One of the components of the intimate mechanisms of the antihypoxic action of pyracetam is therefore its ability to inhibit LPO.

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ANTIHYPOXIC ACTIVITY OF HEME-PEPTIDES OF CYTOCHROME C

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The search for effective antihypoxic agents among natural and synthetic compounds is necessitated by the importance of the hypoxic factor in the genesis and development of diseases of varied etiology. The hypoxic factor also plays an important role in the recovery period after exposure of the healthy individual to extremal factors of varied nature. Among pharmacological agents which increase working capacity may be mentioned antihypoxic agents of the electron carrier group [1], which possess strong electron-acceptor properties, such as, for example, cytochrome c.

There are data in the literature on the antihypoxic properties of cytochrome c [8, 9]. It has proved effective in the treatment of massive blood loss and during resuscitation measures [4, 7].

There is also evidence of the antihypoxic action of a heme-octapeptide obtained from cytochrome c by enzymic hydrolysis [14] This suggests that the clinical effect of cytochrome c is due to the action of heme-peptides, which are its metabolic products.

The aim of this investigation was to compare the antihypoxic activities of several heme-peptides of cytochrome c when given prophylactically and therapeutically in the recovery period after acute hypobaric hypoxia (AHBH) to mice with different types of hypoxic resistance.

EXPERIMENTAL METHOD

In a model of AHBH [2] on male mice (BALB/c × B10CW; CW × ASn × CC57W tetrahybrids) weighing 16-22 g, in the experiments of series I the antihypoxic activity of five heme-containing compounds was compared: hemin (Sigma, USA), heme c, heme-nonapeptide, heme-peptide 1-65,

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TABLE 1. Antihypoxic Activity of Heme-containing Compounds on Model of AHBH, when Given Prophylactically

Series	Parameter	Control	Hemin	Heme c	Heme-nonapeptide	Heme-peptide 1-65	Cytochrome c
A	*n*	12	6	6	6	6	6
	t_{res}, min	7,37±3,12	6,56±4,69	2,23±0,20	6,61±4,69	10,05±4,41	2,31±0,37
	R.u., %	100±42,3	89,0±63,7	80,3±2,71	89,7±63,6	136,4±59,9	31,3±4,97
	HR fraction	1/6	1/6	0	1/6	1/6	0
	K_p	1,0	1,0	0	1,0	1,0	0
B	*n*	10	10	10	10	10	10
	t_{res}, min	10,30±4,35	7,44±3,39	8,01±3,71	19,7±4,32	17,4±4,17	9,85±5,13
	R.u., %	100±42,2	72,2±32,9	77,8±36,0	191,3±41,90	168,9±40.5	95,6±49,8
	HR fraction	3/10	2/10	2/10	6/10	5/10	3/10
	Kp	1,0	0,67	0,67	2,0	1,67	1.0
C	*n*	12	6	6	6	6	6
	t_{res}, min	9,02±3,26	10,89±6,04	6,83±4,81	21,93±4,50**	16,55±6,02	11,17±6,07
	R.u., %	100±36,1	120,7±67,0	75,7±53,3	243,1±49,9	183,5±66,7	123,8±67,3
	HR fraction	3/12	2/6	1,6	4/6	3/6	2/6
	K_p	1,0	1,33	0,67	2,67	2,0	1,33

Legend. Doses of compounds indicated in text, in series C doses are doubled. *$p < 0.05$; in other cases $p > 0.05$; n) number of mice; t_{res}) reserve time, R.u.) relative units; K_p) coefficient of protection.

and cytochrome c. The heme-nonapeptide and heme-peptide 1-65 are regions 14-22 and 1-65, respectively of the amino-acid sequence of cytochrome c from horse heart, respectively, covalently bound with heme. The methods of obtaining them were described in [6, 12]. Heme c was obtained as described in [5]. Cytochrome c was isolated by the method described in [13]. In the experiments of series II cytochrome c was compared with heme-nonapeptide in the recovery period after AHBH in animals of two types: those with low resistance (LR) to hypoxia, which die at an "altitude" of 11,000 m during the first 5 min, and those with high resistance (HR), readily tolerating that "altitude" (without any visible physiological manifestations) for 30 min or more. The antihypoxic activity of these compounds was analyzed at the 4th and 24th hours of the recovery period, which were critical during repeated exposure to hypoxia of the same intensity [2].

The coefficient of protection was determined by the ratio:

$$K_p = \frac{\text{HR fraction (experiment)}}{\text{HR fraction (control)}}.$$

The results were subjected to statistical analysis by the F and t tests, and expressed for convenience of comparison in relative units (in %).

EXPERIMENTAL RESULTS

Table 1 gives data on the antihypoxic effect of the following compounds when given prophylactically: hemin (0.43 mg/kg or $0.66 \cdot 10^{-6}$ mole/kg), heme c (0.54 mg/kg), heme-nonapeptide (1.1 mg/kg), heme-peptide 1-65 (5.42 mg/kg), and cytochrome c (8.30 mg/kg), which were injected intraperitoneally 15 min (series A) and 30 min (series B) before hypoxia, and also in twice the dose 30 min before hypoxia (series C). The doses were equimolar with respect to heme.

The results (series A) are evidence that none of the compounds specified had any antihypoxic activity under the prophylactic conditions chosen, whereas administration of cytochrome c and of heme c actually reduced the animals' resistance to AHBH a little ($p > 0.05$). The increased resistance to AHBH under the influence of heme-peptide 1-65 ($p > 0.05$) was due to its mobilizing action on the LR animals, and it was not truly antihypoxic (by conversion of LR animals into HR), as is clearly revealed by the structure of the samples (the fraction of HR animals), for this criterion is stronger than determination of the reserve time, which is widely used in practice.

A change in the time of prophylactic administration (to 30 min before hypoxia) while the dose remained unchanged (series B) showed definite tendencies in the character of the antihypoxic resistance of the animals, hemin and heme c reduced it somewhat, but heme-peptide 1-65 and, in particular, heme-nonapeptide increased it considerably, as is clear from the change in

TABLE 2. Antihypoxic Activity of Cytochrome c and Heme-Nonapeptide during Recovery Period after AHBH

Compounds	Parameter	HR + 4 h	HR + 24 h	LR + 4 h	LR + 24 h
Control	n	6	5	10	5
	t_{res}, min	4,2±0,49	16,8±2,81	6,13±2,32	14,0±3,81
	R.u., %	100±11,7	100±16,7	100±37,8	100±27,2
	HR fraction	0	4/5	2/10	3/5
	K_p	—	1,0	1,0	1,0
Cytochrome c	n	6	5	4	6
	t_{res}, min	5,17±2,97	13,4±4,07	11,1±5,14	14,6±3,51
	R.u., %	123,1±70,7	79,8±24,2	181,0±83,8	104,3±25,1
	HR fraction	1/6	3/5	2/4	4/6
	K_p	1,0	0,75	2,5	0,83
Heme-nonapeptide	n	6	5	5	6
	t_{res}, min	17,1±2,84*	18,0±2,0	20,0±0,0**	17,25±2,75
	R.u., %	407,1±67,6	107,1±11,9	326,3±0,0	123,2±19,6
	HR fraction	5/6*	4,5	5/5	5/6
	K_p	5,0	1,0	5,0	1,39

Legend. The maximal reserve time was 20 min. *$p < 0.01$, **$p < 0.001$.

the structure of the sample and the value of the coefficient of protection (1.67 and 2.0 respectively).

When the compounds were injected in a double dose 30 min before AHBH (series C) a certain antihypoxic effect of cytochrome c was observed ($K_p = 1.33$), whereas the antihypoxic effect of heme-nonapeptide was considerable ($K_p = 2.67$; $p < 0.05$).

Data on the antihypoxic activity of cytochrome c and heme-nonapeptide in the early stages of the recovery period after AHBH are given in Table 2.

The compounds were injected immediately after the first exposure to hypoxia in doses of 8.3 and 1.1 mg/kg, and resistance to a second exposure to hypoxia was determined in animals of different types after 4 and 24 h. Administration of the compounds immediately after the first hypoxia was due to the fact that a state of relative hyperoxia develops in the animals in this period, and is accompanied by activation of lipid peroxidation.

The results of these experiments showed that cytochrome c has a certain antihypoxic effect at the 4th hour of the recovery period, which is more marked in the case of LR animals ($K_p = 2.5$; $p < 0.2$), whereas no such effect was observed in the later stages of the recovery period (24 h), evidence that this compund has an unimportant role in the formation of antihypoxic resistance in the later stages of the recovery period.

Heme-nonapeptide has a marked antihypoxic action in the early stages of the recovery period ($p_{hr} < 0.01$; $p_{lr} < 0.001$), and in its degree of effectiveness, it is close to the universal antihypoxic agent gutemin; this effect, moreover, is preserved at the later stages in animals of both types.

The results are evidence that the antihypoxic effect of these compounds is determined not only by the time and dose of the substances injected for prophylactic purposes, but also by the background against which these compounds are tested. An essential role is played here by the genetically determined type of metabolism, which is manifested as the formation of two types of animals in the population: HR and LR individuals.

As was pointed out previously [3], the effectiveness of the antioxidants tested for prevention and treatment of hypoxic states lies not simply in the increase of reserve time, but also in the change in population structure due to an increase in size of the fraction of HR animals.

We know that in the early periods after AHBH lipid peroxidation is strongly activated [10]. It has also been shown that heme-peptides possess high peroxidase activity [11] and inhibit lipid peroxidation [15]. It can therefore be tentatively suggested that its antihypoxic activity is linked with its peroxidase activity.

The high antihypoxic activity observed in heme-nonapeptide during prevention and treatment of AHBH suggests that this compound is promising for the correction of hypoxic injuries.

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EXPERIMENTAL STUDY OF THE ANTIHYPOXIC PROPERTIES OF IBUPROFEN AND OTHER NONSTEROID ANTIINFLAMMATORY AGENTS

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KEY WORDS: hypoxia; ibuprofen; orthofen; nonsteroid antiinflammatory agents; inhibitors of prostaglandin biosynthesis.

The pathogenesis of hypoxic states includes several stages, some of them connected with disturbance of the function of the arachidonic acid cascade and with the formation of a number of icosanoids (prostaglandins — PG, prostacycline, thromboxanes, etc.) [2]. In view of data showing that PG can cause disturbances of vascular resistance, which plays an essential role in hypoxic states [3, 6], it was interesting to study the action of various inhibitors of PG biosynthesis, namely nonsteroid antiinflammatory agents (NSAIA) such as ibuprofen, orthofen (diclofenac sodium), acetylsalicylic acid (aspirin), and butadione, on various models of experimental hypoxia.

EXPERIMENTAL METHOD

The effect of NSAIA on the survival time of animals was studied on a model of acute hypoxic hypoxia with hypercapnia [1], using noninbred male albino mice weighing 20-22 g. Each mouse was placed in a vessel with a capacity of 250 ml, with an airtight lid. The presence of an antihypoxic effect was judged by the change in survival time of the mice (in minutes) in the airtight chamber compared with the control. The drugs were given internally and intra-

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