ON THE RELAXING SUBSTANCE OF MUSCLE[1]

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In a previous note (Uchida and Mommaerts, 1963) and abstract (Mommaerts, Seraydarian, and Uchida, 1963), we have reported that cyclic adenosine-3', 5'-phosphate inhibited the turbidimetrically recorded superprecipitation of actomyosin, and that it accelerated this phenomenon in the presence of calcium. From the first observation, it was concluded that the cyclic nucleotide was a relaxing substance and, as such, a component of the relaxing-factor system of muscle; from the second, that it could play a role in the inotropic responses of heart and muscle. However, several inconsistencies were encountered: no relaxing effect was exerted by the nucleotide upon contracted glycerol-extracted muscle fiber preparations, and the ATPase activity of actomyosin was inhibited to a small degree only. Furthermore, the experiments were erratic throughout the entire course of the investigation.

Continuing these investigations, we have aimed at eliminating the accidental introduction of calcium in the experiments, as one obvious source of error. With such precautions, both with and without the use of ethyleneglycol bis (β -aminoethylether)-N, N-tetraacetic acid[2] as

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2 This substance, trade name Chel De, was a generous gift from The Geigy Pharmaceutical Company, Yonkers, New York.

a calcium buffer, we have not been able to reproduce our earlier experiments on the inhibitory effect of cyclic adenylate. This applied to actomyosin as such, or treated with thymol to destroy its contaminating relaxation granules; or to actomyosin to which was added a preparation of phosphorylase-b kinase (Krebs, Graves and Fischer, 1959)[3] which is known to be a site of action of cyclic adenylate in muscle. We cannot consider the relaxing effect as definitively eliminated, but clearly, until we ourselves can again reproduce it, it cannot be accepted as an experimental fact. We did obtain frequent indications of the accelerating effect of the nucleotide in the presence of calcium, but would still require better control over the phenomenon to consider it as established.

In view of the uncertainty this leaves as to the existence of a relaxing substance in general, we deem it desirable to report briefly on our experiments confirming its presence in an aqueous muscle extract after removal of the granular fraction by centrifugation for 90 min. at 150, 000 g. Many of our attempts at its identification corresponded to those of Fuchs and Briggs (1963) and, being negative, will not be described in detail. However, we single out the following crucial points.

The substance passed through a Dowex-2 acetate or formate column (Table I), the eluate having no detectable nucleotide band. A similar material is also elaborated by relaxation granules during incubation with ATP.

No active principle could be extracted from the lyophilized extract by means of chloroform-methanol, ether-ethanol, and other lipid solvents, but the water-extracted residue was also inactive, except after the use of acetone or ether. Thus the substance is not simply a lipid as proposed by Briggs (1963), but its activity may be destroyed by some lipotropic solvent actions, perhaps because of their denaturing effect upon the stabilizing protein (see below).

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3 This enzyme preparation was a generous gift from Professor Edwin G. Krebs of the University of Washington. The samples were dialyzed to remove the glycerol phosphate and ethylene diamine tetraacetate employed in their preparation.

Table I

Passage of relaxing substance through Dowex-2 column; 10 ml. of muscle extract (1 part muscle in 3 parts of 0.1 M KCl, 0.001 M K-oxalate) was passed through a Dowex-2 formate column of 10 x 80 mm. The eluate after the void volume was collected and assayed as follows: 12-15 mg. myofibrils (not thymol- or desoxycholate treated in this particular experiment) in 5 ml. total volume of .088 M KCl; .003 M $MgSO_4$; .003 M ATP; .002 M K-oxalate; 0.05 M Tris acetate buffer pH 6.8. The figures give the fibril volumes, read in arbitrary units, upon centrifugation for 2 min., 1500 rpm. after 5 min. incubation.

	Fibril Volume	Decrease	% Synaeresis
Without ATP	0.50	----	0
With ATP	0.15	0.35	100
ibid., with 0.2 ml. extract	0.49	0.01	3
ibid., with 0.2 ml. effluent	0.48	0.02	6

The activity was found to accompany protein precipitated at 0.7 saturation with ammonium sulfate, and remained largely associated with this protein during subsequent dialysis. Identical with the findings of Fuchs and Briggs (1963), the active principle was separated from the protein by gelfiltration or heating, and then became very labile, showing spontaneous inactivation in a day at 4° C.

Thus, it is clear that relaxing factor system in vitro may to varying degrees be ascribed to the calcium binding properties of a vesicular constituent and to an unidentified relaxing substance.

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