ANTIHYPOXIC EFFECTS OF SOME QUINONES ASSOCIATED WITH RESTORATION OF THE ELECTRON TRANSPORT FUNCTION OF THE RESPIRATORY CHAIN OF THE ISOLATED RAT HEART

A. A. Korneev, O. A. Popova, S. V. Zamula, and L. D. Luk'yanova

UDC 615.23.015.4:612.215].076.9

KEY WORDS: hypoxia; antioxidants with redox properties; respiratory chain; quinones

Many quinone derivatives possess strong redox properties and oxidize various intracellular forms of NADH and NADPH with the participation of menadione reductase, an enzyme widely distributed in the cell, which undertakes the catalytic transfer of reducing equivalents to oxygen [1, 11, 12, 14]. In their presence new pathways are formed for the transfer of reducing equivalents with the involvement of NAD(P)-dependent oxidoreductases and cytochromes of the mitochondrial respiratory chain. As a result of this, concentrations of metabolites in NAD- and NADP-dependent reactions and systems associated with them are redistributed, thus regulating their influence on glycolysis and the tricarboxylic acid cycle. Naturally the effect of these substances ought to be enhanced under conditions stimulating accumulation of incompletely oxidized products. For example, in the case of inhibition of the first enzyme complex of the respiratory chain one derivative of naphthoquinone, vitamin K_3, may restore its function, as a result of shunting of the electron flow in the region NADH — CoQ. Since it is with a block in this region, leading to reduction of the energy-forming function of the cell, that disturbances of energy metabolism begin during hypoxia [6-10], restoration of electron transfer in the respiratory chain and of the coupling function of the second and third points of phosphorylation under these conditions is of fundamental importance.

The aim of this investigation was to study the possibility of using quinones as antihypoxants and cardioprotectors of energizing type, restoring the mechanical function of the myocardium when depressed during hypoxia.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-200 g, and divided beforehand into animals with high resistance (HR) and low resistance (LR) to hypoxia. The heart, isolated by Langendorff's method [4], was perfused with Krebs–Henseleit solution (pH 7.4, 37°C) with glucose (11 mM), oxygenated with a gas mixture of 95% O_2 + 5% CO_2. Moderately severe hypoxia was created by saturating the solution with a gas mixture of 50% O_2 + 45% N_2 + CO_2 (H50). Myocardial contractility was evaluated under isometric conditions, using a type K30 HSE force and displacement transducer with a rigid flat spring. Optimal (by 1.5 times) stretching of the test object was achieved by means of a vernier device, regulating fixation of the transducer in the system. The heart rate (HR) and force of the cardiac contractions (FCC) were recorded. The value of FCC measured by this method is a measure of ATP utilization and is proportional to the ATP concentration in the myocardium [16], and this parameter was therefore used as the criterion of ATP generation, reflecting the level of energization of the myocardium. The value of the tension developed by the heart in unit time (A) also was calculated, as the product of HR and FCC. The oxygen concentration in perfusion fluid flowing into and out of the system was determined polarographically with the aid of a Clark electrode, and the rate of oxygen consumption by the isolated myocardium (Vg) was calculated. The ATP concentration in the myocardial tissue was determined by the luciferol–luciferase method in the modification in [1]. The quinones used included vitamin K_3 menadione (2-methyl-1,4-naphthoquinone) 10^{-5} M, and hydroquinone (10^{-5} M). As standard

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 7, pp. 60-63, July, 1990. Original article submitted November 22, 1989.

 0007-4888/90/0007-0922$12.50

TABLE 1. Effect of Vitamin K_3 and Hydroquinone on Functional-Metabolic Parameters of Isolated Rat Myocardium Under H50 Conditions (percent of normoxic state), $M \pm m$

Experimental conditions	Time of action, min	HR animals								LR animals							
		HR		FCC		effort of heart (A)		Vg		HR		FCC		effort of heart (A)		Vg	
H_{50}	5	95,0	9,8	113,0	10,1	76,7	29,9	27,3	3,0	93,0	5,3	55,0	7,9	52,6	9,4	—	—
	10	91,8	10,3	75,7	9,4	52,7	8,2	27,3	3,0	98,0	8,0	28,0	3,7	25,0	3,6	37,0	1,5
	15	89,8	11,1	56,0	18,6	29,3	10,8	31,3	4,3	105,7	16,1	24,0	2,4	25,2	3,6	39,2	2,0
	20	91,0	9,9	40,3	13,3	27,0	7,6	34,0	5,3	102,6	12,7	26,2	6,3	27,0	6,3	39,2	2,0
H_{50} + vitamin K_3	5	108,0	5,3	116,7	8,3	146,7	23,2	45,0	1,0*	111,0	2,0*	100,0	0,0*	111,3	2,9*	—	—
	10	105,7	3,2	108,3	8,3*	127,0	20,4*	45,0	1,0*	117,3	5,4	70,3	15,2*	60,7	20,5*	47,3	3,3*
	15	104,3	3,8	100,0	0,0*	105,7	4,9*	52,0	3,6*	111,3	4,2	70,3	15,2*	57,3	12,8*	52,7	2,3*
H_{50} + hydroquinone	20	98,7	0,7	100,0	0,0*	98,0	1,0*	52,0	3,6*	95,0	10,4	77,0	8,5*	57,8	19,8	55,3	2,2*
	5	104,0	12,2	108,0	28,1	127,1	50,4	—	—	98,0	8,0	110,1	36,5	120,0	65,5	—	—
	10	98,3	8,0	108,0	28,1	120,2	58,8	43,3	3,4*	99,1	5,0	88,2	8,4*	72,0	6,7*	45,3	1,3*
	15	93,3	10,5	119,4	42,4*	137,0	84,0	46,3	1,7*	100,0	3,4	81,1	16,8*	69,4	26,0*	45,3	1,3*
	20	91,0	12,2	119,4	42,4	133,2	84,0	46,3	1,7*	99,3	3,4	81,1	16,8	69,0	26,9	45,3	1,7*

TABLE 2. Effect of Piracetam and Gutimin on Functional-Metabolic Parameters of Isolated Rat Myocardium under H50 Conditions (percent of normoxic state; $M \pm m$)

Experimental conditions	Time of action, min	HR animals								LR animals							
		HR		FCC		A		Vg		HR		FCC		A		Vg	
H_{50} + piracetam	5	102,0	15,1	122,0	40,2	136,3	62,8	38,0	2,0*	100,0	0,0	100,0	0,0*	100,0	0,0*	—	—
	10	93,7	9,6	128,7	35,9*	147,0	57,6	40,7	3,3*	97,3	1,5	91,7	8,9*	81,7	15,9*	36,7	1,7
	15	90,3	8,7	138,7	33,2*	170,7	56,9*	40,7	3,3*	96,3	2,3	91,7	8,3*	81,7	15,9*	37,3	1,5
	20	89,0	7,5	138,7	33,2*	167,0	56,5*	41,3	3,7	95,3	3,3	76,7	1,7*	61,7	7,3*	37,3	1,5
H_{50} + gutimin	5	100	3	115	19	115	15	—	—	97,1	5,3	113,0	22,1	110,0	27,4*	—	—
	10	100	7	110	18	110	18	41	2,0	88,0	10,6	92,4	1,6*	81,2	8,9	41,0	13,4
	15	100	9	110	18	110	19	41	1,5	87,0	13,3	97,2	6,2*	84,1	18,6	41,0	13,4
	20	100	5	100	13	100	21	41	1,3	113,2	10,6	58,2	1,5*	66,3	2,9*	41,0	13,4

antihypoxants we used piracetam (10^{-4} M) and gutimin (guanylthiourea; 10^{-6} M). All the substances were added to the perfusion fluid simultaneously with the beginning of experimental H50. The time course of the process was studied. Values corresponding to the time immediately before hypoxia were taken as 100%.

EXPERIMENTAL RESULTS

The response reaction and dynamics of the functional-metabolic parameters of the isolated myocardium of HR and LR animals under H50 conditions differed. While the changes in HR of LR animals were virtually not significant, FCC of the myocardium of HR was significantly less than that of LR (Table 1). Conversely, the respiration rate of the HR myocardium was much more strongly depressed than that of LR, evidence of the more active glycolysis taking place in it, compensating for ATP formation during hypoxia. As the writers showed previously [4, 5, 7, 10] the unequal sensitivity of the hearts of HR and LR animals to H50 also was connected with initial differences in aerobic oxidative metabolism of the myocardium and its ability to utilize different energy substrates. In the myocardium of HR rats the succinate-oxidase pathway of oxidation predominated, and could even be activated at low pO_2 values, whereas in the LR myocardium, the NADH-oxidase pathway predominated. Since under H50 conditions rapid inactivation of NAD-dependent oxidation takes place [4, 5], this is reflected primarily in FCC and A of the myocardium of the LR animals.

In the presence of the quinones studied (vitamin K_3 and hydroquinone) the action of H50 on myocardial contractility weakened (LR) or was impossible to detect (HR; Table 1). Since under these circumstances the oxidizing power of the pyridine pool of the myocardium was significantly greater than without them (Fig. 1), and the respiration rate of the myocardium increased (Table 1), while significantly smaller losses of ATP took place in the tissue (Fig. 2), this confirms that the mechanism of action of the test substances is based on the manifestation of their donor—acceptor properties, which were discussed above. It is in fact connected with their shunting of the first complex of the respiratory chain (oxidation of pyridines) and with reduction, on account of this, of its electron-transport (an increase in the respiration rate) and energy-forming (ATP formation) functions.

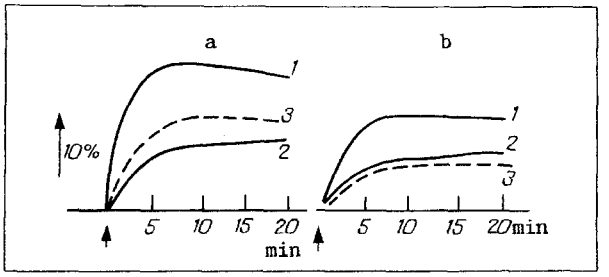

Fig. 1. Relative change in total degree of reduction of pyridines (PNR) of isolated myocardium during H50 for 20 min (50% O_2). 1) Control, 2) vitamin K_3, hydroquinone. Here and in Fig. 2: a) NR, b) HR animals.

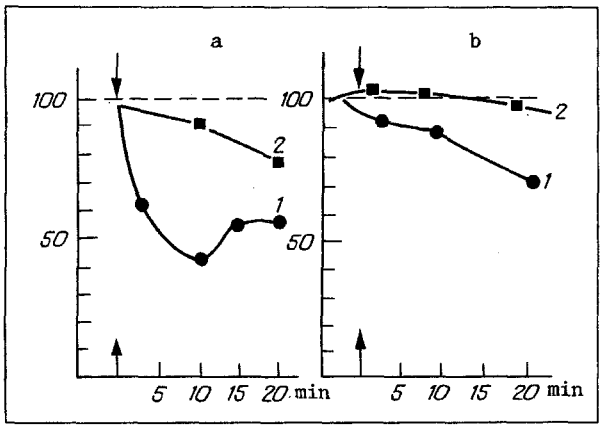

Fig. 2. Time course of ATP concentration (in %, ordinate) in myocardium of isolated contracting rat heart during exposure for 20 min to H50. 1) Control, 2) in presence of vitamin K_3.

Vitamin K_3 preserves its protective properties in the posthypoxic period also, accelerating the return of all the parameters tested to normal. This indicates that the blocking of electron transport in the NADH—CoQ region, formed during hypoxia, persists for some time also into the reoxygenation period, and is one of the causes preventing rapid recovery of the contractile function of the myocardium after exposure to hypoxia. The cytochrome region of the respiratory chain remains intact under these circumstances.

The antihypoxic effects of the quinones studied on FCC and A of the myocardium were quantitatively similar to the action of the standard antihypoxic agents gutimin and piracetam (Fig. 2). However, the latter reduced, and did not oxidize, respiratory carriers of the first enzyme complex (Fig. 1) and restored the respiratory function of the myocardium much less strongly (Table 2), i.e., the mechanism of action of these standard antihypoxants was completely different. In fact, in the modern view, the molecular effects of piracetam are connected with its corrective action on phospholipase activity [14]. It has been suggested that the antihypoxic action of gutimin also is based on membranotropic effects.

Thus vitamin K_3 and hydroquinone are antihypoxants, correcting the work of the respiratory chain in the initial stages of hypoxic (ischemic) damage through direct interaction with it, restoring electron transport as a result under hypoxic conditions from NADH to CoQ and restoring the energy-synthesizing function of the cytochrome region [4-7, 9, 10]. Considering the low toxicity of vitamin K_3, which distinguishes it in this respect from other quinone derivatives [2, 3, 11, 12], its use as a cardioprotector and antihypoxant under hypoxic or ischemic conditions of average severity, may prove to be extremely promising. The antihypoxic action of vitamin K_3 also is manifested at the whole body level. In other countries vitamin K_3 is successfully used for the prevention of certain myopathies, due to congenital insufficiency of the enzyme of the first complex of the mitochondrial respiratory chain of skeletal muscles, namely NADH-cytochrome *c*-reductase.

The high informativeness of the various parameters characterizing the contractile function and oxidative metabolism of the isolated contracting rat heart, which can be used when screening antihypoxants of energizing type, must also be noted.

LITERATURE CITED

1. F. I. Ataullakhanov and A. V. Pichugin, Biofizika, **26**, No. 1, 86 (1981).
2. V. V. Gatsura, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1988), pp. 31-32.
3. G. M. Kolesova, N. G. Kapitanova, and L. D. Raikhman, Pharmacologic Correction of Oxygen-Dependent Pathological States [in Russian], Moscow (1984), pp. 18-19.
4. A. A. Korneev, "Investigation of some oxygen-dependent processes on the isolated contracting heart during hypoxia," Dissertation for the Degree of Candidate of Biological Sciences, Moscow (1985).
5. A. A. Korneev and L. D. Korobkov, Physiological and Clinical Problems of Adaptation to Hypoxia, Hypodynamia, and Hyperthermia [in Russian], Vol. 2, Moscow (1981), pp. 73-76.
7. L. D. Luk'yanova, Physiological Problems in Adaptation [in Russian], Tatu (1984), pp. 128-131.
8. L. D. Luk'yanova, A. A. Korneev, and O. A. Popova, Scientific Methods and Technical Means of Protection of Ischemic Tissues [in Russian], Ustinov (1986), pp. 14-16.
9. L. D. Luk'yanova, Molecular Mechanisms and Regulation of Energy Metabolism [in Russian], Pushchino (1987), pp. 153-161.
10. L. D. Luk'yanova, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1989), pp. 11-44.
11. E. P. Titovets, Metabolic Regulation of Physiological States [in Russian], Pushchino (1986), p. 34.
12. E. P. Titovets, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1988), p. 124.
13. V. I. Torshin, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1988), pp. 125-126.
14. L. Danielson and L. Ernster, Nature, **194**, 155 (1962).
15. J. T. Flaherty et al., J. Molec. Cardiol., **12**, 43 (1980).

MOLECULAR MECHANISMS OF THE MEMBRANE-PROTECTIVE EFFECT OF LITHIUM NICOTINATE IN CHRONIC STRESS

V. I. Kresyun and Ya. V. Rozhkovskii

UDC 616-018.1-008.939.15:576.314]-02:613.863

KEY WORDS: lithium nicotinate; chronic stress; antiradical protection system; erythrocyte membranes.

One of the leading trigger mechanisms in the development of stress-induced injuries is activation of free-radical oxidation (FRO) of lipids, which leads to changes in membrane structures, to disturbance of lipid–lipid and lipid–protein interactions, and modification of the properties of membrane enzymes [3, 9]. The organization of resistance of the body's adaptation to stress can be facilitated and accelerated with the aid of tranquilizers and antioxidants. One representative of these groups is lithium nicotinate, an atypical tranquilizer with a nootropic component of action, whose stress-protective properties are due to its membranotropic activity and its correcting influence on energy metabolism [5].

During this investigation several parameters of the morphological and physiological state of the cell membranes were studied under conditions of chronic stress and its prevention by lithium nicotinate. The total content of phospholipids (PL) and their fractions, total cholesterol (ChS), the ratio ChS/PL, and also the state of structural antioxidants and activity of enzymes detoxicating active forms of oxygen and lipid peroxides, were studied. The erythrocyte membrane was chosen as test object, for it allows the function of the membranes of the body as a whole to be judged [15].

Department of General and Clinical Pharmacology, N. I. Pirogov Odessa Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 7, pp. 63-65, July, 1990. Original article submitted December 27, 1989.

