Functional and Biochemical Correlations in Hypoxic Shock: Cooperative Influence of Regulatory Peptides

S. V. Antonova, M. Ya. Akhalaya, A. A. Baizhumanov, S. V. Shestakova, E. N. Goncharenko, Ya. V. Krushinskaya, A. V. Orlov, N. A. Sokolova, A. A. Kamenskii, S. B. Parin, and I. P. Ashmarin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 400-402, October, 1997
Original article submitted June 3, 1996

In hypoxic shock, serotonin content in the spleen and epinephrine content in the adrenals are lowered. Administration of the peptide mixture FMRFa—thyroliberin—semax 15 min prior to hypoxia significantly prolongs the time of posture loss and abolishes the effect of hypoxia on the level of biogenic amines; the concentration of lipid peroxidation products decreases considerably.

Key Words: *hypoxic shock; serotonin; catecholamines; FMRFa; thyrotropic hormone; semax; lipid peroxidation*

Hypoxic shock leads to considerable changes in tissue contents of biogenic amines and activates lipid peroxidation (LPO), which is accompanied by changes in functional activity. These changes to a certain extent can be associated with activation of the opioid system. It was demonstrated that naloxone, an antagonist of opioid receptors, in some cases elicits a protective effect in hypoxic shock [3]. Some endogenous antagonists of opioids: fragments of adrenocorticotropic hormone (ACTH), thyrotropin releasing hormone (TRH), and FMRFa [6,7,10], can be used for correction of metabolic shifts caused by hypoxic shock. In the organism these peptides are functionally active in small or extremely small doses [2]. It is likely that the protective effect results primarily from cooperative activity of regulatory peptides.

In order to check up this hypothesis we analyzed changes in functional and biochemical parameters of hypoxic shock at different moments of its development and during the after-shock period in intact animals and in those pretreated with a mixture consisting of FMRFa (Phe-Met-Arg-Phe-amide), TRH, and semax (ACTH(4-7)-Pro-Gly-Pro) in subthreshold doses.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weight 180-250 g. The model of acute hypobaric hypoxia was employed. Experimental and control rats were "lifted" at a height of 10,500-11,000 m in a barocamera for 1 min and observed for 5 min. Resistance to hypoxia was estimated by the method [1]. The posture loss time (PLT), i.e., the time from the beginning of hypoxia up to complete relaxation of skeletal muscles, was measured. Control rats were intraperitoneally injected with normal saline (1 ml/kg) 15 min before the beginning of "rise." Experimental rats were given an injection of the peptide mixture or naloxone. Subthreshold doses for each peptide were determined in preliminary experiments by their effects on functional parameters characterizing the development of hypoxic shock. For intra-

Department of Biophysics, Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University; Department of Human and Animal Physiology and Biochemistry, N. I. Lobachevskii Nizhnii Novgorod State University

0007-4888/97/0010-0971$18.00

TABLE 1. Tissue Contents of Biogenic Amines in Rats Subjected to Hypoxia

Group of rats	Serotonin, μg/g	Epinephrine, μg/g	Norepinephrine, μg/g
1 min after hypoxia			
Normal saline	3.6±0.4 (100±11)	368±33 (100±9)	249±19 (100±8)
Peptides	4.1±0.4 (114±11)	348±47 (95±13)	262±27 (106±11)
Hypoxia+normal saline	2.4±0.2 (67±5)**	260±24 (71±6)**	199±11 (80±4)*
Hypoxia+peptides	3.9±0.4 (108±11)	295±38 (80±10)	183±12 (74±5)***
20 min after hypoxia			
Normal saline	3.8±0.2 (100±5)	417±48 (100±11)	298±15 (100±5)
Peptides	3.3±1.0 (87±26)	471±21 (113±5)	304±44 (102±15)
Hypoxia+normal saline	3.6±0.6 (95±15)	438±50 (105±12)	289±21 (97±7)
Hypoxia+peptides	4.0±0.4 (105±10)	459±29 (110±7)	307±15 (103±5)

Note. Serotonin concentration was determined in the spleen; catecholamine concentration was determined in the adrenals. Here and in Table 2: *$p<0.05$, **$p<0.02$, ***$p<0.01$ compared with the control (normal saline). Percentage is given in parentheses.

peritoneal administration these doses were: FMRFa and TRH 0.5 mg/kg and semax 0.05 mg/kg. These doses were used in the peptide mixture. Naloxone was injected intraperitoneally in a dose of 1 mg/kg.

Serum content of LPO products reacting with 2-thiobarbituric acid was determined as described elsewhere [5] using a Specord M-40 spectrophotometer. Serum concentration of malonic dialdehyde in intact rats was 0.53 nmol/ml.

The contents of biogenic amines in the adrenals and spleen were determined by standard methods [8,9].

The concentrations of steroid hormones were measured fluorimetrically [4].

The results were analyzed with the use Fischer—Student *t* test.

RESULTS

In rats treated with the peptide mixture 15 min before hypoxia, PLT was significantly ($p<0.001$) longer than in control rats: 125.7±10.0 *vs.* 45.5±3.31 sec, i.e., the peptides elicited a pronounced protective effect comparable to that of naloxone, an antagonist of opioid receptors, which also significantly prolonged PLT ($p<0.025$).

After 5 min of hypoxia, serotonin content in the spleen and epinephrine content in the adrenals decreased significantly. The peptide mixture had no statistically significant effects on these parameters, but prevented their decrease in response to hypoxia; the contents of serotonin and epinephrine did not differ from the control values (Table 1). Hypoxia led to a 20% decrease in norepinephrine content in the adrenals, and the peptide mixture did not eliminate this effect (Table 1).

The decrease in the contents of biogenic amines was observed for 20 min (Table 1).

Hypoxia did not cause any significant decrease in the content of steroid hormones in adrenals, while the peptide mixture decreased this parameter by 68%. This decrease was observed after administration of the mixture 15 min before hypoxia; the effect lasted 20 min (Table 2).

A significant increase in serum concentration of malonic dialdehyde (192% above the normal level, $p<0.01$) was observed when the changes in the contents of biogenic amines disappeared (on the 20th min). In peptide-treated rats this increase amounted for 66% ($p<0.05$). Without hypoxia, the peptide mixture had no significant effect on the malonic dialdehyde content.

TABLE 2. Adrenal Content of Steroid Hormones in Rats Subjected to Hypoxia

Group of rats	Time after hypoxia termination, min	
	1	20
Normal saline	22.6±1.8 (100±8)	21.2±1.6 (100±7.4)
Peptides	15.3±0.9 (68±6)***	21.1±0.9 (99.5±4.5)
Hypoxia+normal saline	18.4±0.9 (83±5)	23.9±1.2 (112.7±4.9)
Hypoxia+peptides	15.1±0.5 (67±3)***	19.3±0.9 (91.2±5.0)

Thus, our results show that the protective effect of a mixture of FMRFa, TRH, and semax in subthreshold doses on functional parameters (posture loss time) correlates with its influence on the changes in the levels of biogenic amines that accompany stress reaction under conditions of acute hypoxia. The decrease in the concentration of LPO products induced by the peptide mixture supports the concept that antistress and antishock mechanisms underlie the cooperative action of subthreshold doses of the peptides.

REFERENCES

1. N. A. Agadzhanyan and B. N. Torshin, *Byull. Eksp. Biol. Med.*, **102**, No. 12, 683-685 (1986).
2. I. P. Ashmarin and M. A. Kamenskaya, *Itogi Nauki Tekhniki*, Ser. *Fiziol. Cheloveka. Zhivotn.* [in Russian], Vol. 34, Moscow (1988).
3. E. O. Bragin and V. V. Yasnetsov, *Ibid.*, Vol. 47, (1991).
4. Yu. A. Pankov and I. Ya. Usivatova, in: *Methods for Investigation of Some Hormones and Transmitters. New Equipments and Methods* [in Russian], Vol. 3, Moscow (1965), pp. 137-145.
5. T. Asakava and S. Matususita, *Lipids*, **15**, No. 1, 137-140 (1980).
6. G. Coppi and A. Falcone, *Drugs Exp. Clin. Res.*, **18**, No. 5, 173-177 (1992).
7. J. W. Holaday, *Annu. Rev. Pharmacol. Toxicol.*, **23**, 541-594 (1983).
8. G. Metcolf, *Anal. Biochem.*, **57**, No. 1, 316-320 (1974).
9. E. P. Miller and R. P. Maikel, *Life Sci.*, **9**, Pt. 1, No. 13, 747-752 (1950).
10. R. B. Raffa, *Peptides*, **9**, 915-922 (1988).