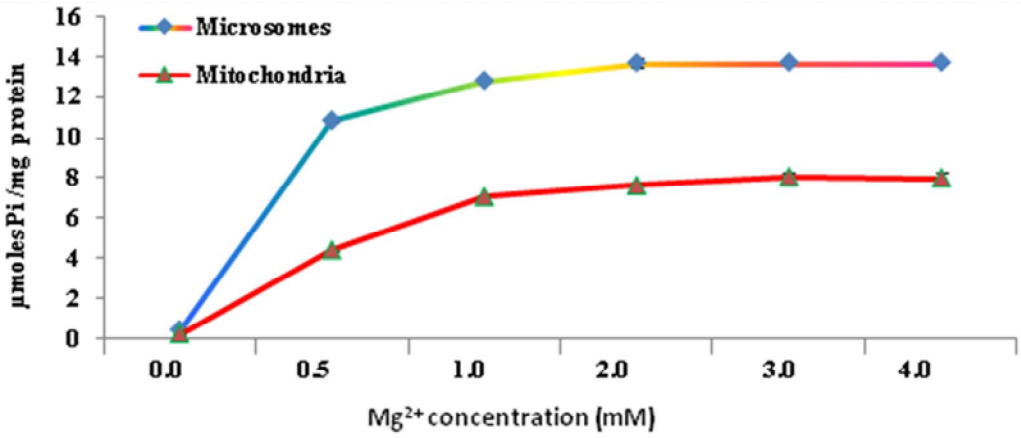

Figure (4): The effect of different concentrations of Mg^{2+} on the ATPase activity of vas deference microsomal fractions (square) and mitochondrial fractions (triangle). Each point is the mean ± S.E .of the mean of ten replicates.

In addition to their enzymatic activity, the microsomal fractions have been shown to possess the capacity to bind calcium in the presence of Mg^{2+} and ATP in some kind of smooth muscle [7, 10, 36]. These properties of the sub cellular fraction seem to be parallel. Therefore, these observations suggest that calcium binding is an active process of physiological significance, since it could contribute to the regulation of myoplasmic free calcium.

It is well known the role of the sarcoplasmic reticulum of the skeletal muscle in the regulation of cytoplasmic free calcium [3, 33, 34, 35]. Based on these findings it has been proposed that the contractile activity of skeletal muscle is controlled by cyclically increasing and lowering the intracellular free calcium level by the sub cellular fractions.

As is thought to be the case in skeletal muscle, the relaxation of smooth muscle is almost certainly also brought about by the removal of calcium from the cytoplasm by an energy-requiring process. The demonstration of an ATP-dependent calcium uptake by various sub cellular fractions isolated from several types of smooth muscle [5, 13, 30, 36] suggests that these structures may be involved in the calcium-regulating system of smooth muscle.

Calcium binding by microsomal vesicles and mitochondrial fractions isolated from vas deferens smooth muscle of the golden hamster is shown in (figure 5).

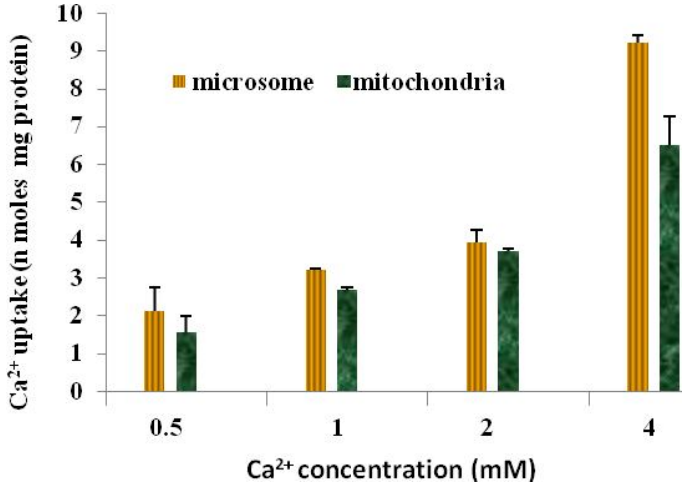

Figure (5): Calcium accumulation by microsomal and mitochondria isolated from vas deferens muscle. Vertical bars represent ± of the mean of ten replicates.

However, the calcium accumulation of the mitochondria is less sensitive to the absence of ATP than that of the microsomes fraction in this kind of smooth muscle. As can been seen from the figure (5) microsomal fraction has a higher binding capacity than the mitochondrial fractions. It has been suggested that calcium uptake by mitochondrial fractions could be due to passive diffusion of the radio calcium into the vesicle coupled with an electrostatic binding of calcium to the vesicle surface [6, 10, 20].

The free calcium concentration in the cytoplasm of smooth muscle is controlled by changes in the rate of calcium supply and the rate of removal. Increased supply is thought to be achieved by increasing the calcium permeability of the plasma membrane and /or by releasing intracellular calcium from its binding sites. Conversely, a decrease in cytoplasmic calcium can be brought about by energy-dependent calcium extrusion and by increasing intracellular binding. This cyclical rise and fall in the free calcium content regulates the contraction-relaxation cycle [1, 2, 9, 38, 39]. Against such a background, the finding that isolated microsomes and mitochondria from the vas deferens possess the capacity to accumulate calcium in the presence of ATP would suggest that these sub cellular structures may well constitute part of the calcium regulation system thereby controlling at least in part the contraction of the vas deferens muscle.

The relevance of mitochondria calcium binding to excitation contraction coupling in smooth muscle is a matter of controversy [40]. Working on the kinetics of calcium uptake by mitochondria from human and rabbit myometrium proposed that the calcium uptake capacity of mitochondria was sufficient to fulfill the requirements of a calcium sink. This view is supported by [10, 11, 41].

In contrast, several investigators have reported that microsomes derived from membrane and sub membrane structures, but not mitochondria, isolated from several types of smooth muscle are capable of calcium sequestration at a sufficient enough rate to account for relaxation [13,39 ,42]. It has been suggested that in relaxed muscle the free calcium concentration is kept at a low level by a calcium pump localized in cell organelles such as microsomal

fractions. Microsomal fractions of smooth muscle contain membrane fragments derived from the plasma membrane and sub membrane vesicles. This is compatible with evidence presented by [4, 8, 26] that in smooth muscle , the calcium pumping located in the periphery of the cell . The findings in this study support such a view since microsomal fractions from vas deferens show Mg^{2+} - Ca^{2+} ATPase activity and also bind calcium. This probably reflects the true functional characteristics of these sub cellular structures in this smooth muscle.

It is relevant here to consider the possible involvement of mitochondria in the contraction relaxation cycle of vas deferens smooth muscles. The bulk of smooth muscle mitochondria are not in direct contact with the plasma membrane or sub membrane structures [4, 44]. Therefore, it is possible that smooth muscle mitochondria may play a role in buffering cytoplasmic calcium under conditions of sustained calcium influx as a reserve store [5, 13, 42, 45].

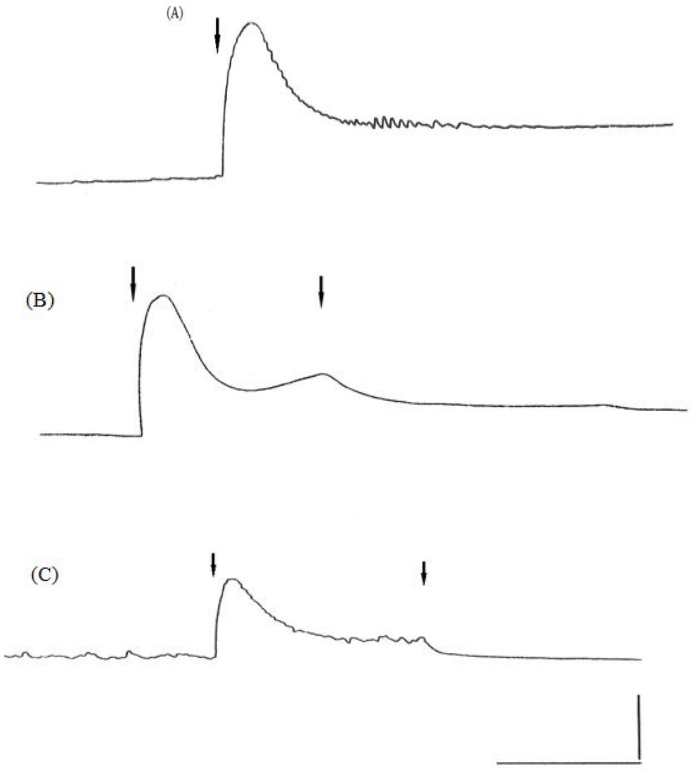

Figure (6): The effect of quinine on the contractile activity of vas deferens muscle induced into contracture by 100 Mm KCl (first arrow). (A) control, (B) 1Mm quinine , (C) 2 Mm quinine (second arrow).

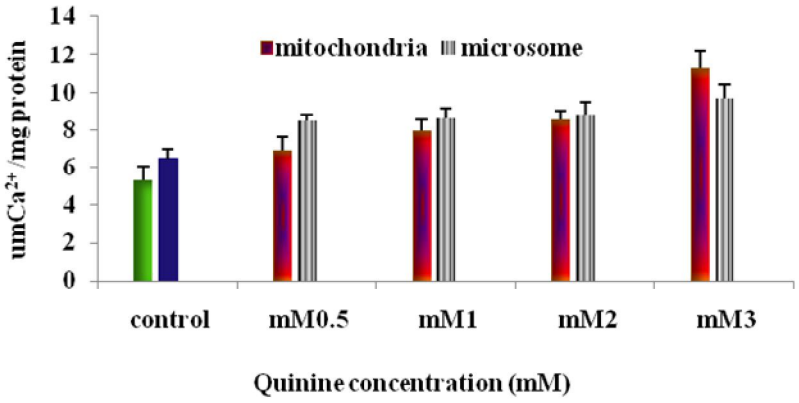

Figure (7): show the mean ± S. E. of ten replicates of the effect of various concentration of quinine on Ca^{2+} uptake by microsomal and mitochondrial fractions of vas deferens smooth muscle.

The effect of quinine on nor adrenaline- induced contracture and KCl – induced contracture of vas deferens preparations are shown in (figures 6) .These records clearly show the inhibitory effects of quinine on tonic components of the induced contractures of this smooth muscle. A bulk of evidences indicate that drugs such as quinine, quinidine and caffeine which alter the contractile state of skeletal muscle do so by their ability to act on compartments known to actively bind calcium [31, 46,47,48].

Quinine at moderate and high concentrations causes contracture of skeletal muscle whereas the same drug brings about a sharp relaxation of smooth muscle [24, 25, 30]. To gain insight into the difference in the excitation-contraction coupling of the two types of muscle and to clarify the possible mechanism by which this drug depresses the contractility of this smooth muscle, the effect of quinine on calcium accumulation by isolated microsomal fractions and mitochondria from vas deferens smooth muscle was investigated.

Calcium uptake by vas deferens microsomes and mitochondria was determined in the presence of various concentrations of quinine .The results of this investigation are shown in figure (7). It is apparent that quinine, while exerting ,an inhibitory action on the mechanical activity of vas deferens smooth muscle enhanced calcium uptake by the sub cellular fractions of this muscle.

In Conclusion, these results may suggest that quinine may inhibit the contractile activity of this kind of smooth muscle at least in part through intracellular calcium handling by inducing calcium sequestration by the subcellular agencies which lead to a decrease of the free Ca^{2+} required for contraction. This hypothesis is supported by findings of other studies [25, 30, 49] which have been shown that quinine inhibit calcium release from intracellular binding sites.

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