MYOCARDIAL PROTECTION BY PANTOTHENIC ACID DERIVATIVES IN HEART MODEL WITH EXPERIMENTAL ISCHEMIA AND REPERFUSION

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To prevent ischemic damage in clinical practice coronary infusions are used and are an effective method of protecting the myocardium against ischemia. With these aims, the preparations used must correct metabolic shifts caused by ischemia [1] and must prevent damage caused by reperfusion [6]. To study the mechanism of ischemic damage under adequate conditions and also to search for new therapeutic agents to protect the heart, a model of experimental ischemia of the isolated heart has been used [10]. The mechanism of the cardioprotective action of propranolol [12], etomoxir [11], hydralazine [13], etc., has been studied in this way. The cardioprotective action of panthetin, a pantothenic acid derivative, was demonstrated previously in the authors' laboratory [8, 15]. Since pantothenic acid deficiency is found in ischemia [2], it is interesting to study pantothenic acid derivatives as potential cardioprotectors and, in particular, to study the effect of pantothenic acid on reperfusion of the heart [14].

The aim of this investigation was to study pantothenic acid derivatives — panthetin (PT), panthenol (PL), and calcium pantothenate (CaPA) — on a model of experimental ischemia and reperfusion of the isolated heart.

METHODS

Experiments were carried out on male Wistar rats weighing 280-320 g under thiopental anesthesia. After removal the heart was cooled in perfusion solution at 0°C and cannulated through the aorta. Perfusion was carried out by Langendorff's method with Krebs—Henseleit solution, containing (mM): NaCl 128.2, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.1, $NaHCO_3$ 20, glucose 10. The solution was oxygenated with carbogen (95% O_2 + % CO_2). The perfusion fluid and chamber of the heart were kept at a constant temperature of 37°C. The heart was perfused under a hydrostatic pressure of 80 cm H_2O (pH 7.4). The experimental program was as follows: stabilization for 15 min, cardioplegia at 0°C for 1 min, ischemia for 30 min, reperfusion for 15 and 30 min. The heart functioned under physiological conditions without stimulation. During perfusion the pH, coronary flow rate (per minute), and ECG were recorded. After the experiment the heart was frozen in liquid nitrogen, using Wollenberger's forceps. Three pantothenic acid derivatives were tested: PT, PL, and CaPA. The preparations were injected intramuscularly and added to the perfusion fluid. Concentrations of lactate [5], ATP [3], CP [9], and conjugated dienes [7] were determined by the corresponding methods [5, 3, 9, 7].

RESULTS

During ischemia lasting 30 min, there was a tenfold increase in the lactate concentration in the myocardium, accompanied by a decrease in STP (16.55% of normal) and CP (22.95% of normal, Table 1). These changes are characteristic of total myocardial ischemia [10]. At the beginning of reperfusion the coronary effluent was acid (from

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TABLE 1. Biochemical Characteristics of Stages of Experimental Ischemia and Reperfusion of the Isolated Heart

Parameter	Stage			
	Stabilization 15 min	Ischemia 30 min	Reperfusion 15 min	Reperfusion 30 min
pH	7,38±0,06	6,95±0,03*	7,26±0,06	7,43±0,04
Coronary flow rate, ml/min	11,80±0,35	9,97±0,47*	10,90±0,62	9,99±0,49
Lactate, μmoles/g	14,22±1,10	22,33±1,20	11,92±0,65	10,11±0,59
ATP, μmoles/g	2,19±0,06	0,69±0,05	—	1,50±0,18
CP, μmoles/g	1,58±0,13	0,73±0,05	—	2,07±0,17
Conjugated dienes, nmoles/mg protein	0,88±0,01	0,84±0,09	1,94±0,35	1,75±1,26
n	11	12	12	10

Notes. Intact rats: ATP = 4.17 ± 0.15 μmoles/g, CP = 3.18 ± 0.34 μmoles/g. Asterisk — determined immediately after resumption of perfusion. pH: $p_{1,2} < 0.05$, $p_{2,4} < 0.05$. Lactate: $p_{1,2} < 0.05$, $p_{2,3} < 0.05$, $p_{2,4} < 0.05$. ATP: $p_{1,2} < 0.05$, $p_{2,3} < 0.05$. CP: $p_{1,2} < 0.05$, $p_{2,4} < 0.05$. Conjugated dienes: $p_{2,3} < 0.05$, $p_{3,4} < 0.05$.

TABLE 2. Effect of Panthetin in Vivo and in Vitro on Experimental Ischemia and Reperfusion of the Isolated Heart

Parameter	Parameter in vivo/in vitro	Ischemia				Reperfusion				
		+1x —	+2x —	+1x +	+2x +	— —	+1x —	+2x —	+1x +	+2x +
pH	7,38±0,06	7,41±0,03	7,37±0,02	7,38±0,03	7,38±0,02	7,01±0,04	7,04±0,08	7,16±0,05*	7,13±0,06*	7,45±0,09*
Coronary flow rate, ml/min	11,80±0,35	7,63±0,49*	7,48±0,54*	8,68±0,49	7,48±0,54*	9,70±0,86	7,80±0,71	8,23±0,75	6,09±0,29*	7,80±0,78
Lactate, μmoles/g	22,33±1,20	14,80±0,93*	12,88±0,96*	9,81±0,94*	10,4±0,92*	9,24±0,80	8,07±0,88	6,59±0,25*	6,08±0,58*	7,45±0,99
Conjugated dienes, moles/mg	2,60±0,30	3,25±0,33	3,81±0,29*	2,93±0,30	4,03±0,25	2,55±0,14	2,44±0,4	4,69±0,30	1,94±0,29*	5,37±0,61*
n	12	11	10	7	8	10	7	4	7	6

Notes. *p < 0.05. Panthetin concentration 25 mg/kg in vivo, 50 μM in vitro (in perfusion fluid).

TABLE 3. Effect of Panthetin, Panthenol, and Calcium Pantothenate on Energy Metabolism and LPO in Myocardium during Reperfusion for 30 min.

Preparation, mg/kg	P				
	pH	Coronary flow rate, ml/	Lactate, μmoles/g	Conjugated dienes, nmoles/mg protein	
Panthetin, 25	7,38±0,03	8,68±0,49	9,81±0,94	1,94±0,29	7
Panthenol, 4,5	7,38±0,03	8,90±0,43	9,72±0,88	1,61±0,13	6
» 9,0	7,23±0,03*	9,55±0,50*	2,85±0,44*	1,27±0,15*	6
» 12,5	7,37±0,04	9,00±0,40	3,96±0,61*	2,52±0,44*	6
Calcium pantothenate, 5.63	7,40±0,03	9,98±0,45	4,88±0,32*	—	6
» 11,25	7,36±0,03	8,94±0,40	4,12±0,27*	—	6
» 16,60	7,16±0,04*	9,06±0,42	2,40±0,31*	—	6

Notes. Concentration of panthetin in perfusion fluid 50 μM, concentration of calcium pantothenate in perfusion fluid 25 μM. *p < 0.05. Compared with reperfusion for 30 min without preparations.

7.38 to 6.95), and the coronary flow rate somewhat reduced. Reperfusion restored the cardiac rhythm without stimulation in the course of 1 min of the experiment. During reperfusion for 15 min the acidity of the coronary effluent was reduced (pH 7.26), the volume increased, and a significant increase in the concentration of conjugated dienes was recorded (from 0.84 to 1.94 mmoles/mg protein), possibly due to a combination of factors: a decrease in activity of antioxidative enzymes, a decrease in the concentration of antioxidants, an increase in the concentration of metabolites with a prooxidative action, and a decrease in the content of high-energy compounds [1].

During reperfusion for 30 min the acidity of the coronary effluent reached its initial level. The lactate concentration in the myocardium fell appreciably to not more than 45 and 23% of the ischemic level, although it was about 4 times higher than its normal concentration. The concentrations of ATP and CP had a tendency to rise, but they did not reach the initial level. The concentration of conjugated dienes remained twice as high as in the period of stabilization.

In order to diminish the severity of these metabolic disturbances due to the action of ischemia on the myocardium we studied the action of pantothenic acid derivatives: panthetin, panthenol, and calcium pantothenate. The effect of PT was studied in ischemia and during reperfusion. The compound was injected intramuscularly in a concentration of 25 mg/kg 24 and 48 h before the experiment, and it was added to the perfusion fluid (50 μM). During ischemia the most important effect of PT was found in the group of rats receiving a single injection (24 h) combined with its addition to the perfusion fluid (Table 2). Under these conditions PT significantly reduced the lactate concentration (57.10%) and the level of conjugated dienes in the myocardium. During reperfusion for 30 min the lowest lactate concentration in the myocardium (62.79%) and conjugated diene concentration (76.08% compared with reperfusion for 30 min without the preparation) were found in this group.

Panthenol and CaPA (intramuscularly, 2 h before ischemia) were tested during reperfusion for 30 min in a series of concentrations, the highest concentrations being equimolar with PT (Table 3). PL was most effective in a concentration of 9.0 mg/kg. During reperfusion for 30 min a stable coronary flow rate was observed and the lactate concentration was normal. An increase in the ATP concentration and the smallest concentrations of conjugated dienes were noted. Incidentally, with PL in concentrations of 4.5 and 12.5 mg/kg it was less effective. CaPA was most effective in a dose of 15.9 mg/kg, with simultaneous addition to the perfusion fluid. With this concentration of CaPA the lowest lactate level (2.4 μmoles/g, equal to the normal level) and also the highest ATP concentration in the myocardium (61.63% of the normal level) were recorded. The results indicate that PL (9.0 mg/kg) and CaPA (15.60 mg/kg) ensured better recanalization of the myocardium in the postischemic period. Evidence of this was given by the low lactate concentration in the myocardium (2.85 and 2.40 μmoles/g PL and CaPA, respectively) and the acidity of the coronary effluent (7.23 and 7.16 for PL and CaPA, respectively). We know from the literature that an increased plasma lactate concentration in the postischemic period stimulates oxidation predominantly of fatty acids in the myocardium, and that 82% of the total CO_2 is formed in their metabolic reactions. Oxidation of fatty acids in the post-ischemic period ensures better renewal of the reserves of high-energy compounds. It has been shown that PL (9.0 mg/kg) and CaPA (15.60 mg/kg) in the post-ischemic period with reperfusion for 30 min increased the ATP concentration by 3.0 and 3.7 times compared with the period of ischemia. Ischemia induces changes in the cell membranes and, in particular, activation of membrane lipid peroxidation, leading to a change in lipid asymmetry, involving disturbance of the membrane ultrastructure [16]. Investigations of the conjugated diene concentrations showed that they rose sharply during reperfusion (Table 1). The observed decrease in the concentration of conjugated dienes after injection of PL indicates better preservation of cardiomyocyte ultrastructure in the postischemic period. Further evidence of the cardioprotective action of these pantothenic acid derivatives is given by the more rapid restoration of the cardiac rhythm in the post-ischemic period and the ECG data. Consequently, the pantothenic acid derivatives studied possess a cardioprotective action on the post-ischemic myocardium. The mechanism of action of these compounds may perhaps be modification of the CoA reserves as a result of increased endogenous synthesis of their precursors and activation of CoA-dependent enzymes in the myocardial mitochondria [4].

It was thus shown on a model of experimental ischemia and reperfusion of the isolated heart that pantothenic acid derivatives, namely PL (9.0 mg/kg) and CaPA (15.60 mg/kg), with simultaneous addition to the perfusion fluid (25 μM) during the post-ischemic period (reperfusion for 30 min) effectively lowered the lactate concentration, increased the ATP concentration, and prevented accumulation of conjugated dienes in the myocardium, ie., that they had a cardioprotective action.

LITERATURE CITED

1. M. V. Bilenko, *Ischemic and Reperfusion-Induced Damage of Organs* [in Russian], Moscow (1989).
2. V. M. Borets, V. V. Mironchik, and L. P. Artaeva, *Intervitamin Relations in Ischemic Heart Disease and Essential Hypertension* [in Russian], Minsk (1988).

3. L. V. Rozenshtraukh, V. A. Saks, and E. P. Anyukhovskii, *Kardiologiya,* No. 4, 80 (1985).
4. L. Ya. Utno, *Byull. Éksp. Biol. Med.*, No. 6, 557 (1991).
5. H. U. Bergmeyer, *Methoden der enzymatischen Analyse,* Berlin (1970).
6. M. M. Gebhard, *Thorac. Cardiovasc. Surg.*, **38**, 55 (1990).
7. K. Herbaczynska-Cedro and W. Gordon-Majszak, *Cardiovasc. Res.*, **23**, 98 (1989).
8. V. A. Korzans, L. J. Utno, and A. G. Moisejonoks, *Latv. ZA Vestis,* No. 7, 87 (1989).
9. W. Lamprecht and I. Trautschold, *Methods of Enzymatic Analysis,* New York (1965).
10. J. Leiris, D. P. Harding, and S. Pestre, *Basic Res. Cardiol.*, **79**, 313 (1984).
11. G. D. Lopaschuk, G. F. McNeil, and J. J. McVeigh, *Mol. Cell Biochem.*, **88**, 175 (1989).
12. T. T. Nielsen, I. P. Bagger, and A. Thomassen, *Br. Heart J.*, **55**, 140 (1986).
13. S. V. Rendig, L. D. Segel, and E. A. Amsterdam, *Cardiovasc. Res.*, **22**, 322 (1988).
14. B. Renstrom, A. J. Liedtke, and S. H. Nellis, *Biochemistry (Washington),* **105**, 27 (1991).
15. L. J. Utno and V. A. Korzan, in: International Society for Heart Research, 3rd Symposium (1986), p. 91.
16. O. J. Verkly, P. J. Andries, and C. T. W. M. Schneijdenberg, *Cell., Biol. Int. Rep.*, **7**, 335 (1990).