IS ADENOSINE-3',5'-PHOSPHATE IDENTICAL WITH THE RELAXING SUBSTANCE?

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It has been reported that microsomes release or produce a relaxing substance (Briggs and Fuchs, 1960; Parker and Gergely, 1960; Nagai et al., 1962). The identification of the relaxing substance has been attempted by several workers (Lorand et al., 1957; Marsh, 1960; Ells and Faulkner, 1961), but their findings have not been accepted widely (Briggs et al., 1959; Bendall, 1960; Parker, 1961; Lorand and Molner, 1962).

More recently, Uchida and Mommaerts (1963) have demonstrated that adenosine-3',5'-phosphate (cyclic 3,5-AMP) inhibits the contraction of actomyosin suspension in the presence of oxalate, and they have considered that cyclic 3,5-AMP is identical with the "relaxing substance".

The present paper describes experiments dealing with the question whether cyclic 3,5-AMP is also effective on glycerol-extracted muscle fibers and myofibrils. It is known that these muscle models show a remarkable relaxation induced by the relaxing substance (Briggs and Fuchs, 1960; Parker and Gergely, 1960; Nagai et al., 1962; Takauji et al., 1962).

The preparations and methods were the same as reported elsewhere (Nagai and Uchida, 1960; Nagai et al., 1962). The control reaction mixture contained 2 mM ATP, 2 mM $MgCl_2$, 2 mM oxalate, 66.7 mM Tris

acetate buffer (pH 7.0) and 82.4 mM K^+. The solution of cyclic 3,5-AMP* adjusted to pH 7.0 with KOH, was added to a final concentration varying from 5×10^{-8} to 5×10^{-3} M.

As shown in Fig. 1, cyclic 3,5-AMP in the concentration of 5×10^{-8} to 5×10^{-3} M scarcely affected the tension of fiber induced by ATP in the presence of 2 mM oxalate.

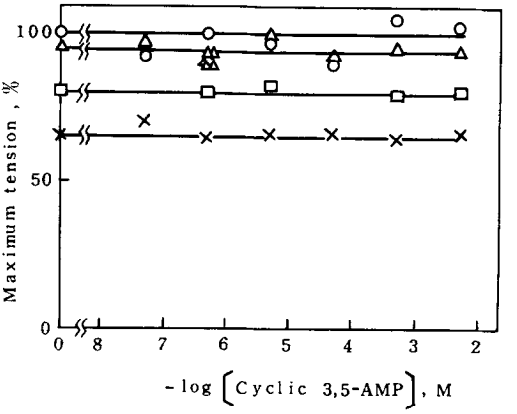

Fig. 1. Effect of cyclic 3,5-AMP on the tension of glycerol-extracted muscle fiber induced by ATP.

Ordinate: the maximum tension of fiber developed without cyclic 3,5-AMP in the presence of 2 mM oxalate was taken as 100. Each point denoted in the case of presence of cyclic 3,5-AMP indicates the maximum tension obtained 20 min after replacement of ATP solution with another ATP solution containing cyclic 3,5-AMP in each concentration. ATP=Mg^{++}= 2 mM; K^+, 82.4 mM; Tris acetate buffer, 66.7 mM (pH=7.0). Oxalate: O, 2 mM; Δ, 5 mM; □, 7 mM; ×, 10 mM. Extraction period of fibers, 2.5 months. Average of maximum tension at 2 mM oxalate, 2750 g / cm^2. Temp., 20°C.

Uchida and Mommaerts (1963) pointed out that the relaxing effect of cyclic 3,5-AMP on actomyosin is seen most readily in the presence of oxalate presumably because of the lowering of the concentration

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of free calcium ions. Accordingly, our experiments were carried out in the presence of oxalate at concentrations exceeding usual level (2 mM). Even when oxalate was increased to 5, 7 or 10 mM, cyclic 3,5-AMP was also found to be without effect on the tension of fiber (Fig. 1). Therefore, this result may indicate that the concentration of free calcium ions in the medium was not responsible for the inability of cyclic 3,5-AMP to relax the glycerol-extracted muscle fiber.

Similar results were obtained on the myofibrillar ATPase activity (Fig. 2).

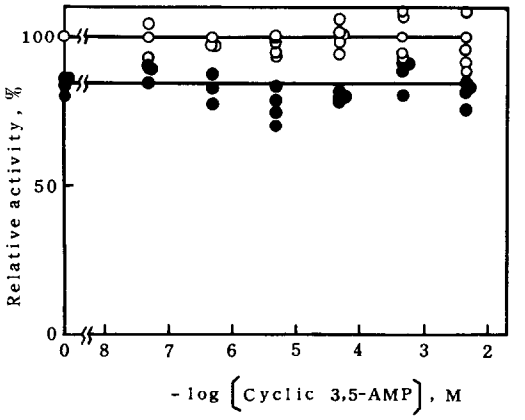

Fig. 2. Effect of cyclic 3,5-AMP on the myofibrillar ATPase activity. Ordinate: the rate of ATP hydrolysis by myofibrils alone in the presence of 2 mM oxalate was taken as 100% activity. Total volume, 2.0 ml. Myofibrils, 0.8 mg of protein / ml. Oxalate: ○ , 2 mM; ● , 5 mM. Conditions otherwise as in Fig. 1.

Moreover, it is to be noted that the glycerol-extracted muscle fiber and myofibrils used in our experiments were relaxed by microsomes or EDTA. It is apparent from these results that cyclic 3,5-AMP has no relaxing effect on these muscle models. Therefore, it may be considered that cyclic 3,5-AMP is not identical with the relaxing substance released or produced by microsomes.

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