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INHIBITION OF SARCOTUBULAR CALCIUM TRANSPORT BY CAFFEINE: SPECIES AND TEMPERATURE DEPENDENCE

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SUMMARY

The effect of caffeine on calcium transport by isolated sarcotubular vesicles from rabbit and frog skeletal muscle was compared. At 25° the calcium uptake by rabbit vesicles which sedimented at 1500–10000 × *g* was only slightly inhibited by caffeine (1–10 mM). Increasing the temperature to 37° caused a marked increase in the caffeine sensitivity of the rabbit vesicles. The lighter vesicular fraction (10000–30000 × *g*) was relatively insensitive to caffeine at both temperatures. Frog vesicles at 25° were as sensitive to caffeine as the rabbit vesicles at 37°. This difference corresponds to the known differential sensitivity of amphibian and mammalian muscle to caffeine contracture. These results are consistent with the hypothesis that caffeine produces contracture through an inhibition of sarcotubular calcium transport.

INTRODUCTION

Caffeine, at concentrations greater than about 2 mM, causes contracture of amphibian skeletal muscle[1]. Since this contracture occurs without depolarization of the cell membrane[2] it has been inferred that caffeine acts at some intermediate step in the excitation–contraction coupling process[1]. The crucial step in the coupling process is the release of calcium from the sarcoplasmic reticulum[1,3]. That caffeine might promote calcium release is suggested by tracer studies showing an enhanced efflux of ^{45}Ca from prelabelled frog muscles in the presence of caffeine[4]. However, attempts to demonstrate a direct effect of caffeine on sarcotubular calcium transport have not met with uniform success.

HASSELBACH AND MAKINOSE[5] and CARSTEN AND MOMMAERTS[6], using oxalate to trap calcium inside the vesicles, failed to detect any appreciable effect of caffeine on ATP-dependent calcium uptake by isolated sarcoplasmic reticulum. Recently, CARVALHO AND LEO[7], extending earlier observations of HERZ AND WEBER[8], reported that in the absence of oxalate the ATP-dependent calcium uptake consists of two fractions; one fraction is tightly bound to the vesicular membrane and is not affected by caffeine while the other is a smaller, more labile fraction which is displaced by caffeine. Their data suggest that the caffeine-sensitive component represents calcium which is translocated across the sarcotubular membrane and exists within the vesicle as Ca^{2+}. This interpretation raises the question of why caffeine does not affect calcium

uptake in the presence of oxalate, where it is clear that most of the calcium accumulated by the vesicles must be translocated across the membrane rather than adsorbed to it[3]. One possible interpretation of these seemingly contradictory observations is that caffeine affects the efflux of calcium from the vesicle rather than active transport across the vesicular membrane. In the presence of oxalate most of the calcium would be trapped inside the vesicle as calcium oxalate and would not readily diffuse out in response, for instance, to an increase in membrane permeability.

In evaluating the published data one must take into account the experimental materials which have been used. Previous investigators[5–7], with one exception[8], have studied subcellular fractions derived from rabbit muscle. Moreover, these studies were conducted at temperatures (20–25°) which are nonphysiological for mammalian muscle. In this temperature range mammalian muscle is highly resistant to the contracture-producing effects of caffeine[9]. Recently FRANK AND BUSS[10] have shown that at the physiological temperature of 37° mammalian muscle is as sensitive to caffeine as amphibian muscle. Taking these facts into account I have reinvestigated the effect of caffeine on oxalate-dependent calcium transport by isolated sarcoplasmic reticulum of rabbit and frog with special emphasis on a possible relationship between temperature and caffeine inhibition.

MATERIALS AND METHODS

Sarcotubular vesicles were prepared from the back and leg muscles of the rabbit and the leg muscles of the frog as described elsewhere[11]. Centrifugal fractions were collected at 1500–10000 and 10000–30000 × *g*. Calcium uptake was measured by the Millipore filtration technique[12], using filters of 0.45 μ pore diameter. Incubations were carried out at either 25 or 37° in a buffer solution (see legend of Fig. 1) to which ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid was added to adjust the free calcium concentration to a physiological level of about 1 μM (ref. 13). In

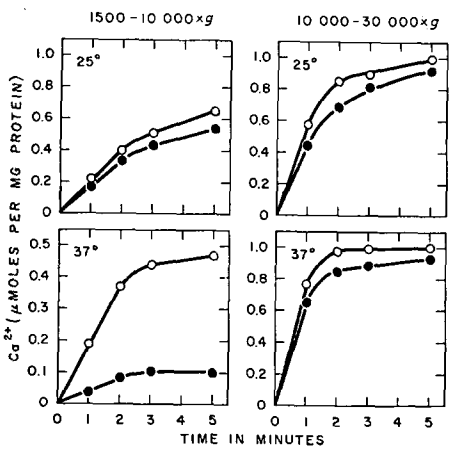
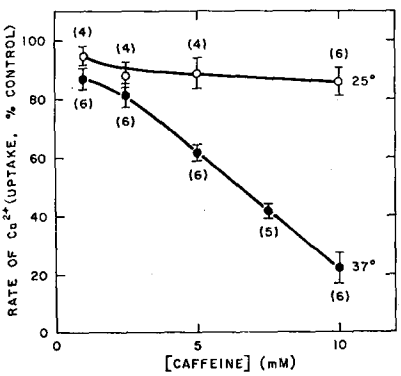


Fig. 1. The effect of caffeine on calcium uptake by subcellular fractions from rabbit muscle. Incubation medium contained 100 mM KCl, 20 mM imidazole (pH 7.0), 4 mM $MgCl_2$, 4 mM potassium oxalate, 0.20 mM $^{45}CaCl_2$, 0.24 mM ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid, 0.02 mg per ml protein, 5 mM ATP, and 0.3 M sucrose. ○, control; ●, 10 mM caffeine.

the experiments with rabbit vesicles 0.3 M sucrose was added to the incubation medium to prevent the rapid deterioration of the vesicles which generally occurred at 37° (unpublished observation). Recent work of E. W. GERTZ AND F. N. BRIGGS (personal communication) has shown that sucrose prevents the release of lysosomal enzymes which takes place at elevated temperatures. The sucrose had no effect on calcium uptake at 25°.

RESULTS AND DISCUSSION

The effect of 10 mM caffeine on the heavy (1500–10 000 × *g*) and light (10 000–30 000 × *g*) vesicular fractions from rabbit muscle at 25 and 37° is shown in Fig. 1. In agreement with previous workers[5,6], there was only a slight effect on either fraction at 25°. Raising the temperature to 37° caused a marked increase in the sensitivity of the heavy fraction to caffeine inhibition. The degree of inhibition of calcium transport was a linear function of the caffeine concentration over the concentration range of 1–10 mM (Fig. 2). At 25° there was an average inhibition of about 10% which was apparently independent of caffeine concentration. As illustrated in Fig. 3, the heavy



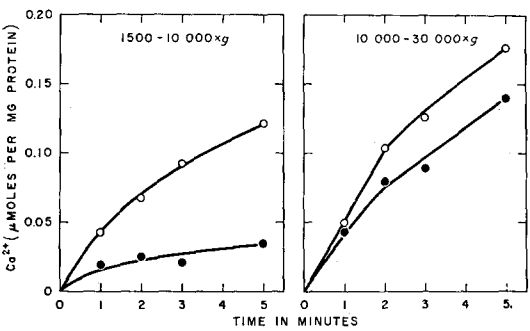


Fig. 2. The rate of calcium uptake by the heavy fraction (1500–10 000 × *g*) of rabbit muscle as a function of caffeine concentration. Measurements were made at 25 and 37°, as indicated. Figures in parentheses indicate number of measurements, and vertical bars indicate standard error. See legend of Fig. 1 for experimental conditions.

Fig. 3. The effect of caffeine on calcium uptake by subcellular fraction from frog muscle at 25°. ○, control; ●, 10 mM caffeine. Experimental conditions were as in Fig. 1 except that the protein concentration was 0.1 mg per ml and the sucrose was omitted.

granule fraction from frog muscle was as strongly inhibited at 25° as the corresponding fraction from rabbit muscle at 37°. Here again, the lighter vesicles did not have the same sensitivity to caffeine. HERZ AND WEBER[8], working with an oxalate-free system, also noted that caffeine caused a greater displacement of calcium from heavy vesicles (650-2000 × *g*) prepared from frog muscle. However, this observation raises the question of whether the caffeine might not be affecting mitochondrial calcium uptake. The fact that the calcium uptake by this preparation was strongly stimulated by oxalate and unaffected by azide and dinitrophenol would tend to support the conclusion of previous workers[14,15] that the calcium uptake by the heavy centrifugal

fraction of skeletal muscle is due predominantly to particles of sarcotubular origin. Further studies combining tissue fractionation and electron microscopy should be useful in localizing more precisely the intracellular site of action of caffeine.

It will be noted that the rates of calcium uptake of the frog vesicles are considerable less than those of the rabbit vesicles. This difference probably reflects differences in purity of the two preparations rather than an intrinsic difference in the calcium-transport system. The frog preparations made in this laboratory tend to be more labile than the rabbit preparations, and it may be that by the time they are assayed (usually the day after preparation), they contain a higher proportion of damaged vesicles.

Thus, with regard to both species difference and temperature variations, sensitivity of the intact muscle to caffeine is associated with a corresponding sensitivity of the calcium-transport system to this compound. These data provide evidence that caffeine produces contracture through an inhibition of sarcotubular calcium transport.

Whether caffeine also enhances the efflux of calcium from the vesicles cannot be decided from these data since the presence of oxalate would presumably obscure such an effect. The results of HERZ AND WEBER[8] and CARVALHO AND LEO[7] suggest that such is the case, although an effect on active uptake in their experiments cannot be excluded. It would seem plausible to suppose that caffeine might affect both uptake and release of calcium. As shown by GUTMANN AND SANDOW[9], under temperature conditions in which caffeine does not cause contracture of mammalian muscle it does cause potentiation of twitch tension. This observation would suggest that caffeine participates in two processes, one of which is temperature dependent in mammalian muscle. On the basis of studies with intact frog muscle BIANCHI AND BOLTON[16] and BIANCHI[17] have suggested that caffeine enhances the release of calcium and also retards the rate of reaccumulation. At higher caffeine concentrations the latter effect would predominate, hence, the prolonged contracture. The results reported here, as well as other data in the literature, are compatible with such a view.

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