in favor of the former, and as a result, a larger number of membrane structures, necessary for functioning of the tissue under conditions of increased loading, can be maintained in the cardiomyocytes. After removal of the inducing factor (in this case, periodic high-altitude hypoxia) the presence of an excessive number of structures becomes energetically disadvantageous for the cell, and they are eliminated through activation of various catabolic processes, of which LPO is one. In fact, on the 3rd day of regression of hypertrophy of the right ventricle, whose weight was doubled, an increase of 56% was observed in the concentration of diene conjugates compared with the hypertrophied heart. Hypertrophy of the left ventricle was slight and its regression was not accompanied by any significant increase in the concentration of LPO products.

The results agree on the whole with our ideas on the role of LPO in regression of hypertrophy of the heart and also, perhaps, of other changes arising during long-term adaptation, and they justify the view that the further study of this problem will be promising.

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CARDIOPROTECTIVE PROPERTIES OF 1,4-DIHYDROPYRIDINE DERIVATIVE GLUTAPYRON IN DEEP HYPOTHERMIA

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A general rule in animals of different species during the primary response to cold is an increase in activity of free-radical lipid peroxidation (LPO) in the myocardium, followed by a decrease in the activity of these processes as the temperature falls to the region of deep hypothermia, with reactivation during continued exposure to deep hypothermia (for 40 min or more) [6, 8]. Intensification of peroxidation during cooling and in deep hypothermia causes changes in the structure of the cell membranes and in 13-lipid interactions [5]. Changes in membrane structure improve access to substrates for pro-oxidants. LPO products found in membrane phospholipids increase permeability of membranes of the sarcoplasmic reticulum for Ca^{++} ions, which in turn induce activation of LPO. The LPO products thus formed inhibit Ca^{++}-ATPase in myocyte membranes [4]. The reaction chain described above ends with an increase in the outflow of Ca^{++} from the myocytes, with a resulting decrease in myocardial contractility [10]. When deep hypothermia is used in cardiac surgery these disturbances of

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TABLE 1. Effect of Glutapyron in Doses of 10 and 40 mg/kg on Parameters of VWC Curve of Mitochondria and MDA Content in Myocardial Mitochondria during Deep Hypothermia (M ± m)

Serial No.	Experimental conditions	L, conventional units	H, $\times 10^{-3}$/mg protein	tan α	MDA concentration, nmoles/mg protein
1	Normothermia (control)	(15) 63,4±5,0	65,3±3,4	0,68±0,04	1,02±0,08
2	Normothermia + glutapyron, 10 mg/kg	(12) 61,9±6,2	68,0±1,3	0,75±0,06	1,24±0,16
3	Normothermia + glutapyron, 40 mg/kg	(12) 37,8±4,6 $p_{3-1}<0,002$	57,8±4,7	0,64±0,05	0,95±0,07
4	Hypothermia 40 min	(12) 63,2±4,9	73,9±3,3	0,94±0,07 $p_{4-1}<0,01$	2,36±0,19
5	Hypothermia 40 min + glutapyron, 10 mg/kg	(16) 63,5±3,0	62,3±2,5 $p_{5-4}<0,02$	0,65±0,06 $p_{5-4}<0,01$	0,91±0,76
6	Hypothermia 40 min + glutapyron, 40 mg/kg	(16) 15,8±3,6 $p_{6-1}<0,001$ $p_{6-4}<0,001$	28,3±5,5 $p_{6-1}<0,001$ $p_{6-4}<0,001$	0,44±0,09 $p_{6-1}<0,05$ $p_{6-4}<0,001$	1,21±0,85

Legend. Here and in Tables 3 and 4, number of animals given in parentheses.

homeostasis require correction, for the outcome of an operation on the heart largely depends on preservation of myocardial contractility.

The creation of methods of preventing disturbances of myocardial metabolism during deep hypothermia must have the following aims: 1) to prevent activation of LPO in the myocardium; 2) to prevent blood changes inducing LPO in the tissues; 3) to preserve activity of reactions involved in energy formation and transport of metabolites which largely determine myocardial contractility under posthypothermic conditions.

This paper describes a study of the action of the 1,4-dihydropyridine derivative glutapyron on LPO and Ca^{++}-ATPase activity and on the formation of high-energy compounds in the myocardium during deep hypothermia.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200 ± 20 g were used. The rats were anesthetized by intraperitoneal injection of a mixture of a 10% solution of thiopental, and 20% solution of γ-hydroxybutyrate, a 0.1% solution of atropine, and water in the ratio of 1:1.6:0.4:1, and in a dose of 0.5 ml/100 g body weight respectively. Morphine also was injected in a dose of 0.5 mg/100 g body weight. For a period of 30 min after induction of anesthesia the rats were cooled by covering them with crushed ice. The mean rate of cooling was 0.6-0.67°C/min. The body temperature was measured witha TPEM-1 electrothermometer. The time of exposure to deep hypothermia began to be counted after the temperature reached 20°C. Two control groups were studied: 1) intact rats; 2) anesthetized rats not exposed to deep hypothermia. At the corresponding stages the animals were decapitated, the myocardium quickly removed, and blood collected in cooled centrifuge tubes. Tests were carried out after exposure to deep hypothermia for 0, 10, 20, 30, 40, 50, and 60 min, in myocardial homogenate and mitochondria and in blood plasma and erythrocytes. The intensity of LPO was determined in blood plasma, erythrocytes, and mitochondria by measuring the malonic dialdehyde (MDA) produced [2] and by a chemiluminescence method. Creatine phosphate was determined in the supernatant after centrifugation at 9,500 rpm [7] and creatine phosphokinase in the mitochondria and plasma [1]. Ca^{++}-ATPase activity was determined in the mitochondria, and the calcium concentration in mitochondria, supernatant, and blood plasma by atomic absorption spectrometry [3]. Mitochondria were isolated by differential centrifugation [9].

EXPERIMENTAL RESULTS

The amplitude of the fast wave (L), characterizing the hydroperoxide content in the system, and the amplitude of the slow wave, reflecting oxidizability of lipids (H), and the tangent of the angle of slope (tan α), the value of which indicates the velocity of lipid peroxidation, were determined from the very weak chemiluminescence (VWC) curves. The results of chemiluminescence analysis showed (Table 1) that injection of glutapyron in a dose of 10 mg/kg lowered the velocity of oxidation and the oxidizability of the lipids

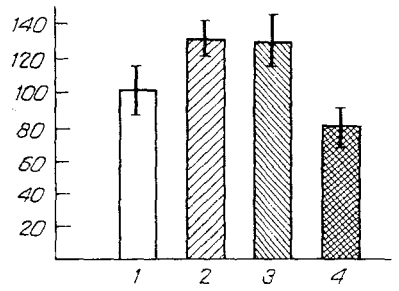

Fig.1. Changes in light sum of VWC of blood plasma during deep hypothermia. 1) Intact rats (control); 2) hypothermia 40 min; 3) hypothermia 50 min; 4) hypothermia 40 min and glutapyron, 25 mg/kg. Ordinate, light sum of VWC (im relative units).

to the control value and did not affect the content of lipid hydroperoxides in myocardial mitochondria during deep hypothermia. In normothermic animals this dose did not affect the parameters of chemiluminescence of mitochondrial lipids.

In a dose of 40 mg/kg, during deep hypothermia glutapyron lowered the hydroperoxide concentration and the oxidizability and rate of oxidation of lipids. In normothermic animals this dose only lowered the concentration of lipid hydroperoxides in the mitochondria (Table 1).

However, as reflected in MDA formation in the myocardium, glutapyron in doses of 10 and 40 mg/kg exhibited equal antioxidant activity. Injection of these doses inhibited MDA formation in the mitochondria, characteristic of deep hypothermia, to the values observed in normothermic animals (Table 1).

Parallel with determination of the intensity of chemiluminescence of the mitochondria, the light sum of chemiluminescence of the blood plasma was measured. Deep hypothermia lasting 40 min increased the light sum of chemiluminescence by 30.7%. Injection of glutapyron reduced the light sum by 40.7% compared with its value during hypothermia and by 22.4% compared with normal (Fig.1). Comparison of these results with changes in the plasma MDA level under the same conditions shows that activation of LPO in the blood plasma did occur, although, judging by the MDA values, this process was less intensive during hypothermia (Table 2). Comparison of the action of glutapyron on the light sum of chemiluminescence and on the blood MDA level shows that although the preparation reduced the light sum, it did not affect the plasma MDA concentration of the hypothermic animals, although the MDA concentration in the erythrocytes was depressed. Changes in the chemiluminescence charcteristics during deep hypothermia thus reflect changes in homeostasis and, in particular, LPO, which is evidently activated by pro-oxidants entering the myocardial cells [11]. During stimulation of LPO, creatine phosphokinase (CPK), responsible for energy transfer from mitochondria to myofibrils is released into the blood stream (Table 3). Loss of this enzyme protein and an increase in the free fatty acids (FFA) concentration in the blood are evidence that simultaneously with a decrease in the rate of oxidation of the principal energy substrate, permeability of the myocyte membranes also is increased. Injection of glutapyron in a dose of 10 mg/kg completely prevented the rise in the blood CPK and FFA levels and caused the creatine phosphate level in the heart muscle to rise.

Administration of glutapyron completely restored mitochondrial Ca^{++}-ATPase activity, when depressed after deep hypothermia to 40 min, by 24.2% but did not afect the mitochondrial calcium concentration (Table 4).

It can be concluded from these results that the cardioprotective action of glutapyron during deep hypothermia is due to its antioxidant activity. Inhibition of LPO takes place in membrane lipids of myocytes and erythrocytes. Antioxidant properties are manifested

TABLE 2. Action of Glutapyron on MDA Concentration in Blood Plasma and Erythrocytes (M ± m)

Serial No.	Experimental conditions	Plasma MDA concentration		MDA concentration in erythrocytes	
		nmoles/liter	%	nmoles/liter	%
1	Intact rats	5,44±0,13	100	33,8±1,6	100
2	Deep hypothermia 40 min	6,00±0,20	110,3 $p_{1-2}<0,05$	35,7±1,3	105,6
3	Deep hypothermia 40 min + glutapyron, 25 mg/kg	6,10±0,20	112,1 $p_{1-3}<0,02$	31,7±1,5 $p_{2-3}<0,05$	93,8

TABLE 3. Effect of Glutapyrone on Raised Blood CPK Level and Blood FFA Level during Deep Hypothermia (M ± m)

Serial No.	Experimental conditions	Plasma CPK activity, μmoles/ml/min	Blood FFA concentration, mg%
1	Normothermia (25)	31,2±8,9	91,4±7,5
2	Normothermia + glutapyron, 10 mg/kg (23)	36,3±7,7	85,3±6,5
3	Normothermia + glutapyron, 40 mg/kg (17)	24,7±6,2	48,0±1,8
4	Hypothermia 40 min (25)	71,0±6,1	105,8±8,0
5	Hypothermia 40 min + glutapyron, 10 mg/kg (17)	40,1±4,2 $p_{5-4}<0,002$	68,6±6,6 $p_{5-4}<0,002$
6	Hypothermia 40 min + glutapyron, 40 mg/kg (17)	57,6±4,0 $p_{6-4}<0,05$	82,9±11,2 $p_{6-4}<0,01$

TABLE 4. Effect of Glutapyron on Calcium Concentration in Blood Plasma, Supernatant, and Mitrochondria and on Ca^{++}-ATPase Activity in Mitochondria during Deep Hypothermia (M ± m)

Serial No.	Experimental conditions	Calcium concentration			Ca^{++}-ATPase activity, μmoles Pi/mg/h
		in blood plasma, μmole/liter	in supernatant, nmoles/mg protein	in mitochondria, nmoles/mg protein	
1	Intact rats (control)	2,64±0,18 (6)	31,18±6,48 (6)	68,91±3,22 (6)	12,06±1,08 (4)
2	Hypothermia 40 min	2,83±0,20 (7)	59,64±8,92 (7) $p_{2-1}<0,05$	51,30±7,31 (7) $p_{2-1}=0,05$	9,14±0,59 (6) $p_{2-1}<0,05$
3	Hypothermia 40 min + glutapyron, 25 mg/kg	2,55±0,08 (12)	20,60±3,00 (12) $p_{3-2}<0,002$	51,63±3,66 (12) $p_{3-1}<0,01$	12,08±0,55 (10) $p_{3-2}<0,001$

both by inhibition of initiation of free-radical oxidative reactions and as stabilization of membrane structures and preservation of function of proteins integrated in membranes. Taken as a whole, these properties ensure that activity of reactions concerned with the formation and transport of high-energy compounds in the myocardium is maintained during deep hypothermia.

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