BIOCHEMISTRY AND BIOPHYSICS

THE EFFECT OF SERUM FROM MYASTHENIC PATIENTS ON FORMATION OF PHOSPHOCREATINE

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The pathogenesis of myasthenia, a severe neuromuscular disease, has not as yet been exhaustively investigated. In all probability myasthenic disorders are not fully accounted for by impairment of synaptic transmission.

A number of investigations have shown that myasthenia is accompanied by impairment of tissue respiration. N. I. Grashchenkov, L. A. Blumenfeld, L. B. Perelman, S. E. Krasovitskaya et al [1] discovered that during the period of myasthenic cachexia the O_2 content of venous blood was increased; administration of proserine* was followed by an appreciable drop in venous blood oxygen which was, evidently, taken up by the tissues. Indirect evidence for this is also provided by oxyhemometric examinations (Rivin) which showed that following administration of proserine there was a decrease in oxygen saturation of arterial blood.

Studies on creatine-creatinine metabolism also furnish evidence for impairment of oxidative processes in the muscles. Creatine may be found in the urine of myasthenic patients. A certain correlation could be observed between the dynamics of urinary creatine and changes in the clinical condition of the patients. According to our data, urinary creatine is only found in patients with marked myasthenic disturbances. Improvement in the clinical state of the patients was often accompanied by diminished excretion of creatine.

The following data can also serve as evidence for changes in tissue respiration in myasthenia. Proserine, a cholinesterase inhibitor, causes accumulation of acetylcholine which, in myasthenia, improves neuromuscular transmission. This is accompanied by accumulation of adrenalin-like substances in the blood. The improvement in the state of myasthenic patients is thus associated with increasing content of adrenalin-like factors which activate respiratory and glycolytic processes in the tissues.

The present investigation is concerned with the study of the effect of myasthenic blood on certain processes of tissue respiration. The working hypothesis adopted was that which suggested the possible presence in the blood of myasthenic patients of a factor eliciting myasthenic disorders. It is known that Torda and Wolff [2] showed that myasthenic serum depresses acetylcholine synthesis. H. Schwarz [3] points out that when serum from myasthenic patients is given to healthy subjects the latter develop muscular weakness. A. Wilson and H. Stoner [4] showed that normal serum did not affect the excitability of the neuromuscular preparation and tetanus values whereas serum from myasthenic patients depressed both the former and the latter. A depressing effect on excitatory processes is also exerted by thymus gland extracts from myasthenic patients but not by such extracts from healthy subjects [5].

METHODS

Oxidative phosphorylation was studied on ground rat muscle which was incubated with phosphate buffer at pH 7.8 in the presence of creatine.

* Russian trade name.

Composition of phosphate buffer: 1.15% KCl; 0.15 M $Na_2HPO_4 \cdot 2H_2O$; 3.82% $MgSO_4 \cdot 7H_2O$; 1.3% $NaHCO_3$. To 2 volumes of this buffer was added 1 volume of NaCl 0.9% and creatine calculated to correspond to 0.15%.

Composition of samples: 3 ml freshly prepared buffer, 0.5 ml serum, 300-500 mg muscle. Incubation – 30 minutes at 30°.

The reaction was terminated in the cold by precipitation with 3 ml ice-cold 5% trichloracetic acid. Inorganic phosphate was precipitated in the cold by an equal volume of magnesia mixture and phosphocreatine phosphorus was determined in 1 ml of centrifugate on incubation with ammonium molybdate. In investigating the effect of boiling 0.5 ml of serum was placed in a flask and brought to the boil before the addition of the remaining reagents to the same flask. The effect of dialysis was determined by preliminary dialysis of 3 ml serum against 3 ml NaCl 0.9% in the cold over a period of 24 hours followed by further determination on 0.5 ml of the dialysate. Respiration was determined in a Warburg apparatus on the same samples.

The rate of phosphocreatine formation served as an index of oxidative phosphorylation. The influence on this process of healthy and myasthenic serum, before and after administration of 2 ml 0.05% proserine to the patient, was studied.

RESULTS

In the first series of investigations the effect of serum from a healthy subject on phosphocreatine formation was studied. It was found that such serum exerted a depressing effect on the process. The degree of such depression is not a constant value but varies within wide limits, depending on the peculiarities of the muscle and rate of phosphorylation under given conditions. Several results of investigation of the effect of serum on phosphocreatine formation are presented in Table 1.

TABLE 1

Phosphocreatine Formation (in γ P per whole sample)

Without addition of serum	With added serum from healthy subject
560	470
425	352
230	105

Depression does not depend on the possible presence of phosphatases in the blood since this depressing effect on phosphorylation was preserved following boiling and dialysis. The factor exerting an inhibitory effect on phosphocreatine formation could be some substrate capable of intercepting part of the adenosinetriphosphate (ATP) phosphorus and so lead to diminished formation of phosphocreatine. Glucose, for example, could be such a substrate. In fact, addition of glucose (instead of serum) in amounts corresponding to its content in blood to the sample in which phosphorylation is taking place also leads to depression of phosphocreatine formation. The following distinctive feature was established: a 2-3 fold increase or 2 fold decrease of the amount of added glucose did not result in a definite change in the degree of phosphorylation depression.

In the next series of experiments a comparative study was made of the effect of myasthenic serum and of donor blood (control) on phosphocreatine formation. In each experiment a parallel and simultaneous investigation of the effect of myasthenic and healthy serum was carried out. Absolutely identical experimental conditions were thus maintained for the blood being tested and for donor blood. In each experiment the value of phosphorylation in the presence of donor serum was taken as 100% and the value of phosphorylation in the presence of myasthenic serum was compared with it. Such a method ensures greater reliability of results than statistic comparison of experimental data owing to the extensive fluctuation of the rate of phosphorylation in different experiments.

It was found that in the majority of cases myasthenic sera exerted a greater depressing influence on phosphocreatine formation than normal sera. This was found in 10 of the 12 myasthenic patients investigated. Most

of the patients were subjected to repeated examinations; a total of 29 experiments was carried out, and increased depression of phosphorylation was found in 24 (Table 2). The average value for phosphocreatine formation in the presence of serum from myasthenic patients as compared with serum from healthy subjects was 87%.

TABLE 2

Phosphocreatine Formation in the Presence of Serum from Myasthenic Patient (in percentage ratio to values obtained with normal serum taken as 100%)

Name	Before administration of proserine	After administration of proserine
	1. Serum from myasthenic patient	
L-va	77	101
T-an	88	103
P-uk	92	110
Z-va	80	89
	2. Boiled serum from myasthenic patient	
L-va	71	110
D-en	83	110
M-di	94	106
T-an	89	—
	3. Dialyzate of serum from myasthenic patient	
F-va	82	—
T-an	92	—

To assess the significance of the discovered phenomenon of increased depression of phosphocreatine formation under the influence of myasthenic serum it appeared to be of interest to determine the change, if any, in the properties of the serum in relation to fluctuations in the patients' condition before and after administration of proserine. Sera samples were therefore taken before proserine and 45 minutes after intramuscular injection of 2 ml 0.05% proserine when the condition of the patients was considerably improved. It was found that under these conditions the rate of phosphorylation reached the control values and sometimes even exceeded them (see Table 2).

The greatest change towards enhanced phosphorylation after administration of proserine was observed in severely ill patients. In their case the increase of phosphorylation reached 20-30% as compared to the values observed prior to proserine injection. Control investigation of the effect of proserine itself or of proserine mixed with serum on the processes of phosphorylation revealed that these factors not only exerted no stimulating influence on phosphocreatine formation on direct action on the test substrate in vitro, but even suppressed this process.

The change in the properties of the serum in patients given proserine provides supplementary indication of the fact that the greater depression of phosphocreatine formation by myasthenic serum reflects functionally important features pertaining to myasthenia. Improvement in the functional state of the patient and liquidation of myasthenic disorders are accompanied by changes for the better in the ability of the myasthenic serum to influence phosphocreatine formation.

The distinctive features of the effect of myasthenic sera on phosphorylation are preserved on boiling the serum and on subjecting it to dialysis. Preservation of these properties despite boiling and dialysis permits the conclusion that the factor responsible for the enhanced depression of phosphorylation is, most probably nonprotein in nature, of low molecular weight and does not belong to the enzyme group of compounds. This factor cannot be glucose either, since addition of glucose to the samples in which phosphocreatine is being formed in the presence of serum from a healthy subject does not lead to increased inhibition. Table 2 presents data of some of the experiments on the formation of phosphocreatine under the influence of myasthenic sera before and after administration of proserine as well as of sera after boiling and of serum dialysate (phosphocreatine formation is given as percentage of the values obtained in control experiments taken, in each given experiment, as 100%).

In connection with the discovered phenomenon of phosphocreatine formation inhibition by serum from patients with myasthenia in a state of myasthenic cachexia, a parallel series of investigations was staged (in collaboration with Ya. K. Smirnov) on the effect of myasthenic sera on processes of phosphocreatine formation and tissue respiration in the same sample. In these experiments (20 observations) no parallelism was found between the effect of myasthenic serum on phosphocreatine formation and on tissue respiration. Myasthenic serum, which exerted an inhibitory influence on the formation of phosphocreatine, did not affect tissue respiration perceptibly under the given experimental conditions. These investigations are being pursued further.

SUMMARY

It was demonstrated that the serum of patients with myasthenia depressed the process of phosphocreatine formation in small pieces of rat's muscle to a greater degree than the serum of a healthy person. Following administration of proserine, when there is a marked clinical improvement in the general condition of the patient, this depressant effect of myasthenic serum is reduced. The factor which depresses the formation of phosphocreatine in myasthenia is preserved after boiling and dialysis.

LITERATURE CITED

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