EFFECT OF ANTIOXIDANT IONOL ON ENERGY METABOLISM AND CARDIAC FUNCTION IN ACUTE HYPOXIA FOLLOWED BY REOXYGENATION

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Antioxidants and, in particular, ionol (dibunol*), have a marked cardioprotective action, including the prevention of arrhythmia in acute ischemia of the heart [4, 5], and preventing damage to the heart arising under reoxygenation conditions after acute hypoxia by restricting the outflow of enzymes from the myocardium [2, 3] and by increasing the rate of recovery of the contractile function [1]. However, the biochemical mechanism of the cardioprotective action of ionol is not clear; in particular, it is not known how this antioxidant affects the energy metabolism of the heart in acute hypoxic hypoxia and subsequent reoxygenation.

The aim of this investigation was to assess the effect of preliminary administration of ionol on the time course of the basic parameters of energy metabolism in acute hypoxic hypoxia and subsequent reoxygenation and to compare changes in energy metabolism under these circumstances with the contractile function of the heart.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g, under pentobarbital anesthesia (50 mg/kg body weight) with an open chest and with artificial ventilation. The animals were divided into two groups. The animals of group 1 (control) received sunflower oil (0.05 ml/100 g per os) once a day for 3 days before the experiments, whereas animals of group 2 received ionol (6 mg/100 g body weight), dissolved in the same volume of sunflower oil, at the same times. The experiments were conducted in two stages. In the first stage biochemical tests were carried out. To study the parameters of energy metabolism the hearts were frozen in situ by application of forceps cooled in liquid nitrogen (the animals were artificially ventilated during this procedure). The hearts were removed 10 min after connection of the animal to the respirator, 3 min after the beginning of exposure to hypoxia, and 5 min after the beginning of reoxygenation. Blood was removed from the hearts which were homogenized to a powder in liquid nitrogen, and a chloride extract was then prepared in the ratio of 1:20; after neutralization of the supernatant, ATP and lactate were determined with the aid of kits from "Böhringer," and creatine phosphate (CP) was determined by the diacetyl method [6]. Glycogen was extracted in 30% KOH on a boiling water bath, and after cooling in 70% alcohol it was hydrolyzed to glucose with amyloglucosidase [11]. Glucose was determined by the glucose oxidase method [7] and phosphorylase by the method in [9]; total activity was measured in the presence of cAMP and the active form of phosphorylase in the absence of cAMP. In the second stage the contractile function of the heart was studied under conditions of hypoxia and reoxygenation, induced by switching off the artifical respiration apparatus for 3 min and restarting it respectively. Cardiac function was studied from the pressure curve recorded inside the left ventricle by means of a "Mingograf-34" electromanometer (Elema–Seimens, Sweden). The following parameters were measured on the pressure

*4-Methyl-2,6-di-tert- butylphenol — Translator.

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TABLE 1. Effect of Ionol on Myocardial Energy Metabolism of Rats during Hypoxic and Reoxygenation Test

Parameter	Control			Ionol		
	1	2	3	4	5	6
	initial level	hypoxia	hypoxia + reoxygenation	initial level	hypoxia	hypoxia + reoxygenation
Total phosphorylase, μmoles P/g/min	30,7±1,5	35,7±1,2 p_{1-2} n.s.	38,6±1,7 p_{1-3} n.s.	40,3±0,9 $p_{1-4}<0,05$	54,6±1,9 $p_{4-5}<0,001$ $p_{2-5}<0,01$	40±2 p_{4-6} n.s. p_{3-6} n.s.
Phosphorylase A	14,6±0,9	30,6±0,47 $p_{1-2}<0,001$	23,4±1,3 $p_{1-3}<0,01$	22,1±0,8 $p_{1-4}<0,01$	46,6±2,1 $p_{4-5}<0,001$ $p_{2-5}<0,01$	13,2±0,7 $p_{4-6}<0,01$ $p_{3-6}<0,05$
PA/P (A + B)	0,47±0,02	0,85±0,02 $p_{1-2}<0,001$	0,6±0,05 $p_{1-3}<0,01$	0,55±0,03 p_{1-4} n.s.	0,88±0,07 $p_{4-5}<0,001$ p_{2-5} n.s.	0,33±0,02 $p_{4-6}<0,001$ $p_{3-6}<0,05$
Glycogen, mg/100 g tissue	428±17	105±5 $p_{1-2}<0,001$	96±4 $p_{1-3}<0,001$	417±8 p_{1-4} n.s.	180±6 $p_{4-5}<0,001$ $p_{2-5}<0,05$	187±7 $p_{4-6}<0,001$ $p_{3-6}<0,05$
Lactate, μmoles/g tissue	1,3±0,1	9±0,9 $p_{1-2}<0,001$	5,0±0,8 $p_{1-3}<0,001$	2,1±0,2 p_{1-4} n.s	4,8±0,8 $p_{4-5}<0,001$ $p_{2-5}<0,01$	2,4±0,3 p_{4-6} n.s. $p_{3-6}<0,01$
Creatine phosphate, μmoles/g tissue	8,55±0,3	0,7±0,06 $p_{1-2}<0,001$	3,3±0,3 $p_{1-3}<0,001$	7,4±0,5 p_{1-4} n.s.	0,35±0,06 $p_{4-5}<0,001$ p_{2-5} n.s.	5,87±0,3 p_{4-6} n.s. $p_{3-6}<0,02$
ATP, μmoles/g tissue	5,8±0,2	2,95±0,2 $p_{1-2}<0,001$	3,64±0,7 $p_{1-3}<0,01$	5,2±0,2 p_{1-4} n.s.	3,4±0,7 $p_{4-5}<0,01$ p_{2-5} n.s.	4,0±0,8 p_{4-6} n.s. p_{4-6} n.s.

Legend. In each series 20 hearts were used; n.s.) not significant, PA) active form of phosphorylase, P(A + B) total phosphorylase.

TABLE 2. Effect of Synthetic Antioxidant Ionol on Contractile Function of the Heart during Hypoxia and Reoxygenation

Parameter	Group of animals	Before hypoxia	Hypoxia			Reoxygenation		
			1 min	3 min	30 sec	1 min	3min	5 min
HR, beat/min	Control ($n = 10$)	259±11,8	210±13	87±13	80±8	115±13	150±12	185±13
	Ionol ($n = 8$)	260±10,6	215±11	90±10	140±9	150±11	170±12	190±14
p					<0,001	<0,05		
P_{dev}, mm Hg	Control	128±12,1	86±8,1	70±8,3	80±5,8	116±6,9	118±6,9	122±10,5
	Ionol	132±8,6	92±7,1	86±8,7	160±6,9	162±6,7	152±7,2	170±8,1
p					<0,001	<0,02	<0,05	<0,05
IFS, mm Hg·min^{-1}·mg^{-1}	Control	69±7,3	15±4,3	8±2,9	12±2,8	22±4,5	43±3,9	41±4,7
	Ionol	75±6,7	16±4,1	10±3,3	50±5,2	50±3,4	61±6,1	70±7,5
p					<0,001	<0,001	<0,02	<0,02
Rate of relaxation, mm Hg/sec	Control	3261±271	2500±214	1350±197	2100±253	2800±274	2600±317	3250±199
	Ionol	3458±263	2571±232	1800±215	5200±249	5500±301	4950±131	5050±236
p					<0,02	<0,02	<0,05	<0,05
Rate of relaxation, mm Hg/sec	Control	1837±114	1536±109	1100±99	1100±113	1750±124	1750±131	2050±112
	Ionol	2254±225	1750±213	1500±221	3750±194	3000±211	2500±205	2900±222
p		>0,1			<0,01	<0,05	<0,05	<0,05

Legend. p) Significance of differences between control animals and animals receiving ionol.

curve: the systolic, diastolic, and developed pressure, heart rate (HR), and intensity of functioning of structures (IFS, in mm Hg·min^{-1}·mg^{-1}), by the formula

$$\frac{P_{dev} \cdot HR}{\text{wt. of left ventricle}},$$

where P_{dev} denotes the developed pressure in the left ventricle. The parameter IFS is the chief determinant of energy consumption, calculated per unit mass of myocardium. Each experiment involved recording contractile function under conditions of relative physiological rest, and then later during hypoxia and reoxygenation.

EXPERIMENTAL RESULTS

The data on the left of Table 1 show that acute hypoxia, in the present experiments just as in those of other workers [10], caused two combinations of changes in the myocardium: activation of the phosphorylase system, a repeated fall of the glycogen content and an even greater rise of the lactate content, and also a tremendous fall (by more than an order of magnitude) of the CP concentration, accompanied by a much smaller (by only 49%) fall of ATP. By the end of the 5th minute of reoxygenation these parameters of energy metabolism had changed in different ways: activity of the phosphorylase system was reduced to subnormal values, the glycogen concentration had not even a tendency to recover, and the lactate concentration had fallen, but still remained 4 times higher than initially. The CP content was increased almost fivefold but remained significantly lower than normal, and finally, the ATP concentration was very slightly increased.

The data on the right side of Table 1 show that the antioxidant ionol definitely affected the time course of energy metabolism during hypoxia and reoxygenation. The first effect of ionol was that, despite the usual hypoxic activation of the phosphorylase system, the glycogen concentration in animals receiving the compound fell significantly less than in the control series, to 180 ± 6 mg/100 g compared with 105 ± 4 mg/100 g ($p < 0.05$); the lactate concentration correspondingly rose not by 7 times, as in the control, but by only 2.3 times. These facts suggest the presence of a more effective system of glycogen synthetase, that prevented reduction of the glycogen concentration despite its hypoxic mobilization. These results are interesting for the explanation of the antiarrhythmic action of ionol, for we know that the probability of onset of arrhythmias and fibrillation of the heart is inversely proportional to the glycogen content in the myocardium [8].

Ionol, as Table 1 shows, did not affect mobilization of the CP and ATP pool. The second marked effect of ionol was realized during reoxygenation, namely: it significantly accelerated restoration of the CP reserves: during 5 min of reoxygenation the CP concentration rose by 10 times, and not by 5 times as in the control. A more rapid decrease of phosphorylase activity and of the lactate concentration also was observed, and the ATP level was restored just as slowly as in the control.

Consequently the main change observed under the influence of ionol in the reoxygenation stage was the more rapid recovery of CP, whose content was 78% greater than in animals not protected by the antioxidant.

To assess the significance of the effects of ionol thus observed, data on the contractile function of the heart during hypoxia and reoxygenation could be important (Table 2). They showed that ionol did not affect depression of the contractile function during hypoxia with respect to any of the parameters studied, but at the same time, it led to a sharp increase in the rate of recovery of the contractile function during reoxygenation; according to parameters such as the developed pressure and the rates of contraction and relaxation, a phenomenon of over-restoration was observed.

Within the context of this description it is important to note that restoration of IFS, parameters of which are determined to a high degree by the myocardial energy metabolism, took place in animals receiving ionol much faster and to a much greater degree than in the control. As a result, the value of IFS toward the end of the 5th minute of reoxygenation was 72% greater than in the control. In other words, IFS in animals protected by ionol, rose toward the end of reoxygenation by the same degree compared with the control as the CP content.

Thus, the protective effect of ionol on energy metabolism under hypoxic conditions is realized mainly at the level of glycolysis, when it ensures maintenance of a higher glycogen content and a lower lactate concentration than in the control. This protective effect in no way prevents depression of the contractile function and does not correlate with it, but conjecturally it may play a role in maintenance of the electrical stability of the heart.

During reoxygenation the protective effect of ionol is manifested chiefly at the level of the creatine kinase system, ensuring rapid recovery of the rate of CP synthesis; this shift, moreover, correlates with the rate of recovery of developed pressure and the rates of contraction and relaxation.

On the whole these data are in conformity with the view that the creatine kinase system and ATP, which is under its control, play the most important role in the depression and subse-

quent restoration of the contractile function of the heart in acute, short-term hypoxia and subsequent reoxygenation [12, 13]. Preliminary administration of the antioxidant ionol guarantees more effective functioning of this system and, correspondingly, the more rapid recovery of the contractile function during reoxygenation.

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GENERATION OF ACTIVE BACTERICIDAL FORMS OF OXYGEN BY LEUKOCYTES CIRCULATING THROUGH THE LUNGS

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Phagocytic leukocytes (granulocytes and monocytes) generate active form of oxygen (AFO): the superoxide anion-radical ($O_2^{\cdot}$), singlet or electronically excited oxygen (I_{O_2}), the hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide, amounts of which increase sharply in response to stimulation of the leukocytes by biologically active substances [10, 13] and during phagocytosis [5, 6, 8, 10, 13]. In the absence of stimulation and phagocytosis AFO generation is miserly [9]. On the one hand, AFO are important bactericidal factors of phagocytosis [5, 8, 9, 11-13], whereas on the other hand they are powerful initiators of free radical lipid peroxidation [4, 5], which has an altering action [2, 4]. On account of these two effects, AFO generated by leukocytes perform a double function: they are involved in the formation of phagocytic antimicrobial defense and in the free radical status of the organism.

The aim of this investigation was to study the possible role of the lungs and other organs in the regulation of bactericidal AFO production by leukocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 18 intact dogs weighing 15-20 kg and two calves, anesthetized with thiopental, and receiving preliminary injections of droperidol, callipsol, trimeperidine, and atropine and maintained on artificial respiration. AFO generation by leuko-

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