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MITOCHONDRIAL CALCIUM RELEASE AS A MECHANISM FOR QUINIDINE CONTRACTURE IN SKELETAL MUSCLE

SATISH BATRA
Department of Pharmacology, University of Lund, Lund, Sweden

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Abstract—When quinidine was added to skeletal muscle homogenates prior to the isolation of the subcellular fractions, the distribution of ^{45}Ca between mitochondria and FSR was drastically altered. ^{45}Ca in the mitochondrial fractions was considerably reduced, whereas it increased by almost an equal amount in the FSR fraction. An identical pattern was obtained when uncouplers of oxidative phosphorylation, instead of quinidine were used. Quinidine caused a marked release of Ca from preloaded mitochondria which remained uninfluenced by the presence of procaine. These results strongly support the argument that quinidine contractures in skeletal muscle are induced as a consequence of Ca release by mitochondria.

It has now been clearly demonstrated that the alkaloid quinidine, like caffeine, is able to induce contractures in skeletal muscle. Quinidine contracture, as caffeine contracture, can be obtained even in a depolarized muscle [1, 2] as well as in a muscle, bathed in Ca-free solution [2]. These observations, together with the evidence that quinidine can readily pass through cell membrane [3], have led to the logical proposal that this drug releases Ca from an intracellular store. It is believed that the source of this intracellular Ca, released by quinidine, is the sarcoplasmic reticulum although no convincing experimental evidence has been available. Recently, using isolated mitochondria and fragmented sarcoplasmic reticulum (FSR) from frog skeletal muscle, evidence was presented showing that the most likely sites from which Ca was released by the action of quinidine were in the mitochondria [4].

In the present study further and more concrete evidence was obtained showing that Ca, during the quinidine contracture, is mobilized from mitochondria rather than from the sarcoplasmic reticulum as hitherto has been assumed. In this endeavour, in the first instance, advantage was taken of the documented evidence that quinidine contracture, in contrast to the caffeine contracture, is not blocked by procaine [5]. Secondly, and more critically, we have examined the effect of quinidine on the distribution of added ^{45}Ca between the fragmented sarcoplasmic reticulum (FSR) and mitochondria in the presence and absence of quinidine. Some of the results have already been communicated in a preliminary form [6].

MATERIALS AND METHODS

The mitochondria and the FSR were isolated from frog (*Rana Temporaria*) skeletal muscle homogenate by differential centrifugation as described previously [7]. Ca uptake by these fractions was studied by Millipore filtration technique. The release of Ca was measured as described previously [4]. The assay medium for Ca uptake consisted of 150 mM KCl, 20 mM imidazole buffer (pH 7.0), 5 mM $MgCl_2$, 1 mM ATP and 0.1 mM $CaCl_2$ with 2.5 μCi ^{45}Ca. Other details have been described previously [4, 7].

The scheme used for studying the distribution of ^{45}Ca between mitochondria and FSR isolated from frog muscle homogenate which was previously incubated with quinidine is described in Fig. 1. After incubation of the homogenate in Ca uptake medium, with or without quinidine, the mitochondrial pellet was obtained simply by centrifugation and the supernatant was passed through the Millipore filters to obtain the FSR. The ^{45}Ca content of FSR could be measured by counting the radioactivity either on the filter or by the difference in the radioactivity of the supernatant before and after filtration. Similarly, mitochondrial ^{45}Ca could be measured in the mitochondrial pellet or by a difference in the activity before and after centrifugation of the homogenate. An excellent agreement was obtained between the two methods of determining ^{45}Ca uptake in the respective fraction.

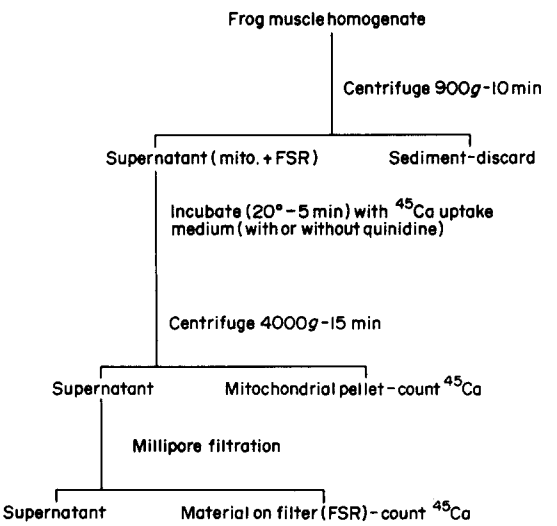

Fig. 1. Scheme of the experimental procedure for investigating the effect of quinidine on the distribution of ^{45}Ca between mitochondria and FSR.

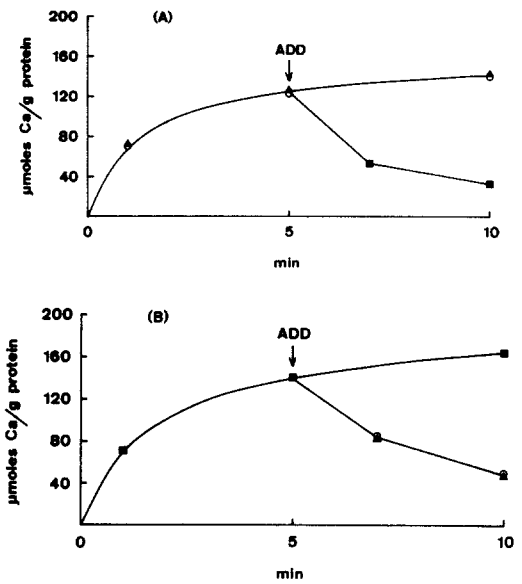

Fig. 2. Effect of procaine on quinidine induced release of Ca by frog skeletal muscle mitochondria. (A), Procaine was present (○) from the start of the Ca uptake or absent (▲) and quinidine was added after 5 min (arrow). (B), Quinidine with (○) or without (▲) procaine was added after 5 min of Ca uptake. The concentration of procaine was 5 mM and that of quinidine 2 mM.

Quinidine sulphate and procaine hydrochloride were purchased from Sigma Chemical Company. FCCP (Carbonyl cyanide-*p*-tri-fluoromethoxyphenyl-hydrazone) was kindly supplied by Dr. P. G. Heytler, of E. I. Du Pont, Wilmington, U.S.A.

RESULTS AND DISCUSSION

The results depicted in Fig. 2 clearly show a substantial Ca release by quinidine in agreement with the previous study [4] in which it was also shown that quinidine, in contrast to caffeine, released little Ca from the fragmented sarcoplasmic reticulum. Procaine, which incidently is able to block caffeine-induced Ca release from sarcoplasmic reticulum [8] (and caffeine contractures, [9]), was unable to influence quinidine-induced Ca release by mitochondria (Fig. 2). The presence of procaine in the medium had no influence either on Ca uptake or, on Ca release induced by quinidine (Fig. 2A).

Quinidine, in concentrations (2 mM) which released considerable amounts of Ca from the isolated mitochondrial fraction (Fig. 2) indeed reduced ^{45}Ca in the mitochondrial fraction which was similar in magnitude to that observed with the use of uncouplers of oxidative phosphorylation (Table 1). These uncouplers also served as the markers for the mitochondrial fraction. Much higher concentrations of the uncouplers than those which usually are effective on the isolated mitochondria [7, 10, 11] were required to manifest their effect in the homogenates. This is probably due to their binding to the extramitochondrial components of the homogenate (but see [12]).

An equally interesting finding in these results was the fact that the presence of quinidine in the homogenate led to an increase in ^{45}Ca content of the FSR isolated from the homogenates (Table 1). Most, but not all, of the Ca that mitochondria failed to accumulate in the presence of quinidine was thus additionally taken up by the FSR. An identical picture was obtained in the presence of the inhibitors of mitochondrial metabolism. Since these results were obtained by the addition of quinidine in a mixture containing mitochondria, FSR and cytosol, much like the intact cell, it is unlikely that in the intact muscle quinidine would act by releasing Ca from the sarcoplasmic reticulum (SR). On the contrary, in that situation a greater amount of Ca would be accumulated by SR in the presence of quinidine as was found with muscle homogenates (Table 1). This would simply be a result of higher Ca concentrations available in the surrounding medium, due to mitochondrial Ca release, and not because of any potentiating effect of quinidine itself on Ca uptake by SR [4]. Interestingly, rather similar results were obtained by Carafoli [12] who studied the *in vivo* effect of uncoupling agents on the incorporation of ^{45}Ca into subcellular fractions of rat liver.

Since the final outcome in the contractile activity of the muscle will be governed by the level of free Ca in the myoplasm, it is important to consider how this parameter would be influenced by quinidine. From the results shown in Table 1, it can be calculated from the values in paranthesis that Ca remaining in the medium, after its accumulation by the mitochondria and FSR, was 3–4 per cent (or 3–4 μM) higher in the presence of quinidine. This increment in myoplasmic Ca concentration would be sufficient to increase the contractile activity of the muscle. The

Table 1. Effect of the presence of quinidine or uncouplers in muscle homogenate on ^{45}Ca distribution between mitochondria and FSR

Additions	^{45}Ca content, 10^{-4} × cpm/mg protein	
	Mitochondria	FSR
Control	346 (46.4%)	225 (29.5%)
Qd (2 mM)	242 (31.8%)	307 (40.3%)
FCCP (25 μM)	234 (30.7%)	310 (40.7%)
DNP (0.2 mM)	270 (35.4%)	288 (37.8%)

Quinidine or inhibitors were added in the homogenate before the separation of mitochondria and FSR (see Fig. 1). Figures in paranthesis represent the fraction as per cent of total Ca (0.1 mM) taken up from the medium.

data in Table 1 represent mean values of eight separate experiments and in every experiment a higher amount of ^{45}Ca remained in the medium in the presence of quinidine.

It is also important to point out that although Ca uptake ability of FSR is only slightly decreased by quinidine, Ca released by mitochondria may not directly or immediately be available to SR for accumulation. In other words it may react with the contractile proteins before and/or during its accumulation by SR.

Quinidine contracture, in contrast to caffeine contracture, has a long and often variable latency period, is sometimes irreversible and is usually biphasic [2, 13, 14]. These special features of quinidine contracture seem to be easily accountable in terms of Ca release by mitochondria and an additional uptake by SR as demonstrated by the present data. There is a delay before contracture is manifest because Ca released by mitochondria is simultaneously being accumulated by SR, and when SR is no longer able to keep up and maintain the low concentration of myoplasmic Ca, a contracture begins to develop but slowly. The biphasic nature of quinidine contracture, a fall in initial contracture output followed by a rise, might reflect an unimpaired Ca uptake capacity of SR initially which exhausts itself finally as it picks up an extra load of Ca, released by mitochondria, in addition to its normal load (also see above).

In conclusion the results presented here strongly support the argument that quinidine contractures in skeletal muscle are induced as a consequence of Ca release by mitochondria. Moreover, the data provide grounds to refute arguments for a release of Ca by sarcoplasmic reticulum during quinidine-induced contracture. Whereas SR may have a domineering role under physiological conditions [7], (also see [11]), mitochondrial Ca transport in skeletal muscle may be an important consideration in studies on the mode of action of pharmacological agents [4, 15].

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