INTRACELLULAR LOCALIZATION OF THE CAFFEINE-SENSITIVE FORM OF Ca^{++}-DEPENDENT ATPase OF THE SARCOPLASMIC RETICULUM

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The key stage in electromechanical coupling in skeletal muscle and myocardial fibers is transmission of the signal in the region of contact between the membrane of the T-system and the terminal cistern of the sarcoplasmic reticulum (SPR). The signal induced by depolarization of the T-system triggers the fast passive outflow of Ca^{++} ions from cisterns of SPR [8]. The nature of the signal triggering the outflow of Ca^{++} ions is not yet known. The study of the mechanism of action of various compounds with a possible modifying action on electromechanical coupling, on the Ca-transporting function of SPR membranes is very important. One potentiator of muscular contraction is caffeine [8]. The potentiating action on caffeine is linked with its ability to induce Ca^{++} outflow from cisterns of SPR, as has been demonstrated on demembranized fibers and isolated vesicles of SPR [6, 9]. The writers showed previously that the receptor for caffeine in SPR membranes is a special form of Ca-dependent ATPase, and it was suggested that the caffeine-sensitive form of ATPase is located in terminal cisterns [1].

This paper describes electron-microscopic investigations which showed that the ATPase sensitive to caffeine is located in the fraction of SPR membranes that is rich in fragments of terminal cisterns. The SPR membrane fraction which does not contain terminal cisterns is insensitive to caffeine.

EXPERIMENTAL METHOD

The caffeine-sensitive fraction of SPR membranes was isolated by a method designed for obtaining highly purified SPR membranes from rabbit white skeletal muscles [3]. To increase the yield of caffeine-sensitive fraction, 10 mM of caffeine was added to the homogenization medium. After centrifugation of the homogenate (10,000g, 20 min) the supernatant was centrifuged at 36,000g for 60 min. The residue of total membrane fraction was extracted for 60 min 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 7.4 (4°C). The membrane suspension was centrifuged at 11,000g for 20 min to sediment the caffeine-sensitive fraction, and then at 40,000g (60 min) to sediment caffeine-insensitive membranes. The residues thus obtained were suspended in medium containing 25% glycerol (by volume), 0.1 mM EDTA, 0.2 mM $CaCl_2$, and 10 mM histidine, pH 7.2 (4°C). For further purification the fractions were layered in a centrifuge tube above 4 ml of the same medium and centrifuged in a fixed angle rotor at 36,000g for 60 min. The caffeine-sensitive fraction was obtained as the residue, and the caffeine-insensitive (light) fraction as a layer of thick suspension in the bottom part of the tube. The fractions thus obtained were suspended and kept in the glycerol medium used for purification. To isolate membranes for microscopic analysis, the extraction medium contained 0.3 M sucrose instead of 0.6 M KCl. This substitution caused no significant change in the effect of caffeine. Transport of Ca^{++} ions was measured with a Ca-selective (Orion 93-20) electrode. ATP hydrolysis was measured simultaneously in the same cell by a pH-metric method [2] (G2222C pH-electrode, from Radiometer). Both parameters were recorded on a two-channel automatic writer. The incubation mixture, 4 ml in volume, contained 100 mM NaCl, 4 mM $MgCl_2$, 2 mM ATP, 20 mM sodium oxalate, 20-40 μM $CaCl_2$, SPR protein 80-120 μg, and 2.5 mM imidazole, pH 7.0 (37°C).

The protein concentration was measured by the biuret reaction. For electron-microscopic analysis the method of ultrathin sections [4] was used.

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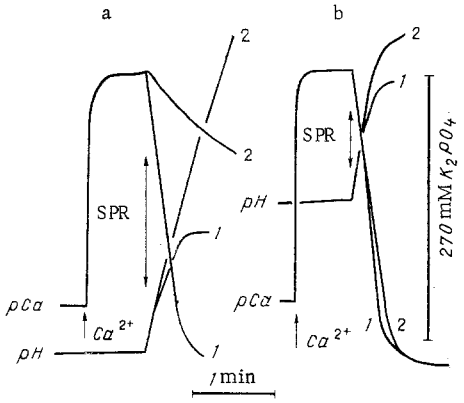

Fig. 1. Effect of caffeine on Ca^{++} transport by caffeine-sensitive (a) and light (b) fractions of SPR. 1) Control, 2) 5 mM caffeine (a), 5 and 10 mM caffeine (b). Additions: SPR protein 80 μg, $CaCl_2$ 30 nM.

EXPERIMENTAL RESULTS

A continuous record of Ca^{++} transport and ATP hydrolysis by different fractions of SPR membranes, obtained by a Ca-electrode and pH-electrode, is illustrated in Fig. 1. As Fig. 1 shows, addition of SPR membranes to the incubation mixture containing Mg-ATP oxalate, and Ca^{++}, led to uptake of Ca^{++} ions accompanied by ATP hydrolysis. After the end of Ca^{++} transport the rate of ATP hydrolysis fell sharply. Continuous recording of Ca^{++} transport and ATP hydrolysis revealed the basic characteristics of the transport process at each moment of time: the rate of Ca^{++} transport, the rate of ATP hydrolysis, and the efficiency of Ca^{++} transport (Ca/ATP). A detailed description of the method will be given in a special publication.

It will be clear from Fig. 1 that addition of 5 mM caffeine to the incubation mixture containing the caffeine-sensitive fraction of SPR membranes led to a decrease in the rate of Ca^{++} transport with no appreciable decrease in the rate of ATP hydrolysis. In the presence of caffeine, SPR membranes likewise were unable to take up all the added Ca^{++}. This is clear from the trace of the Ca-electrode and from the trace of the pH-electrode, which recorded ATP hydrolysis even though Ca^{++} transport had ended.

The results thus demonstrate directly that caffeine uncouples Ca^{++} transport from ATP hydrolysis in the caffeine-sensitive fraction. Caffeine had virtually no action, even in a concentration of 10 mM (Fig. 1), on Ca^{++} transport by vesicles of the light fraction.

Ultrathin sections from preparations of caffeine-sensitive and light fractions, fixed with glutaraldehyde and OsO_4, are illustrated in Fig. 2. As Fig. 2 shows, both fractions consisted of closed membrane vesicles 0.1-0.2 μm in diameter. The morphology of the vesicles differed in the fractions studied. The light fraction consisted mainly of empty vesicles, not containing any formations on the outer surface of the membrane. In the caffeine-sensitive fraction, besides empty vesicles a few containing characteristic "caps" of electron-dense material, could be seen. Asymmetrically distributed electron-dense material was located partly inside the vesicle and partly on its surface, forming a fringe in some cases. In an investigation by Campbell et al. [4], vesicles with this morphology were found in the heavy fraction of SPR. They suggested that vesicles containing electron-dense material are fragments of terminal cisterns, and that the fringe is the residue of material of "pedicles" (projections), forming a junction between the terminal cistern and a tube of the T-system. This hypothesis is based on the results of an investigation [5] which showed that vesicles of this type are formed after destruction of isolated triads. Our observations confirm this hypothesis. Our results show that some vesicles are an intact "diad," i.e., a fragment of a terminal cistern and a flattened fragment of a tubule of the T-system attached to it. It can be clearly seen in this preserved diad that the asymmetrically distributed electron-dense material is in fact localized along the junction of the SPR membrane with the tubule of the T-system.

It can thus be concluded that caffeine does not affect Ca^{++} transport in the light fraction of vesicles, evidently formed from elongated SPR tubules, but it significantly reduces the efficiency of Ca^{++} transport by the membrane fraction rich in fragments of terminal cisterns. The caffeine-sensitive fraction of SPR membranes

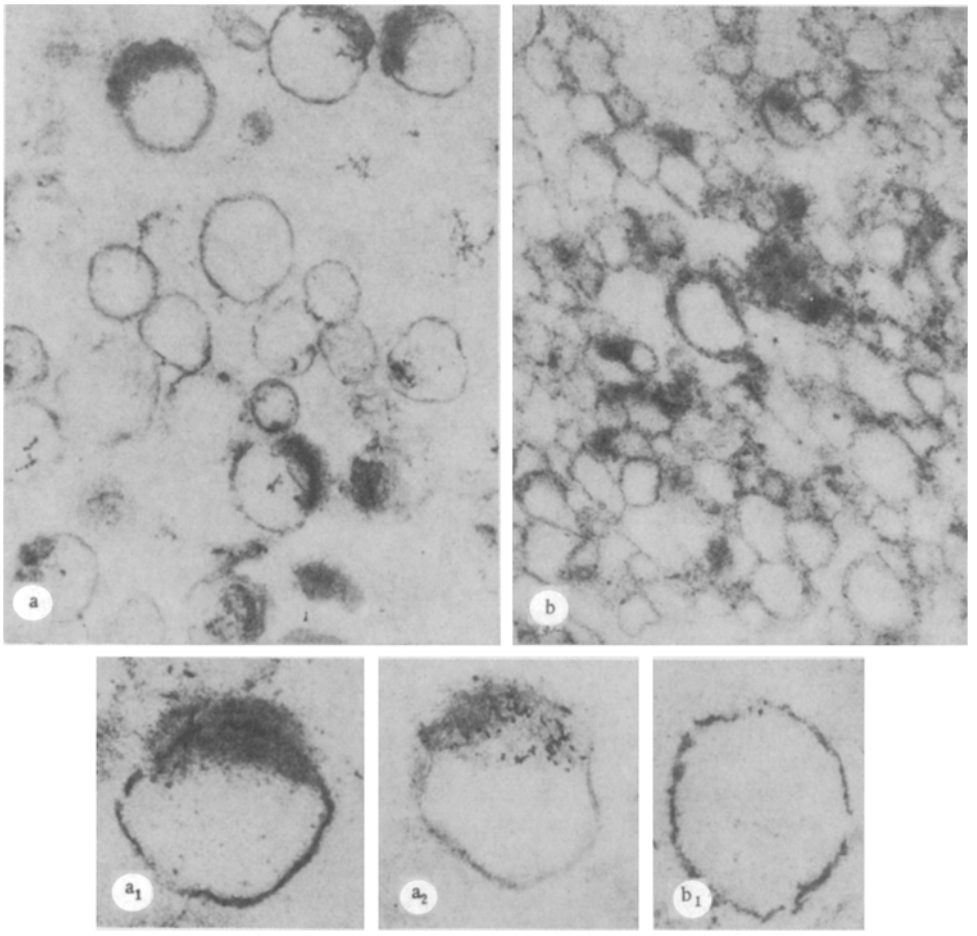

Fig. 2. Caffeine-sensitive (a) and light (b) fractions of SPR. a_1) "Diad," a_2) vesicle with "fringe," b_1) light visicle.

is in fact a fraction of terminal cisterns similar to what is called the "heavy" fraction, isolated by zonal centrifugation [4, 7]. Consequently, the form of Ca-dependent ATPase that is sensitive to caffeine is located in the terminal cisterns, i.e., in those parts of SPR from which Ca^{++} is released during electrical excitation of the muscle. The localization of the caffeine-sensitive form of Ca-dependent ATPase in terminal cisterns provides important experimental grounds for the hypothesis put forward previously, namely that Ca^{++} release from SPR membranes is initiated as a result of interaction of a protein factor, acting like caffeine, with the caffeine-sensitive form of ATPase [1]. This factor may be found within the terminal cisterns of SPR, and is visible on electron-microscopic study as electron-dense material.

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