Alteration of Cyclic 3',5'-Adenosine Monophosphate Action on Adenosine 5'-Triphosphate Induced Muscular Contraction by a Serum Enzyme

The interaction between myosin and actin which results in the formation of actomyosin is catalyzed by the presence of adenosine 5'-triphosphate (ATP). Variations in ATP concentrations influence adenosine 5'-triphosphatase (ATPase) and superprecipitation of actomyosin. Both of these parameters are limited by the availability of the substrate.

Several accessory proteins were reported to play various roles in the functions of the myofibrils. For instance, actinin was shown to play a role in the structure of the Z-band and its attachment to the I-filament. A tropomyosin-tropomyosin factor was shown to be active and to delay the onset of superprecipitation in certain tropomyosin preparations. Troponin is another protein which plays a role in the On-Off control by calcium ions of the contraction cycle.

A serum enzyme was shown to enhance the creatine-phosphotransferase-ATP regeneration and the phosphoenol pyruvate-pyruvate kinase system and muscular contraction. The enzyme is shown in the present studies to reverse excess substrate inhibition and cyclic-3', 5'-adenosine monophosphate (cyclic 3' 5'-AMP) inhibition of the ATP-induced development of tension by glycerinated muscle fibers.

The sensitivity of glycerinated fibers to ATP was shown to be influenced by a toxic glycoprotein from scalded human skin. The degree of inhibition of the ATP-induced muscle contraction was dependent on the concentration of the toxic glycoprotein. Immuno sera and purified immunoglobulin against the toxic glycoprotein prepared by i.m. injections of the glycoprotein neutralized the inhibitory effects of the antigen. When used in combination with the immune serum or with the immunoglobulin, the toxic glycoprotein failed to inhibit the ATP-induced development of tension by glycerinated fibers.

**Materials and methods.** Glycerinated rabbit psoas muscle fibers were prepared according to the method of Szent-Gyorgyi. Actomyosin was prepared from rabbit striated skeletal muscle following the method of Enashe. The serum enzyme was obtained from either human or calf serum following the method described by Hart.

The serum was adjusted to pH 5.0, then made up to 25% saturation with ammonium sulfate. A precipitate was separated by centrifugation at 10,000 g for 30 min at 4°C. The precipitate was dissolved in cold 0.9% sodium chloride and dialyzed exhaustively against 0.9% NaCl. The dialyzed sulfate free solution was then adjusted to pH 4.0 and centrifuged at 15,000 g for 60 min. The precipitate obtained was dissolved in 0.9% NaCl to produce a solution.
with an absorbance at wavelength 280 μm of 0.5, and used as the serum enzyme. Gel filtration through Sephadex G-25 did not influence the enhancing physiological activity of the serum enzyme. Flame spectrophotometry of the serum enzyme indicated either the absence or undetectable traces of either Mg²⁺ or Ca²⁺.

The glycerinated muscle fiber was suspended from a Grass Model 0.3 force transducer in a bathing chamber containing 40 ml M/15 Tris-phosphate buffer of pH 7.5 containing 0.025 mM MgCl₂. In this chamber the fiber was allowed to incubate at room temperature (26°C) for 5 min, or as indicated in the presence or absence of varying amounts of cyclic 3', 5'-AMP or ATP. In certain experiments ATP was added in 0.1 ml aliquots (5% solution) to induce tension. In experiments where Mg deficient medium was employed, development of tension was induced by addition of 0.05 ml of 0.025 mM MgCl₂ solution. All experiments were monitored with a model Grass polygraph with 7 PI preamplifier.

Superprecipitation of actomyosin was determined following the turbidimetric technique in a medium containing 0.06 MKCl, 0.001 M MgCl₂, 0.016 M Tris-phosphate buffer pH 7.0. Actomyosin 0.15 mg and ATP added last to 0.1 mM final concentration in a final volume of 4.0 ml and ionic strength 0.080 μm. The serum enzyme

Fig. 2. Cyclic 3',5'-AMP inhibition of ATP-induced tension. Inhibition of ATP-induced tension of 0.05, 0.5, 1.2 and 1.5 mM (curves a, b, c and d respectively) of ATP, by increasing amounts of cyclic 3',5'-AMP.

Fig. 3. Reversal of cyclic 3',5'-AMP inhibitory action of ATP-induced tension by serum enzyme. The standard ATP-induced tension was employed with 1.5 mM ATP and increasing amounts of cyclic 3',5'-AMP were added alone (a), and in presence of 0.10, 0.25 and 0.50 mg protein of the serum enzyme curves b, c and d respectively.

Fig. 4. Serum enzyme and cyclic 3',5'-AMP action on ATP-induced tension triggered by Mg²⁺. Tracings of tension produced by glycerinated fibers preincubated for 150 sec in Mg²⁺-deficient phosphate buffer containing 0.15 mg protein serum enzyme (a), 0.05 mM cyclic 3',5'-AMP (b), and cyclic 3',5'-AMP and 0.15 mg serum enzyme (c). 5 min after addition of 0.15 ml of 5% ATP solution. Tension was induced by 0.05 ml of 1 mM MgCl₂, followed by 150 sec interval additions of 0.10 ml of the ATP solution. The sharp break in the tracing is a change in polygraph sensitivity from 0.02 to 0.05 mV/cm.