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EFFECT OF CAFFEINE ON Ca^{++}-TRANSPORTING FUNCTION OF VESICLES OF THE RAT MYOCARDIAL SARCOPLASMIC RETICULUM

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Caffeine (1,3,7-trimethylxanthine) is a well known potentiator of contraction of skeletal muscle fibers [5]. A study of the mechanism of action of caffeine has shown that this compound can increase the concentration of ionized Ca^{++} in the myoplasm, by liberating Ca^{++} ions from cisterns of the sarcoplasmic reticulum (SR) [6, 7], and reducing the efficiency of active transport of Ca^{++}-dependent ATPase [1]. An important distinguishing feature of the effect of caffeine is its specificity. It has been shown on isolated SR vesicles that caffeine can inhibit the Ca^{++}-transporting function of an SR fraction enriched with terminal cisterns, but not to affect under these circumstances the fraction of elongated tubules [2]. This suggests that caffeine activates the physiological system for liberating Ca^{++} ions from SR, which is considered to be located in the terminal cisterns. The study of the mechanism of action of caffeine on SR can thus shed light on the mechanism of release of Ca^{++} ions in response to excitation. The specific action of caffeine on the Ca^{++}-transporting function of the terminal cisterns enables it to be used to detect fragments of terminal cisterns in the microsomal fraction from homogenate of muscle tissue, myocardial tissue, for example, in which the microsomal fraction contains relatively large numbers of SR fragments, which makes their morphological identification difficult.

The aim of this investigation was to discover the effect of caffeine on active transport of Ca^{++} by different fractions of SR from the rat myocardium and to demonstrate the specificity of its action.

EXPERIMENTAL METHOD

To obtain microsomal fractions, rat hearts were homogenized on a "Polytron" homogenizer (PT-20 generator) in isolation medium containing 0.3 M sucrose, 5 mM sodium azide, and 10 mM histidine (pH 7.0, at 4°C). The ratio of wet weight of tissue to volume of medium was about 1:4. The homogenate was centrifuged for 20 min at 4300g (the bottom of the tube). The supernatant was recentrifuged at the same speed. Heavy (20 min, 14,000 g) and light (30 min, 48,300 g) fractions of microsomes were sedimented successively from the supernatant. Membrane fractions were washed to remove any contamination with actomyosin by suspending them in medium containing 0.6 M KCl, 0.3 M sucrose, and 10 mM histidine (pH 7.0), after which they were sedimented at 48,300g for 30 min. The residues were suspended in 0.3 M sucrose and 10 mM histidine (pH 7.0) and frozen in liquid nitrogen.

Ca^{++} transport was measured by means of a Ca^{++}-selective electrode (Orion 93-20) in medium containing 100 mM KCl, 15 mM potassium oxalate, 5 mM $MgCl_2$, 2 mM ATP, 5 mM NaN_3, SR protein 20-30 μg/ml, and 10 mM HEPES (pH 6.95, at 37°C). Ca^{++} transport was measured by a nephelometric method on an SP-850 spectrofluorometer in a constant-temperature cuvette, equipped

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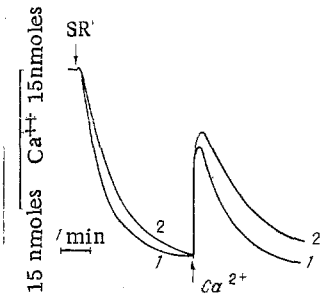

Fig. 1

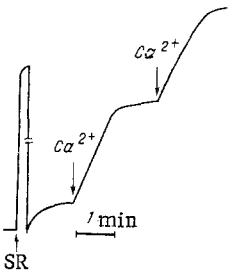

Fig. 2

Fig. 1. Transport of Ca^{++} ions of light microsomal fraction measured by a Ca^{++}-selective electrode. Volume of incubation medium 4 ml. Addition of Ca^{++} in the form of 30 nanomoles $CaCl_2$. Trace 1) control, 2) 10 mM caffeine.

Fig. 2. Transport of Ca^{++} ions of light microsomal fraction measured by nephelometric method. Volume of incubation medium 2 ml. Addition of Ca^{++} in the form of 50 nanomoles $CaCl_2$.

TABLE 1. Effect of Caffeine, Ruthenium Red, and Amethocaine on Ca^{++} Transport by Microsomal Fractions from Rat Myocardium (M ± m; n = 3)

Fraction of microsomes from rat myocardium	Rate of Ca^{++} transport, μmoles/min/mg protein						
	control	caffeine 5-10 mM	effect of caffeine	ruthenium red (3 μM)		amethocaine 0,2 mM	
				control	caffeine (5 mM)	control	caffeine (5 mM)
Light	0,53	0,37	30±2	0,64	0,53	0,59	0,59
Heavy	0,45	0,30	38±6	0,64	0,43	0,49	0,49

with a vibrator. The wavelengths of exciting and emitted light were 540 nm, and the width of the slits 1 nm. The incubation medium was of the same composition as that used to measure with the Ca^{++}-selective electrode.

Protein concentration was determined by the biuret reaction. Reagents: ATP was from Reanal (Hungary), caffeine, HEPES, ruthenium red, and NaN_3 were from Serva (West Germany), and sucrose from Sigma (USA).

EXPERIMENTAL RESULTS

To reveal the effect of caffeine a direct method of measuring transport of Ca^{++} ions with the aid of the Orion 93-20 Ca^{++}-selective electrode was used; as was shown previously, because of the high sensitivity, short reponse time, and absence of a damaging action on the membrane this electrode is suitable for this purpose [3]. The kinetics of Ca^{++} ion transport by fragments of rat myocardial SR (light fraction), measured with the Ca^{++}-selective electrode, is illustrated in Fig. 1. It will be clear from Fig. 1 that soon after addition of the preparation of SR membranes to the incubation medium, Ca^{++} transport ceased. This was due to exhaustion of Ca^{++} ions in the incubation medium, for after addition of Ca^{++} ions and a response of the electrode, uptake of Ca^{++} ions could again be observed. The process of active transport recorded by the Ca^{++}-electrode is associated with functioning of the SR fragments, it is not inhibited by sodium azide, and is not observed in the absence of oxalate. The rate of Ca^{++} transport, measured by the Ca^{++}-selective electrode, was about 0.5 μmole/min/mg protein. Addition of 10 mM caffeine to the incubation medium led to a decrease in the velocity of Ca^{++} transport. The degree of inhibition of Ca^{++} transport by caffeine in the light fraction of SR averaged about 30%.

To study the specificity of action of caffeine, its action on the heavy and light fractions of SR membranes was compared and the action of blockers of the caffeine effect (ruthenium red and amethocaine) also was investigated. Since these two compounds can influence the potential of the Ca^{++}-electrode, to measure transport of the Ca^{++}-selective electrode a nephelometric method, based on the fact that on precipitation of crystals of calcium oxalate in-

side vesicles of SR, the scattering of light by the suspension of SR membranes increases [4], was used. The kinetics of the change in intensity of the scattering of light in the incubation medium after successive additions of SR membranes and $CaCl_2$ is illustrated in Fig. 2. As Fig. 2 shows, after the addition of Ca^{++} ions an increase in the intensity of scattering of light, developing over a period of time, was observed. After a certain time this increased ceased and could be resumed by the addition of further Ca^{++}. This shows that the optical response is limited by the number of Ca^{++} ions. The magnitudes of responses to two successive additions of Ca^{++} were virtually equal, evidence of a linear relationship between the quantity of Ca^{++} taken out and the magnitude of the optical response. As a result, it was possible to calculate the velocity of Ca^{++} transport, by using for calibration purposes the magnitude of the optical response to an added quantity of Ca^{++}. Control experiments showed that an increase in the intensity of scattering of light was associated with accumulation of calcium oxalate inside the vesicles of SR, for it was not observed in the absence of ATP and was prevented when alamethicin (3 μg/ml) was added to the incubation medium. Data on the effect of caffeine, ruthenium red, and amethocaine on transport of Ca^{++} ions, measured by the nephelometric method, are given in Table 1. They show that the rate of Ca^{++} transport, determined by this method, was about 0.5 μmole/min/mg protein for the light fraction, and the magnitude of the caffeine effect was about 30% for 5 and 10 mM caffeine, in agreement with data obtained by the use of the Ca^{++}-selective electrode. Table 1 also shows that the effect of caffeine was somewhat stronger when it acted on the heavy fraction. However, no marked differences in the effects of caffeine, such as were characteristic of SR fractions from skeletal muscles [2], were observed on SR fractions from the rat myocardium. Nevertheless, it is possible to speak of the specificity of action of caffeine on SR of the rat myocardium, because its effect was virtually completely abolished by ruthenium red and amethocaine in the same concentrations as those which block the inhibitory effect of caffeine on Ca^{++} transport in the fraction of terminal cisterns from rabbit skeletal muscles [3]. Addition of ruthenium red not only prevents the action of caffeine, but also stimulates Ca^{++} accumulation. Ruthenium red evidently inhibits the caffeine-induced outflow of Ca^{++} from SR vesicles and blocks other pathways of calcium leakage.

The results are evidence that the total fraction of SR membranes from the rat myocardium contains fragments of terminal cisterns, numbering, to judge from the effect of caffeine, about 30%. The fact that caffeine exerts a specific action on Ca^{++} transport by myocardial SR points to a common mechanism of the release of Ca^{++} ions from SR of the myocardium and skeletal muscles in response to excitation.

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