DIFFERENCES IN REACTIONS OF THE RAT AND GUINEA PIG HEART TO ISCHEMIA AND REPERFUSION

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UDC 616.12-005.4-036.82-092.9-092

KEY WORDS: ischemia of the heart; contracture of the myocardium; extracellular K^+ and pH during ischemia.

Total normothermic ischemia of the rat heart is accompanied by loss of contractility and the development of myocardial contracture, which is expressed as an increase in the diastolic pressure in the isovolumic ventricle. The rate of development of the contracture and its maximal value under the influence of different factors depend on the degree of fall of the ATP concentration [6] and, other conditions being the same, they determine the degree of recovery of contractile function during reperfusion. Meanwhile the guinea pig heart, despite the absence of contracture during ischemia for 10 min [9], is characterized by severe arrhythmia during reperfusion.

To explain the role of factors determining the dynamics of ischemic contracture and its importance, the response of the rat and guinea pig hearts to ischemia and reperfusion was compared under identical conditions. Besides measurement of the isovolumic pressure, the time course of accumulation of K^+ and H^+ ions in the extracellular space, which reflects the degree of disturbance of energy formation in ischemia [3, 7, 10], also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on the hearts of rats and guinea pigs weighing 200-300 g, anesthetized with urethane (1.6 g/kg, intraperitoneally). The isolated hearts were perfused through the aorta at a temperature of 37°C and at the rate of 10 ml/min/g with Krebs' solution (pH 7.33 ± 0.02), saturated with carbogen. A small latex balloon with constant volume was introduced into the left ventricle. The pressure in the isovolumic balloon, reflecting tension in the fibers of the ventricle, was measured with a Gould Statham P23Db electromanometer and recorded on Gould Statham SP1405 or Gould Brush 2400 instruments. To measure extracellular K^+ activity, a combined electrode with K^+-selective valinomycin membrane [8] was inserted into the wall of the left ventricle to a depth of about 1 mm. Potentials were measured with an F223A electrometer (WPI) and recorded on a "Linear" automatic writer. The extracellular pH was measured by a combined electrode about 4 mm in diameter, introduced into the chamber of the right ventricle (OP-211/1 Radelkis pH-meter). Total ischemia of the isolated hearts was produced by stopping perfusion, and during the period of ischemia (15 min) the air temperature in the chamber was kept constant (36°C).

EXPERIMENTAL RESULTS

In the experiments of series I the effect of ischemia and reperfusion was studied during contractions at the spontaneous frequency, which was the same in rats and guinea pigs before ischemia (Table 1). During ischemia the fall of frequency in guinea pigs took place twice as quickly as in rats (in 2.1 ± 0.1 and 3.2 ± 0.4 min, respectively, $P < 0.05$). Ischemia was accompanied by an early rise of diastolic pressure in the left ventricle of the rats: after 5 min it had increased by 6 ± 2 mm Hg, and after 15 min of ischemia by 22 ± 5 mm Hg. In the experiments on guinea pigs' hearts the rise of diastolic pressure was not significant, even after 15 min of ischemia.

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0007-4888/86/0004-0423$12.50

TABLE 1. Parameters of Contractile Function of Rats' (n = 11) and Guinea Pigs' (n = 9) Hearts Before and After Ischemia

Parameter	Group of animals	Before ischemia	After ischemia	
			15 min	30 min
Heat rate, beats/min	Rats	231±11	210±13	236±12
	Guinea pigs	235±8	226±7	236±10
Developed pressure, mm Hg/sec	Rats	92±5	109±5*	101±6
	Guinea pigs	77±4	52±4**	65±5
Maximal rate of rise of pressure, mm Hg/sec	Rats	1900±350	2120±400	2180±420
	Guinea pigs	1380±120	900±100*	1210±110
Maximal rate of fall of pressure, mm Hg/sec	Rats	1070±160	1300±190	1270±200
	Guinea pigs	1350±160	840±100*	1090±100

Legend. *P < 0.05, **P < 0.01 compared with initial value.

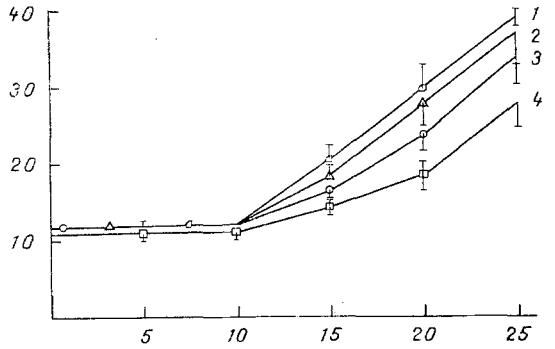

Fig. 1. Dynamics of diastolic pressure in isovolumic rat heart before ischemia and during 15 min of total ischemia, at 36°C, and during electrical stimulation of the heart at different frequencies. Abscissa, time (in min); ordinate, diastolic pressure (in mm Hg). Frequency of electrical stimulation: 1) 8 Hz, 2) 6 Hz, 3) 4 Hz, 4) 2 Hz. Results of 5-7 experiments given in each group (M ± m).

In the animals of both groups the beginning of reperfusion was associated with the onset of arrhythmia. In six of nine experiments on guinea pigs' hearts fibrillation developed, and lasted on average 110 ± 43 sec. Fibrillation was not observed in experiments on rats' hearts. Restoration of a regular rhythm took place earlier in rats (after 4.6 ± 1.2 min) than in guinea pigs (after 17.2 ± 3.2 min of reperfusion, P < 0.01); the heart rate in both groups of experiments returned virtually to its initial value after 15 min. The contractile function of the rats' heart also was restored sooner and more completely (Table 1). After this same duration of ischemia virtually complete recovery of the force of contractions of the isolated papillary muscles of the rats was observed [2].

The development of ischemic contracture in the rats' hearts could be connected with the slower lowering of the heart rate. In this connection, experiments of series II were performed on rats' hearts, whose frequency of contractions before and during ischemia was controlled by means of an electrical stimulator at frequencies of between 2 and 8 Hz. A frequency of 2 Hz was imposed against the background of atrioventricular blockade. It will be clear from Fig. 1 that the rate of development of contracture was maximal during stimulation with a frequency of 6-8 Hz and minimal at a frequency of 2 Hz. The original intensity of the contractile function (the product of developed pressure and heart rate) at a frequency of stimulation of 6-8 Hz was greater than at 2 Hz, even though the developed pressure in the latter case was approximately twice as great. After 5 min of complete ischemia, differences in the intensity of the contractile function at frequencies of stimulation of 2 and 6 Hz were still present, for the developed pressure was reduced about equally (tenfold). The reduction of the developed pressure to zero took place faster at a frequency of stimulation of 6-8 Hz (after 7-8 min of ischemia) than at 4 Hz (after 13 min) or with 2 Hz (16th minute of ischemia).

Consequently, the higher intensity of contractile function during the first 5 min of ischemia at a high frequency of stimulation was accompanied by a faster rate of increase of con-

TABLE 2. Changes in Diastolic Pressure and in K^+ Concentration and pH of Extracellular Medium of Rats' Hearts (n = 5) and Guinea Pigs' Hearts (n = 5) during Ischemia and Electrical Stimulation with a Frequency of 4 Hz

Parameter	Group of animals	Initial value	Ischemia	
			5 min	15 min
Diastolic pressure, mm Hg	Rats	13±1	18±1	48±3
	Guinea pigs	12±1	11±1**	12±1**
K^+ concentration, mM	Rats	6,0	8,2±0,2	9,4±0,3
	Guinea pigs	6,0	10,5±0,6*	13,6±0,8**
pH	Rats	7,24±0,05	6,71±0,06	6,41±0,04**
	Guinea pigs	7,22±0,03	6,59±0,04	6,25±0,04*

Legend. *P < 0.05, **P < 0.001 compared with corresponding values for rats.

tracture. This means that the absence of ischemic contracture in the experiments of series I on guinea pigs' hearts could in fact be connected with the more rapid fall of the heart rate.

In the experiments of series III the contraction rate of the rats' and guinea pigs' hearts was therefore controlled at 4 Hz by means of an electrostimulator, and the dynamics of K^+ and H^+ accumulation in the extracellular space during ischemia was studied at the same time. Before ischemia the systolic pressure in the rats and guinea pigs was 80 ± 2 and 82 ± 2 mm Hg, respectively, whereas during ischemia it fell to zero after 11 min in the experiments on rats' hearts and after 7 min in the experiments on guinea pigs' hearts. The diastolic pressure in the guinea pigs' hearts was unchanged during ischemia, whereas in the rats' hearts it rose by 35 ± 3 mm Hg (Table 2).

Changes in developed and diastolic pressure were thus similar in principle to those in the experiments of series I. They show that the more rapid decrease in intensity of contractile function in the initial period of ischemia was combined with the development of a smaller contracture. This is in agreement with data showing a decrease in the degree of contracture and in the degree of lowering of the ATP concentration, if the intensity of contractile function before or in the initial period of ischemia was reduced under the influence of inhibitors of Ca^{++} inflow and of hypocalcium, hyperpotassium, or hypermagnesium solution [4, 6]. A situation of this kind is characteristic of guinea pigs' hearts: Contracture did not appear during 10 min of ischemia [9] or during 15 min in the present experiments.

The lowering of the frequency and force of contractions while the ATP concentration remains relatively high may take place as a result of intracellular acidosis and accumulation of extracellular K^+ [1, 7, 10]. As a result, the membrane potential was lowered and the maximal rate of depolarization and the duration of the action potential were reduced [8]. In the present experiments accumulation of K^+ in the extracellular space of the guinea pigs' hearts took place about twice as quickly as in rats, during the first minutes of ischemia, and after 15 min the difference with respect to K^+ was combined with higher extracellular acidosis (Table 2). On a similar object, and with ischemia of the same duration, an increase in extracellular K^+ activity to the same level as in the present experiments was combined with lowering of the transmembrane potential to −46 mV [8]. Great accumulation of K^+ and changes in transmembrane potential of the myocardium connected with it in guinea pigs may therefore be one cause of the more rapid decline of contractile function, of delayed ATP consumption, and weakening of contracture during ischemia.

Abolition of this protective mechanism at the beginning of reperfusion, when K^+ and H^+ ions accumulated in the extracellular space are quickly removed [1], is accompanied by increased penetration of Ca^{++} into the cells [4]. Under these conditions the longer action potential and other differences in the Ca^{++} transport system in the myocardial cells of guinea pigs probably contribute to their greater overloading with Ca^{++} ions and the onset of fibrillation. This means that absence of ischemic contracture is still no guarantee of better recovery of function during reperfusion.

LITERATURE CITED

1. V. I. Kapel'ko, V. N. Titov, N. A. Novikova, et al., Kardiologya, No. 3, 14 (1983).
2. M. R. Mukumov, Yu. S. Lyakhovich, S. A. Isaeva, et al., Patol. Fiziol., No. 5, 27 (1984).
3. L. N. Cherkashchenko, A. Ya. Shargorodskaya, and V. F. Portnoi, Patol. Fiziol., No. 1, 28 (1980).
4. M. M. Bersohn and K. I. Shine, J. Mol. Cell. Cardiol., 15, 659 (1983).
5. S. C. Dennis, D. M. Jellon, F. Frasch, et al., Int. J. Cardiol., 2, 461 (1983).
6. D. J. Hearse, P. B. Garlick, and S. M. Humphrey, Am. J. Cardiol., 39, 986 (1977).
7. H. J. Hirche, C. Franz, L. Bös, et al., J. Mol. Cell. Cardiol., 12, 579 (1980).
8. A. G. Kleber, Circ. Res., 52, 442 (1983).
9. H. Narita, N. Taku, M. Sato, et al., Jpn., Heart J., 24, 277 (1983).
10. J. Weiss and K. I. Shine, Am. J. Physiol., 12, 318 (1982).

STATE OF LYMPHOPOIESIS IN MICE WITH ALLOXAN DIABETES

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UDC 616.379-008.64-092.9-07:616.155.32-007.1

KEY WORDS: alloxan diabetes; lymphopoiesis.

Diabetes mellitus is one of the commonest diseases of the endocrine system in man. Among the many structural and functional disturbances observed in this diseases, changes in the immunity system are not the least important [1, 5-7]. Lowering of the resistance of the body to infectious diseases, and the associated development of angiopathies [3], etc. are a characteristic feature of diabetes [9]. Experimental observations have shown that lymphopoiesis may be disturbed in this disease. For instance, marked inhibition of lymphophoiesis has been found in the thymus of pancreatectomized rats [8] and of mice with alloxan diabetes [4].

Accordingly, in the investigation described below, the state of lymphopoiesis was studied in animals with alloxan diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 20-25 g, in some of which alloxan diabetes was induced by a single subcutaneous injection of a 4% solution of alloxan hydrate (from Lachema-Chemapol, Czechoslovakia), in a dose of 400 mg/kg on an empty stomach. The group of diabetic animals consisted of mice in which the blood sugar concentration determined by the orthotoluidin method standardized in the USSR, when tested twice on the 3rd and 14th days after injection of alloxan, was not below 14 mM (250 mg%). To rule out any possible toxic effect of alloxan itself on lymphopoiesis, a control group of 13 mice was formed, into which the compound was injected in the same dose as into the diabetic mice, but these mice did not develop diabetes because of their individual resistance. The blood sugar concentration in these animals on the 14th day after injection of alloxan did not differ significantly from that in intact healthy mice.

The total leukocyte count, the absolute and relative lymphocyte counts, and the number of T-, B-, and O-cells separately, identified by the cytochemical reaction for acid phosphatase, detected by the method of Goldberg and Barka [2], were determined in the peripheral blood of all the mice 2 weeks after injection of alloxan. After blood analysis, all the animals were given an intraperitoneal injection of ^{3}H-thymidine (All-Union "Izotop" Combine, specific activity 925 GBq/mmole) in a dose of 40 MBq/kg, 1 h before the animals were killed by cervical dislocation. The weight of the lymphoid organs, their relative weights, and their cell composition, and the number of myelokaryocytes in the femoral diaphysis were determined

Department of Pathophysiology, Tomsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Vasil'ev.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 101, No. 4, pp. 407-409, April, 1986. Original article submitted January 25, 1985.

 0007-4888/86/0004-0426$12.50