after the injections of amines. Partial purification and estimation of activity of γ-amylase in liver and heart were carried out as described previously[2].

Stimulation by adrenaline of γ-amylase activity in rat liver is not reproduced by equimolar doses of other amines which, in contrast to adrenaline, markedly inhibit hydrolysis of glycogen by the acid α-glucosidase in liver (Figure 1). Inhibitory effect on γ-amylase in heart muscle is observed in experiments with all the monoamines tested (Figure 1). This effect is prevented by monoamine oxidase inhibitors in doses causing strong inhibition of enzymatic deamination of monoamines[6]. Monoamine oxidase inhibitors also prevent the effect of noradrenaline and tryptamine on γ-amylase in liver (Figure 2).

The data obtained suggest that the inhibitory effects of adrenaline on γ-amylase, exhibited also by other monoamines, are possibly caused by the deaminated metabolic products. The stimulatory effect of adrenaline on liver γ-amylase is specific for the hormone, and may not be prevented by inhibition of monoamine oxidase activity in liver.

Выводы. Норадреналин, дофамин, тирамин и триптамин тормозят γ-амилазу печени и сердца крыс. Ингибиторы моноаминоксидазы предупреждают этот эффект.

V. Z. Gorkin, V. N. Synyukhin, I. A. Popova, V. S. Orlova and E. L. Rosenfeld

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moskwa G-117 (USSR), 8 February 1971.

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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[1].

Several accessory proteins were reported to play various roles in the functions of the myofibrils. For instance, α-actinin was shown to play a role in the structure of the Z-band and its attachment to the I-filament[2–4]. A troponin-tropomyosin factor was shown to be active and to delay the onset of superprecipitation in certain tropomyosin preparations[5]. Troponin is another protein which plays a role in the On-Off control by calcium ions of the contraction cycle[6,7].

A serum enzyme was shown to enhance the creatine-phosphotransferase-ATP regeneration and the phosphoenol pyruvate-pyruvate kinase system and muscular contraction[8]. 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[9]. The degree of inhibition of the ATP-induced muscle contraction was dependent on the concentration of the toxic glycoprotein[10]. 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[10].

Materials and methods. Glycerinated rabbit psoas muscle fibers were prepared according to the method of Szent-Gyorgyi[11]. Actomyosin was prepared from rabbit striated skeletal muscle following the method of Ebashi[12]. The serum enzyme was obtained from either human or calf serum following the method described by Hakim[8]. 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

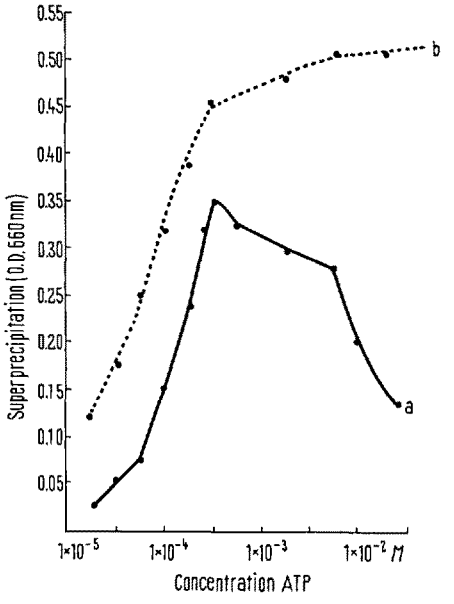

Fig. 1. Action of serum enzyme on the superprecipitation of actomyosin. a) Superprecipitation of actomyosin in a system containing 0.05 *M* KCl, 1.0 m*M* $MgCl_2$, 0.016 *M* *Tris*-phosphate buffer of pH 7.0 and 0.15 mg/ml of actomyosin in a total volume of 4.0 ml and ionic strength of 0.080 µm. The final ATP concentrations employed are indicated on the abscissa. Superprecipitation of actomyosin assayed in presence of 0.25 mg protein of serum enzyme produced the data of curve b).

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 Mg^{++} or Ca^{++}.

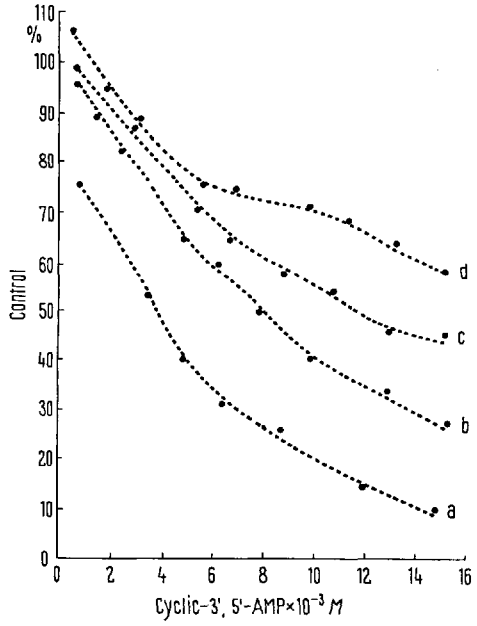

Fig. 2. Cyclic 3′,5′-AMP inhibition of ATP-induced tension. Inhibition of ATP-induced tension of 0.05, 0.5, 1.2 and 1.5 m*M* (curves a, b, c and d respectively) of ATP, by increasing amounts of cyclic 3′,5′-AMP.

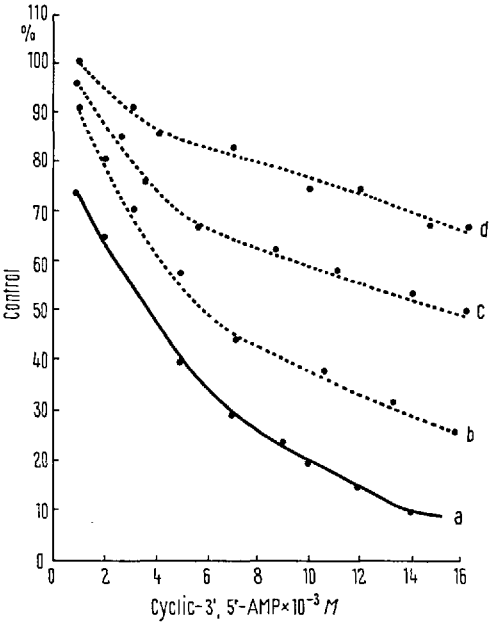

Fig. 3. Reversal of cyclic 3′,5′-AMP inhibitory action of ATP-induced tension by serum enzyme. The standard ATP-induced tension was employed with 1.5 m*M* ATP and increasing amounts of cyclic 3′,5′-AMP were added alone (a), and in presence of 0.10, 0.25 and 0.50 mg protein of the serum enzyme curves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

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 m*M* $MgCl_2$. 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 m*M* $MgCl_2$ solution. All experiments were monitored with a model Grass polygraph with 7 Pl preamplifier.

Superprecipitation of actomyosin was determined following the turbidimetric technique[12] in a medium containing 0.06 MKCl, 0.001 *M* $MgCl_2$ 0.016 *M* *Tris*-phosphate buffer pH 7.0. Actomyosin 0.15 mg and ATP added last to 0.1 m*M* final concentration in a final volume of 4.0 ml and ionic strength 0.080 μm. The serum enzyme

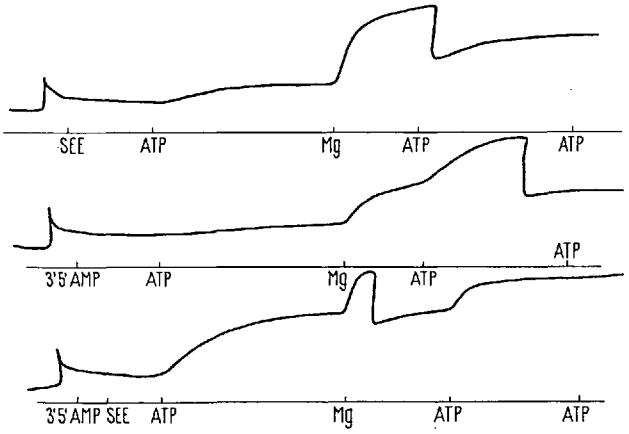

Fig. 4. Serum enzyme and cyclic 3′,5-AMP action on ATP-induced tension triggered by Mg^{++}. Tracings of tension produced by glycerinated fibers preincubated for 150 sec in Mg^{++}-deficient phosphate buffer containing 0.15 mg protein serum enzyme (a), 0.05 m*M* cyclic 3′,5′-AMP (b), and cyclic 3′,5′-AMP 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 m*M* $MgCl_2$, 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.

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was added in amounts shown in the figure. The transmittance changes in the actomyosin suspension upon the addition of ATP was done in 1 × 1 cm glass cell of 4 cm height in the optical path of Beckman DU2-spectrophotometer at 660 μm wavelength and 27 °C.

Results and Discussion. Variations in the ATP concentration and their effect on actomyosin superprecipitation and development of tension by glycerinated fibres have been studied. At the low ATP concentration, both parameters are limited by the availability of ATP. That is, the addition of more ATP increased both the magnitude and the rate of superprecipitation and the development of tension by glycerinated fibers. At high substrate levels, the phenomenon of excess substrate inhibition occurs. Both superprecipitation and tension development are inhibited by the addition of excess substrate. In Figure 1 curve a) shows the variations in ATP concentrations and their effect on actomyosin superprecipitation, curve b) shows the effect of 0.25 mg protein of the serum enzyme in the assay system.

Addition of cyclic 3′, 5′-AMP into the assay system affected actomyosin superprecipitation and development of tension by glycerinated muscle fibers in a comparable manner. If glycerinated fibers are incubated with cyclic 3′,5′-AMP for 5 min before the addition of ATP, the contraction of the fibers is inhibited to a degree dependent on the concentration of the cyclic nucleotide. Figure 2 shows the concentration dependence of the cyclic 3′, 5′-AMP inhibition of the development of tension at 0.05, 0.5, 1.2 and 1.5 m*M* ATP concentrations. The data suggest that the inhibitory effect of the cyclic nucleotide is complex and varies inversely with ATP concentration.

If glycerinated fibers are incubated first with the serum enzyme and then cyclic 3′, 5′-AMP is added and incubation continued for 5 min before the addition of ATP, the contraction of the fiber is inhibited to a degree dependent on the concentration of both the serum enzyme and the cyclic nucleotide. When no serum enzyme is added (curve a, Figure 3) the contraction of the fiber is inhibited to a degree dependent on the concentration of the cyclic 3′, 5′-AMP at 0.05 m*M* ATP. The effect of 0.10, 0.25 and 0.50 mg protein of the serum enzyme on the cyclic 3′, 5′-AMP inhibition of the development of tension at 0.05 m*M* ATP is shown by curves b, c, and d respectively.

The upper tracing a) of Figure 4 shows the response of a glycerinated fiber preincubated in Mg^{++}-deficient *tris*-phosphate buffer containing the serum enzyme. Weak tension developed slowly after addition of ATP. If Mg^{++} was then added, full tension developed. Additional amounts of ATP led to development of maximum tension.

Tracing b) of Figure 4 shows the response by glycerinated fibers preincubated in the Mg^{++}-deficient *tris*-phosphate containing cyclic 3′, 5′-AMP. Addition of ATP caused development of a very weak tension. The magnitude of tension induced by addition of magnesium was approximately $^1/_4$ that produced in tracing a).

Tracing c) shows the response of glycerinated fibers preincubated in the Mg^{++}-deficient *tris*-phosphate to which cyclic 3′, 5′-AMP and the serum enzyme were added. Addition of ATP produced an enhanced development of tension. Addition of Mg^{++} resulted in further development of tension.

Résumé. Les effets d'une enzyme du sérum humain sur la contractilité de la fibre musculaire glycerinée, et sur la superprécipitation de l'actomyosine sont démontrés. Les effets produits sur l'inhibition par excess du substrat et sur l'inhibition par l'adénosine 3′, 5′-monophosphate cyclique des deux parameters, suggèrent que l'enzyme du sérum est un facteur qui contrôle la contraction musculaire.

A. A. Hakim

Biochemical Research Laboratories of the I.T.R., 259 D.M.P. School of Medicine, University of Illinois at the Medical Center, Chicago (Illinois 60680, USA), 31 August 1970.

The Effect of 5-Hydroxytryptophan on the Efflux of Noradrenaline from Brain Slices

Recently the possibility of reciprocally acting 'catecholaminergic' and 'serotoninergic' systems in the brain has been proposed[1,2], whereby not the absolute level of a given amine in a given mechanism (e.g. sleep[3], aggression[4,5], motor activity[6], psychoses[7]) is important, but the relative levels of available transmitter.

Starting from this suggestion, we have compared the effects of different additives on the efflux of noradrenaline (NA) from brain slices. Two systems were studied: efflux of exogenous NA previously taken up from the incubation medium, and – with the possibility in mind that a part of the uptake may not be specifically localised – efflux of NA newly synthesized from L-DOPA.

Experimental. The di- and mesencephalon ('mid-brain') from male Füllinsdorf albino rats were sliced[8] and princubated for 30 min in Krebs-Ringer bicarbonate glucose supplemented medium containing pyridoxal phosphate (10^{-5} *M*)[9], under an atmosphere of 95% O_2–5% CO_2, either with NA or DOPA (both 10^{-3} *M*). The tissues were washed and resuspended in an incubation medium containing the compound under investigation, and aliquots of tissue were taken at intervals. After perchloric acid extraction of the tissue, NA was determined after alumina absorption by a modified fluorescence method[10], serotonin (5-HT) also by fluorescence[11]; protein was determined in the precipitate[12].

Results. NA was readily taken up or synthesized from DOPA. After washing, the level of NA in the tissue

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