MODIFICATION OF THE CONTRACTILE RESPONSES OF ACTOMYOSIN BY CYCLIC ADENOSINE 3', 5' PHOSPHATE *

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Research on the so-called relaxing factor system of muscle (summarized by Gergely, 1959) has indicated two possible mechanisms of the in vitro relaxation effects; a sequestering of Ca ions (Hasselbach and Makinose, 1961; Ebashi, 1961), and a production of a relaxation substance (Briggs and Fuchs, 1960; Parker and Gergely, 1960). The relative significance of these several effects, in vitro and especially in vivo, cannot yet be assessed, and the chemical nature of the relaxing substance has not as yet been elucidated.

This paper deals with an investigation of the possible role of cyclic adenosine-3', 5'-phosphate (referred to as cyclic AMP) in these phenomena. Our inquiry was prompted by the consideration that the relaxing factor is produced by a particulate fraction in the presence of ATP and that, if no other substrate were required, a formation of cyclic AMP would be the obvious possibility (cf. Sutherland et al., 1962). We have found that this substance is formed in relaxation factor preparations, that it can profoundly alter the contractile responses of actomyosin, and that its effects are strongly modified by Ca-ions.

Several types of results were obtained, not all of which will be described in detail at this occasion (cf. Mommaerts et al.,

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1963). These showed that under some circumstances the contraction of actomyosin systems with ATP were strongly promoted by cyclic AMP, whereas in other experiments an inhibitory effect occurred. The results varied with the electrolyte composition of the medium, and the latter type of response was changed into the former by the addition of calcium ions. This variety of effects ranges beyond what has hitherto been described for the relaxation substance.

Simpler results were obtained when oxalate was present in the assay medium, which presumably acted by stabilizing the concentration of free calcium ions at a low level. As is shown in fig. 1, the effect of cyclic AMP then became indistinguishable from that of the relaxation substance in the usual assay systems; its actions were still marked at concentrations as low as 10^{-9} M. Calcium ions immediately counteracted the relaxation and could reverse it to a contraction-promoting effect.

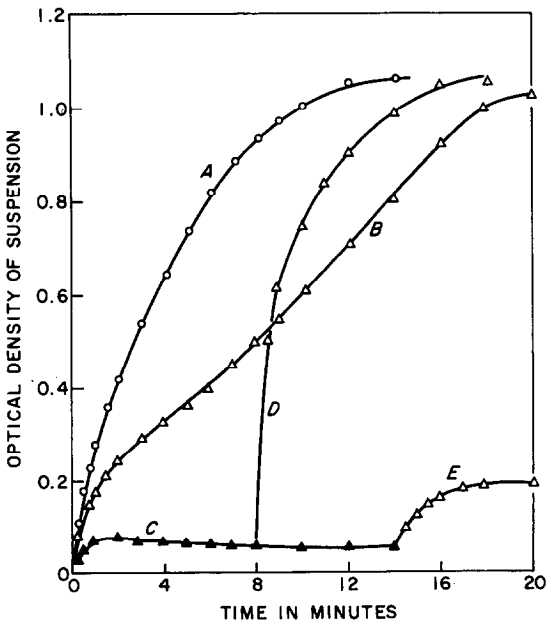

Fig. 1. Contraction of actomyosin suspension as measured by optical density increase at 620 mμ, over the original value of about 0.15. Temperature 25°C. Actomyosin concentration 0.96 mg. per ml. in 3 ml. reaction mixture composed of 0.05 M tris-acetate buffer pH 6.7, 0.0015 M ATP, 0.002 M $MgCl_2$, and other additions as indicated. K-ion concentration was between 0.056 and 0.060 M. Curve A: without other additions, B: with 0.002 M oxalate; these constitute the control experiments showing contraction and its slowdown in the presence of oxalate. Curve C: inhibition of contraction by 3.8×10^{-6} M cyclic AMP. Curves D and E: as C, relaxation released by the addition of 66×10^{-6} M $CaCl_2$ after 8 minutes, and 6.6×10^{-6} M $CaCl_2$ after 14 minutes, respectively.

Since there are indications of the participation of a cofactor (Briggs et al., 1959), and since the present investigation was restricted to the responses of actomyosin suspensions, it is not justified to state that all phenomena observed in relaxing factor systems are due to cyclic AMP only. We wish to consider, however, that cyclic AMP is identical with what has become known as the "relaxing substance".

We do not propose, however, that the active state in muscle is established and terminated by an inactivation and reformation of cyclic AMP. Rather, we hold that the substance sensitizes actomyosin towards small changes in the free calcium concentration, and that, in keeping with physiological evidence (Mommaerts et al., 1961), it is through the calcium ion that contraction is elicited and abolished. We furthermore propose that changing levels in cyclic AMP are responsible for the variations in contractile strength occurring physiologically in the myocardium, as related to the staircase effect or adrenergic potentiation (Mommaerts and Langer, 1963). Thus it appears that cyclic AMP, in addition to its role as a metabolic regulator (Rall and Sutherland, 1961), may be the basis of physiological control systems of wide significance.

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