boundary zone with the ischemized myocardium can be explained by an improvement in the blood supply of this zone through vasodilatation and augmentation of the collateral blood flow [3], on account of a rise in the pressure gradient between the perfused and unperfused areas of myocardium in the boundary zone. The fact that nitroglycerin exerts its strongest action on the inner layers of the myocardium was probably the results of selective action of the drug on vessels of the subendocardium [11].

The results of this investigation thus provide additional evidence on the mechanism of the therapeutic action of nitroglycerin in short-term ischemia. Even in transient disturbances of the coronary circulation (of the spasm type, for example, when treatment is aimed mainly at abolishing the spasm) the effect of the drug on the functional state of the myocardium bordering on the ischemized zone must be borne in mind.

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EFFECT OF GLUTAMIC AND ASPARTIC ACIDS ON METABOLISM AND FUNCTION OF THE HYPOPERFUSED HEART

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UDC 616.12-005.4-092.9-06:616.12-008.9-02:615.31:547.466.63/.64

KEY WORDS: amino acids; cardiac function; ischemia; ATP; creatine phosphate; amino-acid ammonia.

An urgent problem at the present time is the search for substances capable of preventing the fall in the level of adenine nucleotides and creatine phosphate caused by ischemia. The use of exogenous amino acids, which can improve the contractile function of the heart in ischemia [2, 3], seems a promising way of achieving this goal. The mechanism of the protective effect of amino acids is not yet clear.

The object of the present investigation was to study whether improvement in the contractile function of the heart is connected with a rise in the ATP and creatine phosphate levels and also with intensification of detoxication reactions of the ammonia which accumulates during ischemia [6, 7].

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(Presented by Academician of the Academy of Medical Sciences of the USSR E. I. Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 15-17, July, 1982. Original article submitted December 23, 1981.

0007-4888/82/9407-0866$07.50

TABLE 1. Effect of Glutamic and Aspartic Acids on Concentrations of Adenine Nucleotides and Creatine Phosphate (in μmoles/g wet weight of tissue) in Rat Heart during Hypoperfuction (M ± m)

Experimental conditions	ATP	ADP	AMP	Total adenine nucleotide pool	Creatine phosphate
Initial state (6)	4,10±0,43	0,91±0,10	0,21±0,05	5,22±0,28	5,80±0,32
Hypoperfusion for 10 min (4)	3,05±0,45 [a]	1,45±0,15 [a]	0,61±0,08 [a]	5,11±0,21	2,25±0,30 [a]
Hypoperfusion for 40 min (8)	1,42±0,38 [a]	0,74±0,20	0,38±0,08	2,54±0,17 [a]	1,15±0,41 [a]
Hypoperfusion with glutamic acid for 40 min (6)	2,95±0,46 [a, b]	0,88±0,31	0,29±0,07	4,02±0,22 [a, b]	3,78±0,42 [a, b]
Hypoperfusion with aspartic acid for 40 min (8)	2,36±0,35 [a, b]	0,83±0,28	0,32±0,06	3,51±0,19 [a, b]	3,20±0,31 [a, b]

Legend. Here and in Table 2: a) Difference compared with initial state significant at $P < 0.05$ level, b) the same, compared with ischemia, at $P < 0.05$ level; number of animals in group given in parentheses.

TABLE 2. Effect of Glutamic and Aspartic Acids on Concentrations of Amino Acids (in μmoles/g wet weight of tissue) in Rat Heart during Hypoperfusion (M ± m)

Experimental conditions	Aspartic acid	Glutamic acid	Glutamine	Asparagine	Alanine	Ammonia	Urea (total content in tissue and perfusion fluid)
Initial state (6)	0,98±0,05	3,68±0,25	4,58±0,22	0,28±0,2	2,20±0,10	1,62±0,15	1,52±0,11
Hypoperfusion for 10 min (4)	0,90±0,06	3,40±0,30	5,05±0,30	0,32±0,03	2,28±0,12	1,86±0,17	—
Hypoperfusion for 40 min (8)	0,85±0,05	2,62±0,28 [a]	5,20±0,35	0,38±0,05	2,64±0,16	2,34±0,20 [a]	2,28±0,17
Hypoperfusion with glutamic acid for 40 min (6)	1,28±0,08 [a, b]	5,26±0,48 [a, b]	5,94±0,42 [a]	0,49±0,04 [a]	2,15±0,15 [b]	2,80±0,20 [a]	2,44±0,22 [a]
Hypoperfusion with aspartic acid for 40 min (8)	2,35±0,10 [a, b]	4,55±0,38 [b]	5,48±0,32	0,63±0,06 [a, b]	2,21±0,16	2,21±0,25	3,17±0,27 [a, b]

EXPERIMENTAL METHODS

Experiments were carried out on isolated hearts of male Wistar rats weighing 250-300 g. The hearts were perfused through the aorta at the rate of 10 ml/min with 30 ml of Krebs–Henseleit bicarconate buffer containing 5.5 mM glucose, saturated with carbogen (95% O_2 + 5% CO_2) at 37°C and pH 7.4. After atrioventricular blockage the heart was stimulated at a frequency of 120 stimuli/min and the pressure was recorded inside the left ventricle by means of a latex balloon filled with liquid, a P50 transducer, and an SP1405 monitor (Gould Statham). The rate of perfusion was reduced to 3 ml/min after 15 min. The duration of hypoperfusion was 40 min. The action of glutamic or aspartic acid was studied during a decrease in the coronary blood flow by injecting them into the perfusion fluid at the 10th minute of hypoperfusion. The concentration of amino acids in the perfusion fluid was 10 mg/ml. After hypoperfusion the heart was rinsed with Krebs–Henseleit buffer to remove unreacted amino acids, frozen with Wollenberger's forceps, cooled in liquid nitrogen, and weighed. The concentration of amino acids and ammonia in protein-free tissue extracts was determined by means of the Liquimet-111 amino-acid analyzer. To determine ATP, ADP, AMP, creatine phosphate, and urea enzymic methods [1] were used. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The stable level of pressure developed by the heart (84 ± 10 mm Hg/g wet weight of tissue) fell quickly during the first few minutes of hypoperfusion by more than half, to reach 24 ± 8 mm Hg/g wet weight of tissue after 40 min. The diastolic pressure rose sharply after 20 min of hypoperfusion, and at the end of perfusion it was three times higher than initially ($P < 0.01$), i.e., contracture developed. Perfusion with glutamic acid prevented the development of contracture (Fig. 1), but did not significantly change the level of pressure developed at the end of hypoperfusion (34 ± 2 mm Hg/g wet weight of tissue). Aspartic acid had a much

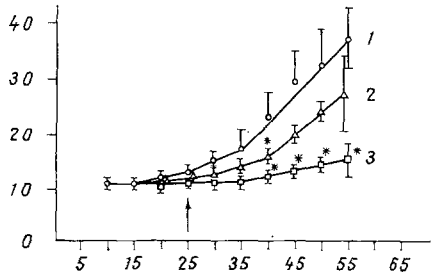

Fig. 1. Effect of glutamic and aspartic acids on time course of diastolic pressure of isolated isovolumic rat hearts during hypoperfusion. Abscissa, time of perfusion (in min); ordinate, diastolic pressure (in mm Hg). 1) Hypoperfusion without amino acids; 2) hypoperfusion with aspartic acid; 3) hypoperfusion with glutamic acid. Heart perfused during first 15 min at rate of 10 ml/min, during next 40 min at rate of 3 ml/min. A 1% solution of amino acid was injected at 10th minute of hypoperfusion (arrow). *) Differences compared with hypoperfusion without amino acids significant at the $P < 0.05$ level.

weaker action on contractile function: the degree of contracture (Fig. 1) and the pressure developed (40 ± 4 mm Hg/g wet weight of tissue) in its presence were not significantly different from the corresponding values during hypoperfusion without amino acids.

Lowering of the contractile function of the heart after hypoperfusion for 10 min was combined with a fall in the creatine phosphate level by more than half (Table 1). Injection of glutamic or aspartic acid into the perfusion solution maintained a higher level of ATP and creatine phosphate and preserved more of the total pool of adenine nucleotides. After hypoperfusion for 40 min with glutamic acid the concentrations of ATP and creatine phosphate were 2 and 3 times higher respectively than in the absence of amino acids (Table 1).

Addition of glutamic acid significantly raised the ATP level during hypopherfusion and prevented the development of contracture of the heart. Feedback between the ATP concentration in the myocardium and the degree of contracture was demonstrated previously [4]. The effect of aspartic acid on the concentrations of high-energy phosphates was similar but weaker, so that the nature of action of the amino acids used can be considered to be similar. To study the mechanism of the protective effect of amino acids, the concentration of metabolites of nitrogen metabolism directly connected with them was studied.

Reduction of the coronary flow caused characteristic changes in amino-acid and ammonia metabolism, reflecting inhibition of oxidative phosphorylation and stimulation of anaerobic metabolism. Evidence of this was given by the accumulation of alanine in the myocardial tissue, and a decrease in the combined content of aspartic and glutamic acids, with a simultaneous increase in ammonia formation. Addition of glutamic and aspartic acids abolished the deficit of the tissue concentration of these amino acids (Table 2). The increase in their concentration when the ATP level was raised led to intensification of energy-dependent binding of ammonia, with the formation of glutamine, asparagine, and urea. However, the level of ammonia ions in the tissue remained high, despite the increase in the concentration of bound forms of ammonia. The alanine level fell to its initial value during perfusion with the amino acids (Table 2).

Since the metabolism of glutamic and aspartic acids is closely linked with the formation of α-ketoglutarate and oxaloacetate, an increase in the tissue concentrations of these amino acids could facilitate maintenance of the function of the tricarboxylic acids and of

the malate-aspartate shunt, which is depressed in ischemia [5]. Evidence of activation of transamination in the mechanism of improvement of the function of the ischemic heart is given by disappearance of this effect under the influence of hydroxyaminoacetic acid, an inhibitor of cytoplasmic and mitochondrical transaminases [3]. Under conditions of ischemia amino acids thus improve contractile function by maintaining the natural compensatory reactions of energy metabolism through preservation of amino-acid pools and subsequent activation of oxidative and substrate phosphorylation in the mitochondria, and not by lowering the ammonia level.

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EFFECT OF AN ANTIOXIDANT ON RESISTANCE OF THE UNTRAINED ANIMAL TO MAXIMAL PHYSICAL EXERCISE

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UDC 616.127-008.931-02:613.863

KEY WORDS: antioxidant; physical exercise.

During maximal physical exertion by untrained animals and humans the ATP and creatine phosphate concentrations in the skeletal muscles fall regularly, glycolysis is activated, the lactate concentration in the blood rises considerably, and the pH of the plasma falls [1, 9]. One possible cause of the insufficient power of the system for aerobic resynthesis of ATP, which is the basis of the changes mentioned above, is damage to cellular and, in particular, mitochondrical membranes. Such injuries have in fact been demonstrated after maximal physical exertion and are manifested as the appearance of enzymes in the blood stream, due to increased liberation of cytosol enzymes through the plasma membrane into the blood plasma, and also to destruction of the outer membrane and cristae of mitochondria [2]. At least two possible causes of the disturbance of the membrane mechanisms of oxidative phosphorylation during extremal physical exertion are now known. The first is that, as a result of hypoxemia and tissue hypoxia, lipid peroxidation (LPA) and phospholipases in the mitochondria [6] are activated, so that the membrane becomes permeable for protons and, in accordance with the chemo-osmosis theory, it disturbs oxidative phosphorylation. The second cause is a fall in pH which, as has recently been shown, through the same mechanisms of activation of phospholipases and LP, switched oxidation of NADH to the so-called pathway, and thereby substantially reduces the efficiency of utilization of substrates and oxygen for ATP synthesis [8]. This means that one way of increasing the efficiency of oxidative phosphorylation during extremal physical exertion and increasing resistance to such loads is by the rational use of inhibitors of LPO and phospholipases.

The object of the present investigation was to study the effect of preliminary administration of antioxidant M-1, an inhibitor of peroxidation, on the maximal duration of standard physical exertion by untrained animals, on their blood enzyme levels, and on the blood lactate concentration.

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(Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 17-19, July, 1982. Original article submitted January 13, 1982.

0007-4888/82/9407-0869$07.50