CARDIOPROTECTIVE ACTION OF FRUCTOSE-1,6-DIPHOSPHATE

L. N. Sernov, Yu. B. Rozonov, O. A. Sokolova, T. V. Shal'neva, G. V. Snegireva, and V. V. Gatsura

UDC 615.22:547.455.632].015.4.076.9

KEY WORDS: fructose-1,6-diphosphate; mechanism of action; myocardial infarction; metabolic acidosis

The positive action of phosphorylated hexoses in myocardial ischemia has been demonstrated by several investigations [3, 5, 9]. Of these compounds, the one which is most highly justified theoretically is fructose-1,6-diphosphate (FDP), an intermediate in the Embden—Meyerhof cycle, which enables two ATP molecules, utilized in the initial stages of glycolysis, to be spared, thus increasing the potential for glycolytic energy production.

In the investigation described below the antiischemic action of FDP was studied.

EXPERIMENTAL METHOD

In experiments on rats weighing 250-350 g, anesthetized with pentobarbital sodium (40 mg/kg body weight), using a differential indicator method [6] the effect of FDP on the size of the zones of ischemia and necrosis of the myocardium was studied 4 h after ligation of the descending branch of the left coronary artery at the level of the lower border of the auricle of the atrium. In experiments on conscious male Chinchila rabbits, with an occluder implanted beforehand on the coronary artery, the effect of FDP on the threshold of myocardial ischemia was assessed. This effect was manifested as the appearance of signs of ischemia on the precordial ECG, during gradual and measured occlusion of the coronary artery [4]. In the experiments on rabbits FDP was injected intravenously, slowly, in a dose of 100 mg/kg, after establishment of the stable threshold of ischemia, whereas in the experiments on rats it was injected in doses of 100 and 300 mg/kg (25% of the dose was injected quickly after ligation of the coronary artery, the remaining 75% was injected slowly during 1 h, by means of a micropump). The efficacy of FDP under conditions of compensated and decompensated metabolic acidosis was evaluated on rat hearts, isolated by Langendorff's method and perfused with Krebs—Henseleit solution, and with an imposed frequency of contractions (200 beats/min). For this purpose, after the end of a 15-min period of adaptation, two different versions of experiments were carried out. In the first, subsequent perfusion was carried out with unoxygenated Krebs—Henseleit solution, whose high buffer capacity compensated the developing metabolic acidosis. In the second variant of the experiments, unoxygenated "buffer-free" Krebs—Henseleit solution, in which the sodium bicarbonate was replaced by the equimolar concentration of sodium chloride, was used for perfusion. The formation of metabolic acidosis was established on the basis of measurements of the pH of the outflowing perfusion fluid, assuming constancy of the pH (7.4 units) of the "inflowing" solution. The criterion of myocardial function was the duration of contractions of the isolated heart with an amplitude recorded by an isometric transducer, which was not less than 10% of its initial value. Concentrations of NADH, ATP, and glucose-6-phosphate in the myocardium and concentrations of lactate, pyruvate, and glucose in perfusion fluid flowing from the isolated heart were determined by enzymic methods. In each series of experiments there were at least five observations.

EXPERIMENTAL RESULTS

The experiments showed (Fig. 1) that FDP, in a dose of 100 mg/kg, caused a decrease in size of the zone of necrosis after liquation of the coronary artery in the rats. For instance, whereas in the control series of experiments the zone of necrosis

Laboratory of Cardiovascular Agents, All-Union Research Center for Safety of Biologically Active Substances, Staraya Kupavna, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Napalkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 172-173, February, 1991. Original article submitted April 18, 1990.

 0007-4888/91/0002-0204$12.50

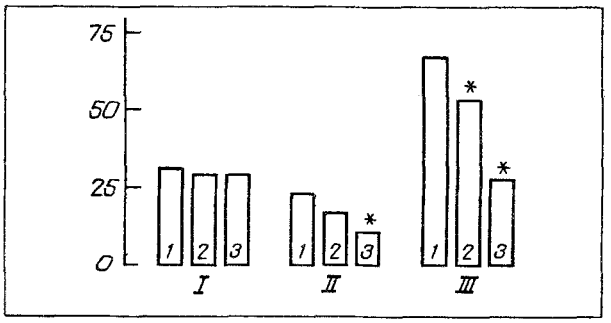

Fig. 1. Effect of fructose-1,6-diphosphate (FDP) on size of zone of ischemia and zone of necrosis 4 h after occlusion of coronary artery in rats. I) Zone of ischemia (in per cent of total mass of myocardium); II) zone of necrosis (in per cent of total mass of myocardium); III) zone of necrosis (in per cent of zone of ischemia). 1) Control; 2) FDP (100 mg/kg); 3) FDP (300 mg/kg). Asterisk indicates significant differences from control at $p < 0.05$ level.

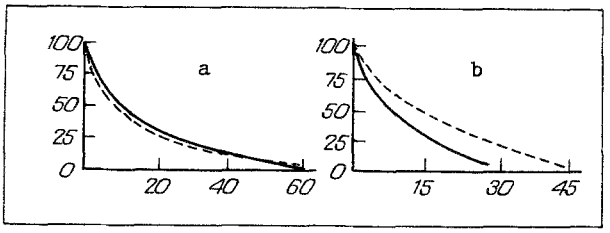

Fig. 2. Effect of fructose-1,6-diphosphate (FDP, 10^{-4} M) on amplitude of contractions of isolated hypoxic rat heart. a) Compensated acidosis, b) decompensated acidosis. Abscissa, time from beginning of experiment (in min); ordinate, amplitude of contractions (in per cent of initial value). Continuous line — control, broken line — FDP.

after 4 h amounted to 68 ± 4.3% of the zone of ischemia, against the background of FDP this was reduced to 54 ± 5.0%. With an increase in the dose up to 300 mg/kg, the cardioprotector action of FDP was intensified, as was shown by a decrease in the zone of necrosis to 28 ± 5.5% of the zone of ischemia.

The presence of a cardioprotector action of FDP in acute myocardial infarction served as the basis for assessment of its prophylactic antiischemic effect. However, experiments on rabbits showed that FDP had no effect in any of the six experiments on the threshold of myocardial ischemia. In all experiments the degree of constriction of the coronary artery at which electrocardiographic signs of ischemia appeared remained the same before and after injection of FDP.

Analysis of the results indicates that the positive effect of FDP is observed only under the conditions of ischemic heart damage. It can accordingly be postulated that the appearance of the cardioprotective action of FDP is limited by the degree of permeability of the cardiomyocyte sarcolemma, which is increased in acute myocardial ischemia. One of the leading causes of the increased permeability of the cell membranes in myocardial infarction is the developing regional metabolic acidosis [1, 7, 8], which penetration of FDP inside the cell.

The validity of this hypothesis is confirmed by the results of experiments on the isolated rat heart (Fig. 2). Investigations showed that in compensated metabolic acidosis FDP did not affect the functional activity of the hypoxic myocardium, but proved to be effective against decompensated metabolic acidosis, when it prolonged the period of functional activity from 27.0 ± 2.9 min in the control to 44.3 ± 2.4 min.

The mechanism of this phenomenon is evidently connected with incorporation of FDP into the glycolytic cycle, for biochemical investigations of the action of FDP revealed an increase in concentrations of ATP and glucose-6-phosphate in the myocardium, reduction of the degree of excess lactate in the outflowing perfusion fluid, and a decrease in the glucose consumption of the heart muscle (Table 1).

TABLE 1. Effect of Fructose-1,6-Diphosphate (FDP) (10^{-4} M) on Course of Metabolic Acidosis in Isolated Rat Heart

Experimental conditions	pH	Perfusion fluid			Myocardium	
		lactate, mmole/liter	pyruvate, mmole/liter	glucose, %	ATP, mmole/g	glucose-6-phosphate, mmole/g
A. 15 min of perfusion with oxygenated solution	7,40	0,500±0,050	1,448±0,19	100	2,13±0,23	0,098±0,016
B. 20 min of perfusion with "buffer-free" unoxygenated solution	6,82±0,04	0,823±0,100*	0,673±0,11*	−6,9±1,4	1,34±0,22*	0,299±0,056*
C. 20 min of perfusion with "buffer-free" unoxygenated solution containing FDP	7,04±0,04	0,663±0,270	0,150±0,11*°	+8,8±6,4	1,97±0,18*	0,35±0,120

Legend. Asterisk indicates significant differences from A, $p < 0.05$; circle indicates significant differences from B, $p < 0.05$.

It is interesting to note that reduction of the energy formation deficit was accompanied by an antiacidotic effect, i.e., FDP abolished to some degree the factor causing increased permeability of the cell membranes and its penetration inside the cell. The membrane-stabilizing effect FDP also is confirmed by our data showing an inhibitory action of FDP on lipid peroxidation in the ischemic myocardium and on its ability to reduce permeability of the tissue-blood barrier [2].

Thus FDP, in a certain sense, is a preparation with a selective type of action, the "target" for which consists of ischemic cardiomyocytes with a definite level of disturbance of cell membrane permeability. It must be pointed out that, with its antiacidotic action, and its ability to stimulate glycolytic energy production, FDP can restore normal permeability of the sarcolemma, and this may lead to termination of its subsequent entry inside the cell.

LITERATURE CITED

1. V. V. Gatsura and A. F. Sidorenko, Usp. Fiziol. Nauk, **17**, No. 9, 38 (1986).
2. T. A. Grimova and E. A. Gubarev, Recent Advances in Experimental Pharmacology of the Cardiovascular System [in Russian], Voronezh (1981), pp. 67-74.
3. V. V. Pichugin, S. A. Sumin, and V. V. Gatsura, Kardiologiya, **5**, 80 (1980).
4. Yu. B. Rozonov and T. V. Morozova, Byull. Éksp. Biol. Med., No. 10, 460 (1989).
5. L. N. Sernov, G. V. Snegireva, and V. V. Gatsura, Kardiologiya, **29**, No. 8, 66 (1989).
6. L. N. Sernov and V. V. Gatsura, Byull. Éksp. Biol. Med., No. 5, 534 (1989).
7. Y. Kagiyama, J. L. Hill, and L. S. Gettes, Circulat. Res., **51**, No. 5, 614 (1982).
8. P. A. Pool-Wilson, J. Molec. Cell Cardiol., **10**, 511 (1978).
9. P. Rossi, A. Guarraggi, and F. Anfossi, Minerva Cardioangiol., **20**, 22 (1972).