CORRECTION OF CONTRACTILE FUNCTION AND METABOLISM OF THE ISCHEMIC DOG MYOCARDIUM WITH EXOGENOUS GLUTAMIC ACID

O. I. Pisarenko, E. B. Novikova,
L. I. Serebryakova, O. V. Tskitishvili,
V. É. Ivanov, and I. M. Studneva

UDC 616.127-005.4-085.272.6:547.466.64[-07:]616.127-009.1+616.127-008.9

KEY WORDS: myocardial ischemia; reperfusion; glutamic acid.

One approach to the study of the problem of maintaining cardiac function when the blood supply to the heart is inadequate and during its restoration is to attempt to influence energy formation in the myocardium. It has been shown that glutamic acid (GA) can help to maintain the pool of high-energy phosphates [5] and to reduce disturbances of cardiac function [1, 6] when the oxygen supply to the myocardium is limited. We also know that utilization of GA by the human heart is increased in chronic myocardial ischemia [4]. However, many aspects of the action of GA remain unexplained.

The aim of this investigation was to study the action of GA on function of the dog's heart during temporary ischemia in the intact organism, and changes arising under these circumstances in the coronary arteriovenous difference of various compounds associated with its metabolism in the myocardium arising under these circumstances.

EXPERIMENTAL METHOD

Experiments were carried out on 15 mongrel dogs of both sexes weighing 15-20 kg, under anesthesia (urethane 500 mg/kg, and chloralose 50 mg/kg, intraperitoneally). The chest was opened in the fourth intercostal space and the anterior descending and circumflex coronary arteries were dissected, and glass cannulas inserted into them for perfusion from the coronary artery of a donor dog. A catheter with inflatable cuff was introduced into the coronary sinus. The partial pressure of oxygen (pO_2), pH of the arterial blood, and animal's temperature were kept within physiological limits. GA (1% solution), neutralized with NaOH to pH 7.4, was injected intravenously by means of a peristaltic pump at the rate of 3 mg/kg/min. The coronary blood flow was recorded with an electromagnetic flowmeter (Biotronix, USA), and the coronary perfusion pressure and end-diastolic and systolic pressures in the left ventricle were recorded with electromanometers, and the first derivative of the intraventricular pressure was determined with a Contractility Calculator (Siemens-Elema, West Germany). An eight-channel automatic writer (Mingograph-804, Siemens-Elema) was used for recording. Values of pO_2 and pH in samples of arterial and coronary venous blood were determined with a blood gas analyzer (Radiometer, Denmark), and hemoglobin was determined by means of a Sicca-Hemometer (Denmark). Concentrations of GA, alanine, glutamine, and ammonia in protein-free plasma extracts were determined with a Liquimat III amino-acid analyzer (Labotron, West Germany). To determine lactate and pyruvate, standard enzymatic methods were used [4]. Blood samples were taken during adequate perfusion of the coronary arteries, at the end of the 2nd minute of restriction of the coronary blood flow, and at the end of the 2nd minute of reperfusion, before and during administration of GA. Hemodynamic parameters were recorded continuously. In the control, physiological saline was injected instead of GA. After the experiment, the perfused weight of the myocardium was determined. The experimental results were subjected to statistical analysis by Student's t test for paired observations.

EXPERIMENTAL RESULTS

Reduction of the coronary blood flow on average by 58% caused weakening of cardiac contractility after only 2-3 sec. Toward the end of the 2nd minute of ischemia the systolic

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR E. I.Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 280-282, September, 1985. Original article submitted February 3, 1984.

0007-4888/85/0009-1173$09.50

TABLE 1. Concentrations of Ammonia, Glutamine, and Alanine in Arterial and Venous Coronary Blood (M ± m)

Substance	Experimental conditions	GA	Arterial blood, μM	Venous coronary blood, μM	Uptake, %
Ammonia	Normal	—	13,3±0,4	13,0±0,8	2,9±1,2
		+	12,5±0,3	12,3±0,5	1,6±0,8
	Ischemia	—	13,5±0,2	14,7±0,6	—8,6±2,8+
		+	12,6±0,6	11,3±0,4*+	1,1±1,4+
	Reperfusion	—	12,2±0,4	12,4±0,6	—1,7±0,8
		+*	10,7±0,3*	10,3±0,2*+	3,6±1,3+
Glutamine	Normal	—	571,7±15,8	566,1±17,4	0,8±0,1
		+	649,7±21,4	647,8±23,5	0,2±0,1*
	Ischemia	—	560,5±11,1	563,8±13,2	—0,6±0,1*
		+	643,2±12,0*	666,8±18,7*+	—1,8±0,2*+
	Reperfusion	—	547,8±12,7	546,1±14,9	0,3±0,1*
		+*	648,0±20,5*	646,9±18,6	0,1±0,1*
Alanine	Normal	—	338,9±18,5	331,2±17,1	2,5±0,4
		+	360,7±22,0	361,8±19,8	0,5±0,9
	Ischemia	—	373,2±12,6	376,1±14,0	—1,4±0,3*
		+	413,2±17,6*	453,3±18,1	—10,9±1,8*+
	Reperfusion	—	342,3±14,0	339,3±18,6	0,5±0,3*
		+*	423,8±24,3*	420,5±20,4	0,8±0,6

Legend. Values shown were obtained in series of 8-10 experiments. Uptake equals $(A - V/A) \times 100\%$, where A denotes concentration in arterial blood, and V concentration in coronary venous blood. Asterisk indicates significant difference from normal without administration of GA ($P < 0.05$), plus sign significant difference from corresponding parameter during ischemia and reperfusion without administration of GA ($P < 0.05$).

pressure in the left ventricle was reduced by 22%, and the parameter dp/dt by 29% (Fig. 1). The product of systolic pressure and heart rate (double product) was reduced by 31%. The end-diastolic pressure was increased from 2.0 ± 0.5 to 6.1 ± 0.9 mm Hg. The oxygen consumption of the left ventricular myocardium was reduced from 8.1 ± 0.6 to 3.4 ± 0.8 ml/min/100 g. After restoration of the blood flow all parameters gradually returned to their original values. At the 6th minute of reperfusion the systolic pressure in the left ventricle was 9% lower, and dp/dt was 17% lower than initially (Fig. 1). The end-diastolic pressure and myocardial oxygen consumption returned to normal by this time.

Reduction of the coronary blood flow against the background of injection of GA caused significantly less inhibition of the contractile function of the heart. For instance, the systolic pressure in the left ventricle at the 2nd minute of ischemia had fallen by 9% instead of 22%, and dp/dt by 16% compared with 29% (Fig. 1). No significant differences were observed in changes in the end-diastolic pressure, which rose from 2.6 ± 0.6 to 5.2 ± 1.0 mm Hg. The myocardial oxygen consumption in the period of ischemia was reduced, just as during ischemia induced without injection of GA. However, the double product was reduced significantly less during ischemia when accompanied by GA injection, by 16% compared with 31% (Fig. 2). Thus the higher contractile activity of the left ventricle during ischemia when GA was administered occurred without any change in oxygen consumption.

Injection of GA also was reflected in recovery of cardiac contractility during reperfusion. At the 6th minute of reperfusion the systolic pressure in the left ventricle and dp/dt returned to their original values, whereas during the same period, but without injection of GA, these parameters were 10% and 18%, respectively, lower than initially (Fig. 1). The oxygen consumption of the heart during reperfusion was the same in the presence or absence of GA. Administration of GA thus improved the contractile function of the myocardium during ischemia and contributed to its more rapid and complete recovery during reperfusion without any additional utilization of oxygen.

Turnover of GA and Its Metabolites. After administration of GA its concentration in the plasma quickly reached a constant level, 6 times higher than initially after 5 min. Its percentage uptake was unchanged at 7.7 ± 1.6. During ischemia uptake diminished, although it still remained twice as high as during ischemia without GA administration (4.6 ± 0.9 and 2.1 ± 0.6%, respectively). Reperfusion did not affect GA uptake. Ischemia caused excretion of ammonia from the heart, followed by uptake of ammonia in the presence of GA, which normally did not affect the ammonia concentration in arterial and venous coronary blood (Table 1). In response to the increased ammonia formation during ischemia, glutamine synthesis in the myocardium also increased. GA stimulated this process and increased glutamine excretion from the

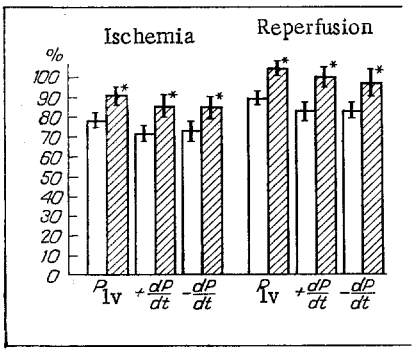

Fig. 1

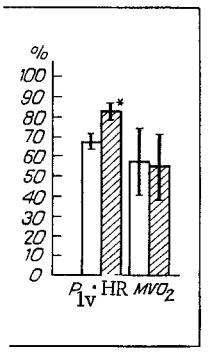

Fig. 2

Fig. 1. Effect of GA on parameters of left ventricular function during ischemia (A) and reperfusion (B). P_{1V}) Systolic pressure in left ventricle; dp/dt) maximal rate of rise (+) or fall (—) of pressure in left ventricle. Values expressed as percentages of normal (adequate perfusion of coronary arteries). Unshaded columns — without GA, shaded columns — with GA. Asterisk indicates significant difference from data obtained without GA ($P < 0.05$).

Fig. 2. Effect of GA on double product and oxygen consumption of left ventricular myocardium during ischemia. P_{1V}•HR) Product of systolic pressure in left ventricle and heart rate; MVO_2) myocardial oxygen consumption. Remainder of legend as to Fig. 1.

heart threefold. The most significant changes were found in alanine turnover. Whereas normally the heart assimilated very small amounts of alanine, during ischemia it was excreted. GA stimulated its formation even under normal conditions, but during ischemia it increased its excretion more than threefold. During reperfusion, GA did not affect alanine uptake (Table 1).

Lactate assimilation was unchanged by GA both under normal conditions and during reperfusion, but in the period of ischemia its elimination from the heart fell significantly (from -3.45 ± 1.87 to -1.4 ± 0.92%). Under normal conditions pyruvate uptake by the heart was independent of GA administration and it averaged 11%. During ischemia pyruvate uptake fell to 3%, and during GA administration it doubled (to 6.4 ± 1.1%). However, the lactate/pyruvate ratio in blood from the coronary sinus was unchanged during ischemia in the presence of GA, on account of a tendency for uptake of both these substances by the myocardium to be increased simultaneously.

The most important result of the investigation was the discovery of the normalizing action of GA on contractility of the heart during ischemia and reperfusion of the myocardium in anesthetized dogs. The highest level of contractility was achieved under these circumstances without the use of additional oxygen. Data obtained previously related to the effect of GA on the myocardium of the isolated heart or on fragments of myocardium in the presence of oxygen deficiency [6, 9]. The existing data suggest that exogenous GA, on entering cells of the ischemic myocardium, abolishes the glutamate deficiency and thereby maintains the course of compensatory energy-forming reactions [1, 3, 5, 6]. Coupling of GA metabolism with glycolysis and with reactions of the Krebs' cycles is effected by alanine- and aspartate-aminotransferases, for the protective action of GA is abolished by aminohydroxyacetic acid, and inhibitor of transaminase [6]. The increase in alanine formation and pyruvate utilization during ischemia under the influence of GA indicates that alanine aminotransferase participates in the chain of glutamate conversion. Alanine formation from pyruvate ought to reduce the concentration of the latter that is converted into lactate, and that could contribute to the reduction of tissue acidosis, i.e., could prevent inhibition of phosphofructokinase and the fall in the rate of glycolysis [7]. In the present experiments a tendency was noted for lactate production to fall and for the pH of the coronary venous blood to rise during ischemia under the influence of GA. The possibility cannot be ruled out that substrate phosphorylation in mitochondria, activated by GA, may also be an important mechanism maintaining contractile function during ischemia [8, 10]. It is evidently the improvement in the energy supply to the myocardium under the influence of GA that makes ATP-dependent synthesis of glutamine possible in ischemia, which is associated with reduced elimination of ammonia from the heart, which must be accomp-

anied by a corresponding fall in its concentration in the myocardial tissue. This must also have a beneficial effect on myocardial function, because an excess of ammonia can block several mitochondrial processes [2].

LITERATURE CITED

1. J. Bittl and K. L. Shine, Am. J. Physiol., 245, H406 (1983).
2. N. Katunuma, M. Okada, and Y. Nishi, Adv. Enzym. Reg., 4, 317 (1966).
3. H. L. Lazar, G. D. Buckberg, A. J. Mangarano, et al., J. Thorac. Cardiovasc. Surg., 80, 350 (1980).
4. G. H. Mudge, R. H. Mills, H. Taegtmeyer, et al., J. Clin. Invest., 58, 1185 (1976).
5. O. L. Pisarenko, E. S. Solomatina, I. M. Studneva, et al., J. Mol. Cell. Cardiol., 15, 53 (1983).
6. E. E. Rau, K. I. Shine, A. Gervais, et al., Am. J. Physiol., 236, 4873 (1979).
7. J. Rovetto, J. T. Whitmer, and J. R. Neely, Circ. Res., 32, 699 (1973).
8. T. Sanborn, W. Gavin, S. Bercovitz, et al., Am. J. Physiol., 237, 535 (1979).
9. H. G. Shertzer and J. Cascarano, J. Exp. Zool., 207, 337 (1979).
10. H. Taegtmeyer, M. B. Peterson, V. V. Ragavan, et al., J. Biol. Chem., 252, 5010 (1977).

HEMODYNAMIC CHANGES IN IMMOBILIZATION STRESS

L. S. Ul'yaninskii, O. S. Medvedev, A. M. Bunyatyan, D. D. Matsievskii, and N. I. Rozhanskaya

UDC 613.863-02:612.766.2]-07:616.1-008.1-092.9

KEY WORDS: hemodynamics; immobilization stress.

Hemodynamic disturbances in emotional stress may be due mainly to changes in cardiac output (CO) [7, 8] and in total peripheral resistance (TPR) [3, 10, 11]. Marked disturbances of regulation of blood pressure (BP), possibly leading to death of the animals, have been found [5, 9] in rats with immobilization stress (IS). However, the hemodynamic mechanisms of these disturbances have not yet been studied.

Changes in hemodynamic parameters of rats differing in resistance to IS and correlation between cardiac and vascular components of the hemodynamics during development of the terminal state in the course of immobilization were studied in the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on 75 rats (Wistar, August, and noninbred, 25 of each). A model of IS was used (immobilization of the animals in a confined chamber for 30 h). BP was recorded in all experiments by means of a catheter introduced into the abdominal aorta through the caudal artery by the method in [4], and the ECG was recorded in standard lead II. As a first step, ultrasonic blood flow transducers were implanted on the ascending aorta of 20 of these animals (Wistar and August). By using an ultrasonic measuring technique it was possible to measure the linear velocity of the blood flow in the ascending aorta (VBA), and the stroke volume (SV) and CO could be determined by the use of electronic integrators [1, 2]. TPR also was calculated.

EXPERIMENTAL RESULTS

In unrestrained rats at rest, no differences in hemodynamic parameters could be found between the three different lines. The mean BP was 117.5 ± 3.5 mm Hg, the heart rate (HR)

Laboratory of Experimental Cardiology, P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR. Laboratory of Experimental Pharmacology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Bioengineering Laboratory, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 282-285, September, 1985. Original article submitted August 17, 1984.

0007-4888/85/0009-1176$09.50