PHARMACOLOGY AND TOXICOLOGY

Energizing and Antihypoxic Effects of Energostim

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It is demonstrated that energostim, an aqueous mixture of NAD^+, cytochrome *c*, and inosine possesses pronounced antihypoxic activity. Energostim increases by 26-fold the survival rate of rats with low resistance to hypoxia under conditions of hypobaric hypoxia. Administration of energostim before the "rise" in a pressure chamber prevents a decrease in the brain content of ATP in rats with high resistance to hypoxia under the conditions of maximum hypoxia and increases it in rats with low resistance to hypoxia. Energostim has no effect on the survival rate of high-resistant rats in acute hypobaric hypoxia and on the brain content of macroergic substances in low- and high-resistant rats when agony is not developed on "the critical height" and there is no deficiency of macroergic substances.

Key Words: *antihypoxants; resistance to hypoxia; survival rate; macroergic substances; brain; energostim*

The electron-transporting function of the respiratory chain is suppressed in hypoxia at high levels of reduction in the cell. This is associated with impaired electron transport on the first enzyme complex [4] and labilization of membranes and dissociation of cytochrome *c* and coenzyme Q from the inner mitochondrial membrane [12], leading to disturbances in aerobic oxidation and a decrease in the intracellular contents of ATP and creatine phosphate. It can be suggested that a lower degree of reduction in the cell, similar to prevention of the loss of cytochrome *c* or coenzyme Q, will produce protective effect on energy production in hypoxia.

Previously, it was shown that pretreatment with NAD^+, which lowers redox potential in the cell during hypoxia, elicits a pronounced antihypoxic effect [1]. Coenzyme Q and cytochrome *c* produce a similar effect [2,5-11]. The combination of NAD^+—cytochrome *c* exhibits a higher protective activity [3], although this combination, as well as separate application of each substance, does not eliminate the deficiency of adenine nucleotides developing during hypoxia. However, the addition of inosine to this combination, which presumably stimulates *de novo* synthesis of adenine nucleotides, almost completely restores energy homeostasis upon physical overload of the heart [3]. Based on these observations, we decided to evaluate the protective activity of a mixture containing NAD^+, cytochrome *c* and inosine (energostim) on energy production in cells under conditions of acute hypoxic hypoxia.

MATERIALS AND METHODS

Outbred male albino rats weighing 180-200 g were tested for resistance to acute hypoxia. For this purpose the rats were "raised" on a critical height of 11,500 m in a pressure chamber at a rate of 50 m/sec (hypobaric hypoxia), and the time of posture loss and the survival time (ST) before the second agonal inspiration were recorded. The ST of low-resistant (LR) rats was <3 min; the ST of high-resistant (HR) rats was >10 min. The experiments were performed

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3-4 weeks after the test. Energostim — an aqueous mixture of inosine (80 mg/kg), NAD^+ (0.5 mg/kg), cytochrome *c* (10 mg/kg) — was injected intraperitoneally 15 min before the "rise." γ-Hydroxybutyric acid (GHBA, 300 mg/kg) was used as a reference preparation [6]. Control rats were injected with distilled water. The effect of energostim on the time of posture loss and ST was estimated, and the coefficient of protection (C_p) was calculated from the following formula: $C_p=ST_{exp}/ST_{control}$. Antihypoxic effect of an energostim was examined in two experimental series: 1) the animals remained at the critical height to agony and 2) during a period equal to 80% of the mean ST for the given sample. Immediately after hypoxia, the rats were frozen in liquid nitrogen, the brains were collected, and the brain contents of ATP, ADP, and AMP were determined. Adenine nucleotides were measured by high-performance liquid chromatography on Zorbach-Zax columns (Du Pont) using a step gradient. The sum of the adenine nucleotides (ATP+ADP+AMP) and the energy charge were calculated. The results were analyzed by Student's *t* test.

RESULTS

Administration of energostim 15 min before the rise in a pressure chamber produced different effects on the survival rate of LR and HR rats under conditions of acute hypobaric hypoxia. In LR rats, it prolonged the time of posture loss and ST, by 2 and 26 times, respectively, compared with the control. This indicates that energostim considerably increased the resistance of LR rats to hypoxia. The protective activity of energostim was 4.6-fold higher than that of the antihypoxant GHBA. However, similar to GHBA, energostim had no appreciable effect on the time of "posture loss" and ST in HR rats (Table 1). Thus, judging from survival rate of LR rats under conditions of acute hypoxia, energostim acts as an antihypoxant much more potent than GHBA.

Since energy metabolism is affected by hypoxia, we examined the effects of energostim on the brain pool of macroergic substances in rats subjected to acute hypoxia. It should be noted that the initial brain contents of these substances were practically the same (Table 2).

When the animals were left on the critical height until agony, the ATP and ADP contents as well as the total adenine nucleotide content and the energy charge significantly decreased, while the AMP content increased (Table 2). The relative changes in these parameters in LR and HR rats were practically the same.

Administration of energostim 15 min before the rise in a pressure chamber not only prevented a decrease in brain ATP content, sum of adenine nucleotides, and energy charge in agonizing rats, but increased these parameters compared with control rats (Table 2). The contents of ADP and AMP remained at the control level. In HR rats, the effect of energostim was the same as that of GHBA, while in LR rats its effect was higher (Table 2).

When the time during which the rats were on critical height was shortened by 20% (agony was not developed) the brain contents of ATP and AMP, and the sum of adenine nucleotides increased particularly in LR rats (Table 2). However, the ADP content decreased to the same degree as in agony (Table 2). Enhanced synthesis of ATP probably reflects the activation of urgent compensatory mechanisms responsible for the maintenance of energy homeostasis at the early stages of hypoxia that precede a decrease in the contents of macroergic substances in severe hypoxia during agony.

In this case energostim administered 15 min before acute hypobaric hypoxia produced no substantial effect on the adenine nucleotide pool. The increase in ATP content, the sum of adenine nucleotides, and the energy charge were preserved (Table 2). Consequently, energostim did not activate compensatory mechanisms responsible for the maintenance of energy synthesis in the brain. In GHBA-treated rats, the ATP content was the same as in the controls, i.e., energostim probably inhibited compensatory reactions at this stage of hypoxia (Table 2).

Thus, the energizing effect of energostim is realized only when the deficiency of macroergic sub-

TABLE 1. Effects of Energostim and GHBA on the Time of Posture Loss, Survival Time (ST), and Coefficient of Protection (C_p)

Experimental conditions	LR			HR		
	time of posture loss, min	ST, min	C_p	time of posture loss, min	ST, min	C_p
Control	0.40	1.5±0.6	—	1.3	23.0±3.1	—
Energostim	0.75*	39.0±4.5**	26.3**	1.0	29.4±3.9	1.3
GHBA	0.44	6.9±1.2	4.6**	0.4	21.0±2.5	0.9

Note. *$p<0.01$, **$p<0.001$ compared with the control.

TABLE 2. Effect of Energostim and GHBA on the Brain Contents of Macroergic Substance, Sum of Adenine Nucleotides and Energy Charge in LR and HR rats

Experimental conditions, group of rats		ATP	ADP	AMP	Sum of adenine nucleotides	Energy charge
		μmol/g wet weight				
Control	LR	2.29±0.221 (100)	0.78±0.055 (100)	0.25±0.018 (100)	3.33±0.108 (100)	0.81±0.021 (100)
	HR	2.65±0.342 (100)	0.65±0.180 (100)	0.18±0.018 (100)	3.56±0.181 (100)	0.86±0.021 (100)
Agony						
Acute hypobaric hypoxia						
	LR	1.53±0.182 (67)*	0.62±0.044 (79)	0.35±0.022 (140)*	2.52±0.079 (76)*	0.73±0.042 (90)
	HR	1.64±0.181 (62)*	0.53±0.210 (82)	0.24±0.210 (133)*	2.42±0.058 (68)*	0.79±0.039 (92)
Acute hypobaric hypoxia+ energostim	LR	3.16±0.341 (138)*	0.80±0.046 (103)	0.26±0.023 (104)	4.22±0.110 (127)*	0.85±0.027 (105)
	HR	2.86±0.381 (108)	0.64±0.180 (99)	0.19±0.016 (106)	3.69±0.082 (104)	0.86±0.017 (100)
Acute hypobaric hypoxia+ GHBA	LR	2.52±0.280 (110)	0.67±0.050 (86)	0.30±0.024 (121)	3.49±0.109 (105)	0.82±0.027 (101)
	HR	2.84±0.420 (107)	0.61±0.200 (94)	0.19±0.016 (106)	3.64±0.075 (102)	0.86±0.027 (100)
Agony was not developed**						
Acute hypobaric hypoxia						
	LR	3.02±0.481 (132)	0.63±0.271 (81)	0.29±0.152 (116)	3.94±0.129 (118)	0.85±0.025 (105)
	HR	3.02±0.521 (114)	0.55±0.120 (85)	0.21±0.090 (117)	3.78±0.259 (106)	0.87±0.017 (101)
Acute hypobaric hypoxia+ energostim	LR	3.14±0.510 (137)	0.62±0.242 (79)	0.27±0.142 (106)	4.03±0.100 (121)	0.86±0.030 (106)
	HR	3.21±0.580 (121)	0.56±0.131 (84)	0.22±0.091 (122)	3.99±0.138 (112)	0.87±0.029 (101)
Acute hypobaric hypoxia+ GHBA	LR	2.20±0.331 (96)	0.92±0.391 (118)	0.23±0.100 (92)	3.35±0.176 (101)	0.80±0.020 (99)
	HR	3.15±0.551 (119)	0.62±0.161 (95)	0.19±0.081 (106)	3.96±0.156 (111)	0.88±0.021 (102)

Note. *$p<0.05$. Percent of changes compared with the control is given in parentheses. **Time of during which rats were left on a height of 11,500 m was 80% of the ST for this sample.

stances occurs at certain stages of acute hypoxia. Taking into consideration the composition of energostim, it can be suggested that its protective effects are realized via 1) normalization of intracellular redox potential under the action of NAD^+ and restoration of electron transport in the NAD—coenzyme Q complex, which is suppressed at the early stages of hypoxia [4], and 2) restoration of the function of the cytochrome link of respiratory chain, which is provided by exogenous cytochrome *c*. Further investigations are necessary to clarify these issues and evaluate the role of inosine in these processes.

Nevertheless, it should be noted that energostim is a potent antihypoxic agent increasing the resistance of predominantly LR rats to acute hypoxia and correcting the energy metabolism disorders in the brain much more effectively than GHBA. Thus, energostim is a prospective candidate for a drug protecting the organisms against adverse effects of hypoxia. Further investigations are necessary to elucidate the mechanisms of its action.

REFERENCES

1. V. V. Gatsura, in: *Pharmacological Correction of Energy Metabolism in Ischemic Myocardium* [in Russian], Moscow (1993), p. 252.
2. M. D. Draguzya, S. E. Manoilov, I. V. Oblapenko, *et al.*, in: *Special and Clinical Physiology Hypoxic States* [in Russian], Vol. 1, Kiev (1979), pp. 54-58.
3. N. V. Karsanov, L. E. Gogiashvili, and E. V. Selikhova, *Vopr. Med. Khimii*, **39**, No. 2, 50-55 (1993).
4. L. D. Luk'yanova, in: *Pharmacological Correction of Hypoxic States* [in Russian], Moscow (1991), pp. 11-44.
5. M. D. Mashkovskii, *Drugs* [in Russian], Vol. 2, Moscow (1995), pp. 66-68.
6. L. D. Luk'yanova (Ed.), *Methodological Recommendations for Screening of Antihypoxants* [in Russian], Moscow (1990).
7. L. N. Sernov and S. V. Zyuzina, *Byull. Eksp. Biol. Med.*, **121**, No. 5, 533-535 (1996).
8. H. Ishida, K. Hosone, and M. Okuda, *J. Mol. Cell. Cardiol.*, **13**, No. 2, 64 (1981).
9. Y. Karlsson, C. Sylven, and E. Jansson, *Mol. Cell. Biochem.*, **84**, 59-60 (1988).
10. M. Miyahare, E. Okimasu, and H. Mikasa, *Biochim. Biophys. Acta*, **233**, No. 1, 139-150 (1984).
11. N. Susuki, T. Nakamura, H. Ishida, *et al.*, *Chem. Pharm. Bull.*, **33**, 2896-2903 (1985).
12. A. Toleikis, *J. Mol. Cell. Cardiol.*, **12**, No. 1, 169 (1980).