9. J. Weil and C. J. Epstein, Am. J. Hum. Genet., 30, 98A (1978).
10. J. Weil and C. J. Epstein, Am. J. Hum. Genet., 31, 478 (1979).
11. G. Winchester, Nature, 303, 660 (1983).

ACTIVATION OF THE CAFFEINE SITE OF THE SARCOPLASMIC RETICULUM AT LOW MAGNESIUM ION CONCENTRATION

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Studies of skeletal muscle fibers deprived of their membranes have shown that contraction of these fibers can be induced by a reduced Mg^{++} concentration in the medium. This is connected with the release of Ca^{++} ions from the sarcoplasmic reticulum (SR), which takes place under these circumstances [7]. Contraction of such fibers and Ca^{++} release can also be induced by caffeine [4]. Caffeine releases Ca^{++} ions from the isolated fraction of terminal cisternae of SR [8]. It has been shown in experiments on terminal cisternae that caffeine has an uncoupling action on active Ca^{++} transport. Caffeine does not act on active Ca^{++} transport in the longitudinal tubules of SR [3]. Hence the importance of a study of the effect of Mg^{++} ions on active transport of Ca^{++} by different fractions of SR.

EXPERIMENTAL METHOD

The caffeine-sensitive fraction of SR membranes was isolated from white muscles of rabbit hind limbs. The issue was homogenized as described previously [2]. To increase the yield of membranes 10 mM caffeine was added to the homogenization medium. The total membrane sedimented by centrifugation from 10,000g to 36,000g was extracted in the cold in medium containing 0.6 M KCl, 0.1 mM EDTA, 0.2 mM $CaCl_2$, human serum albumin (0.6 mg/ml), and 5 mM histidine (pH 7.2). The membrane suspension was centrifuged at 11,000g (20 min) to sediment the caffeine-sensitive fraction, and then again to 40,000g (60 min) to sediment caffeine-resistant membranes (light fraction). The residues thus obtained were suspended in medium containing 25% of glycerin (vols. 5), 0.1 mM EDTA, 0.2 mM $CaCl_2$, and 10 mM histidine (pH 7.2 at 4°C). For further purification the fractions were layered in a centrifuge tube above 4 ml of the same medium and centrifuged at 36,000g (60 min). The caffeine-sensitive fraction was obtained as the residue, and the light fraction as a thick suspension in the lower part of the tube. ATPase activity and the mean efficiency of Ca^{++} transport by SR membranes were determined by pH-metry [1]. The incubation medium contained 100 mM NaCl, 4 mM $MgCl_2$, 20 mM sodium oxalate, 25 μM $CaCl_2$, 2 mM ATP, 20-30 μg/ml of SR protein, and 2.5 mM imidazole (pH 7.05 at 37°C). The protein concentration was measured by the biuret reaction.

EXPERIMENTAL RESULTS

Previously, using an apparatus whereby ATP hydrolysis and Ca^{++} transport by SR fragments could be recorded simultaneously by means of Ca-selective and pH-electrodes, introduced into the same cell, the writers showed that caffeine inhibits uptake of Ca^{++} ions without affecting ATP hydrolysis, i.e., it has an uncoupling action on active Ca^{++} transport [3]. In that way the effect of caffeine can be estimated quantitatively by its effect on the mean efficiency of active Ca^{++} transport (Ca/ATP), which can easily be determined by pH-metry. A pH-metric record of ATP hydrolysis during active Ca^{++} transport by the fraction of terminal cisternae and longitudinal tubules of SR is shown in Fig. 1. Clearly, if the oxalate concentration in the incubation in medium was 20 mM, caffeine reduced the efficiency of active Ca^{++} transport by the fraction of terminal cisternae by about two-thirds but had relatively little

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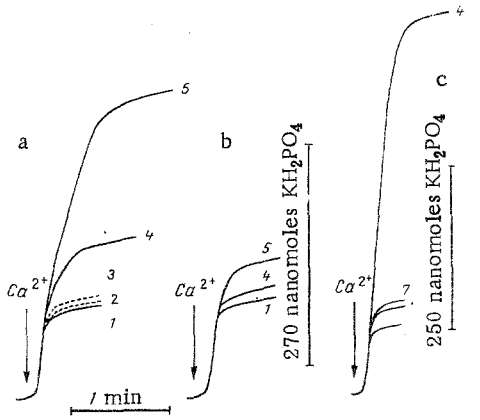

Fig. 1. Effect of caffeine on efficiency of Ca^{++} transport by fraction of terminal cisternae (a, c) and by fraction of longitudinal tubules (b). 1) Control, 2) amethocaine 0.2 mM + 5 mM caffeine, 3) ruthenium red 3 μM + 5 mM caffeine, 4) caffeine 5 mM, 5) caffeine 10 mM, 6) 10% DMSO, 7) 10% DMSO + 5 ml caffeine. Concentration of sodium oxalate in incubation medium: a, b) 20 mM, c) 5 mM.

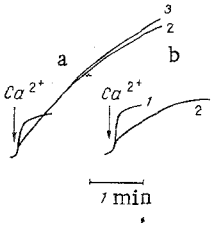

Fig. 2. Kinetics of ATP hydrolysis during active Ca^{++} transport by fraction of terminal cisternae (a) and longitudinal tubules (b) with Mg^{++} concentration of 4 mM (1) and 0.1 mM (2, 3) in presence of 5 mM caffeine.

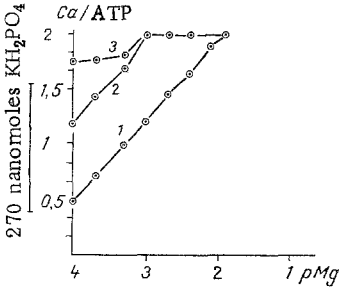

Fig. 3. Dependence of efficiency of Ca^{++} transport by fraction of terminal cisternae on Mg^{++} ion concentration. 1) Control, 2) amethocaine 0.2 mM, 3) ruthenium red 3 μM.

effect on the efficiency of Ca^{++} transport by the fraction of longitudinal tubules. The mean value of Ca/ATP in the incubation medium used was 1.73 for the fraction of terminal cisternae. Under the influence of 10 mM caffeine this value was reduced to 0.45. Corresponding values for the fraction of longitudinal tubules were 1.8 and 1.37.

A pH-metric trace of ATP hydrolysis by fractions of terminal cisternae and longitudinal tubules with different Mg^{++} concentrations shown in Fig. 2. It will be clear from Fig. 2 that if the oxalate concentration in the incubation medium for the fraction of longitudinal tubules was 20 mM, lowering the Mg^{++} concentration thus had the same effect on Ca^{++} transport by SR fragments as caffeine, i.e., there was a decrease in Ca/ATP, and this decrease was most marked on the fraction of terminal cisternae. This conclusion is confirmed by data obtained during a study of dependence of the effect of caffeine on Mg^{++} concentration. As Fig. 2 shows, if the Mg^{++} concentration was 0.1 mM caffeine had no uncoupling action on Ca^{++}

transport. The absence of effect of caffeine in the presence of low Mg^{++} concentrations is not due to the fact that Mg^{++} ions are essential for the action of caffeine, but to the fact that at a reduced Mg^{++} concentration activation of the caffeine site takes place, and the addition of caffeine to the incubation mixture now produces no further action. This is shown by experiments in which blockers of the action of caffeine were used.

Investigations have shown that the outflow of Ca^{++} from isolated SR vesicles, induced by caffeine, can be prevented by the local anesthetic amethocaine and by the specific blocker of Ca^{++} ion transport into mitochondria, ruthenium red [5, 6]. A comparative study was therefore made of the action of ruthenium red and amethocaine on the uncoupling effect of caffeine and lowering of the Mg^{++} ion concentration. As Fig. 1 shows, ruthenium red in a concentration of 3 mM and amethocaine in a concentration of 0.2 mM completely abolished the uncoupling effect of caffeine. The action of amethocaine and ruthenium red on the value of Ca/ATP in the absence of caffeine, and with different Mg^{++} concentrations, is shown in Fig. 3. Lowering the Mg^{++} concentration in the incubation medium from 12 to 0.1 mM led to a decrease in Ca/ATP from 2 to 0.5. The fall in the value of Ca/ATP with a decrease in the Mg^{++} concentration was largely prevented by the action of amethocaine and ruthenium red. In the presence of Mg^{++} in a concentration of 1 mM amethocaine and ruthenium red increased the value of Ca/ATP to 2, i.e., to its highest possible value having regard to the stoichiometry of action of Ca-dependent ATPase, which transfers two Ca^{++} ions for each act of ATP hydrolysis.

Yet another effective blocker of the uncoupling action of caffeine was discovered, namely dimethyl sulfoxide (DMSO). The action of caffeine was completely prevented by the addition of 10% DMSO to the incubation medium (Fig. 1). The same concentration of DMSO completely prevented the uncoupling effect of lowering the Mg^{++} concentration. The value of Ca/ATP in the presence of 10% DMSO was 2 in the presence of low (0.1 mM) and high (4 mM) Mg^{++} concentrations. The mechanism of activation of the caffeine site on a fall in the Mg^{++} concentration is not yet clear. If it is assumed that activation of the caffeine site is the result of changes in the structure of this site on a fall in the Mg^{++} concentration, it is difficult to understand how this effect can be blocked by such widely different compounds as ruthenium red, amethocaine, and DMSO. However, considering that these compounds also block the effect of caffeine, it is logical to suggest that the fraction of terminal cisternae contains a caffeine-like factor which interacts with the caffeine site when the Mg^{++} ion concentration falls. The isolation and identification of such a factor would be of great interest becuase it would shed light on the mechanism of release of Ca^{++} ions during excitation of muscles.

LITERATURE CITED

1. V. B. Ritov, Biokhimiya, 36, 393 (L971).
2. V. B. Ritov and M. K. Murzakhmetova, Biokhimiya, 43, 415 (1978).
3. M. Endo, Physiol. Rev., 57, 71 (1977).
4. N. Miyamoto and E. Racker, J. Membrane Biol., 66, 193 (1982).
5. S. Ohnishi, J. Biochem. (Tokyo), 86, 1147 (1979).
6. E. Stephenson, Am. J. Physiol., 240, 1 (1981).
7. A. Weber, J. Gen. Physiol., 52, 760 (1968).