7. F. Z. Meerson, Adaptation, Stress, and Prophylaxis [in Russian], Moscow (1981), pp. 200-215.
8. É. A. Migas, Byull. Éksp. Biol. Med., No. 5, 541 (1976).
9. É. A. Migas and T. N. Nilova, Byull. Éksp. Biol. Med., No. 9, 316 (1980).
10. E. V. Moreva and V. V. Bul'on, Farmakol. Toksikol., No. 6, 625 (1979).
11. V. S. Raitses, T. N. Semenyuk, and L. I. Aftanas, in: Stress and Adaptation [in Russian], Kishinev (1978), pp. 191-192.
12. N. R. Shatunov and A. I. Sytinskii, in: The Nervous System [in Russian], Vol. 3, Leningrad (1962), pp. 12-16.
13. L. Eroglu and S. Atamer-Simsek, Arzneimit.-Forsch., 30, 2115 (1980).
14. B. J. Hirschowitz, Annu. Rev. Pharmacol., 19, 203 (1979).
15. P. A. Shore, A. Burkhalter, and V. Cohn, J. Pharmacol. Exp. Ther., 127, 182 (1959).

EFFECT OF HYPOXIC AND HYPERCAPNIC ATMOSPHERE AND LOW AMBIENT TEMPERATURE ON FUNCTION OF THE HYPOTHALAMUS—PITUITARY—THYROID SYSTEM IN RATS

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KEY WORDS: ambient atmospheric factors; temperature; hypothalamus—pituitary—thyroid system; physiological state.

Exceptionally close attention is currently being paid to the creation of conditions modifying reactivity of warm-blooded animals. The physiologically most adequate conditions, similar to those leading to the development of natural hypobiosis, are those attainable through the combined use of an altered atmosphere and low ambient temperature. It has been shown that these factors can be used to obtain deep hypothermia [12, 14], to create a stable state of depressed vital activity and of artificial hypobiosis [11], and to develop increased resistance of the organism to the action of stress stimuli of various kinds by measured cooling of rats once or twice under conditions of increasing hypoxia and hypercapnia [3]. However, the pathophysiological mechanisms of interaction between organism and altered atmosphere have not yet been adequately studied. In particular, despite the importance attached to the hypothalamus—pituitary—thyroid system in the formation of resistance [1, 6, 9, 15], there are no data on the characteristics of response of this division of the neuroendocrine system of animals during cooling under conditions of hypoxia and hypercapnia.

This paper describes a study of the dynamics of hypothalamus—pituitary—thyroid function of rats during cooling in a modified atmosphere.

EXPERIMENTAL METHOD

Experiments were carried out on 239 noninbred male rats weighing 180-200 g. The experiments were carried out in the fall and winter at the same time of day. The animals were cooled to a rectal temperature of 20.4 ± 0.03°C by the method of Giaja and Andjus [12, 14]. The O_2 and CO_2 concentrations in the pressure chamber and the rectal temperature of the rats were measured before and after exposure. Material (hypothalamus) for histologic study was taken after decapitation of the animals 20, 50, 70, 90, and 120 min after the beginning of exposure and 48 h after its end. Blood was taken at the 20th, 70th, and 120th minutes of cooling and 3 h after cooling. To assess the state of thyroid and pituitary function the

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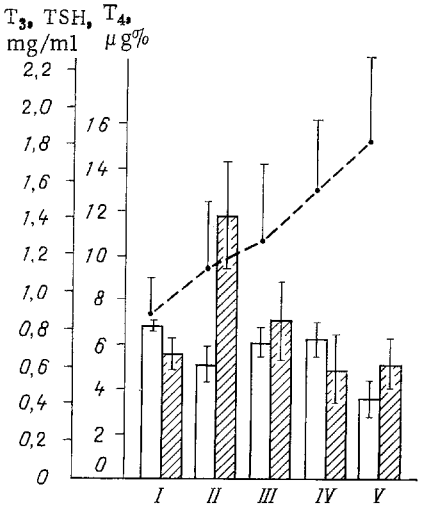

Fig. 1. Effect of cooling of rats under conditions of hypoxia and hypercapnia on serum levels of total T_4 (unshaded columns), T_3 (shaded columns), and TSH (broken line). I) Control; II) 20th minute of combined exposure; III) 70th minute; IV) 120th minute; V) 3 h after exposure. Ordinate, concentrations of T_4 (in μg%) and of T_3 and TSH (in mg/ml).

serum concentration of total thyroxine (T_4), total triiodothyronine (T_3), and of thyrotrophin (TSH) were determined by the use of standard kits for radioimmunoassay: TSHK (TSH), TETRAK (T_4), and TRIK (T_3) (from CEA-IRE-Sorin, France). Radioactivity in the samples was measured by means of an LKB-1280 scintillation Gamma-counter (Sweden). Standard curves were calculated and hormone concentrations in the samples determined in a logit-log coordinate system by the computer of the Gamma-counter used for the measurements, based on a program specially developed for this computer. To detect levels of activity of the neurosecretory cells (NSC) of the supraoptic (SON) and paraventricular (PVN) nuclei the hypothalamus was fixed in Bouin's fluid. Paraffin sections were stained by the Gomori–Gabe method and counterstained by Heidenhain's method or with methylene blue by Maiorova's method [4]. The volume of the nuclei and nucleoli was measured in NSC by means of a screw ocular micrometer (MOV-15) under a total magnification of ocular 15 and objective 90. The volume of the nuclei was calculated on the assumption that they have the shape of an ellipsoid of rotation ($V = (\pi/6)Dd^2$), and the volume of the nucleoli was calculated by the formula for the volume of a sphere. The data were subjected to statistical analysis and the significance of differences was determined by Student's t test.

EXPERIMENTAL RESULTS

The serum T_4 concentration fell in the first 20 min of exposure to 5.1 ± 0.4 μg% (control 6.7 ± 0.16 μg%; $P < 0.05$; Fig. 1). The blood T_3 level rose significantly to 1.42 ± 0.13 ng/ml (control 0.66 ± 0.4 ng/ml; $P < 0.05$). The TSH concentration did not change significantly and after 20 min it was 1.12 ± 0.16 ng/ml (control 0.88 ± 0.09 ng/ml; $P > 0.05$) (Fig. 1). The fall in the serum T_4 concentration suggests that thyroid function was depressed in the initial period of exposure. The increase in the blood T_3 concentration evidently does not reflect any change in secretory activity of the thyroid but depends in this case on intensification of extrathyroid T_3 neogenesis. The volumes of the nuclei of NSC in SON and PVN at these times were increased compared with the corresponding parameters of intact rats to 757.5 ± 10.1 μ^3 (control 688.6 ± 11.8 μ^3) and 752.9 ± 12.2 μ^3 (control 686.0 ± 10.6 μ^3) respectively ($P < 0.001$; Fig. 2). The volumes of the nucleoli, on the other hand, were significantly reduced in SON to 18.5 ± 0.3 μ^3 (control 22.0 ± 0.5 μ^3) and in PVN to

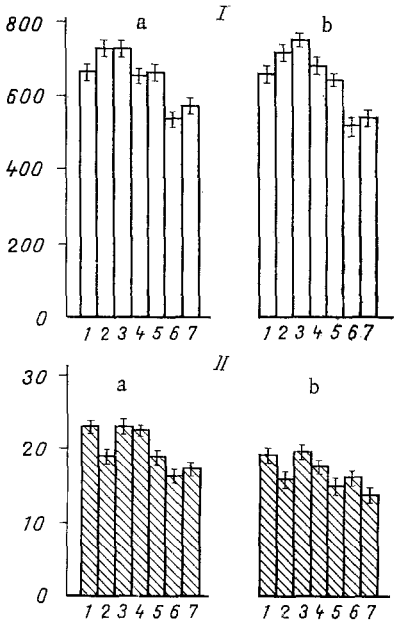

Fig. 2. Effect of cooling rats under conditions of hypoxia and hypercapnia on size of nuclei (I) and nucleoli (II) of NSC of anterior hypothalamus. a) SON; b) PVN. 1) Control; 2) 20th minute, 3) 50th minute, 4) 70th minute, 5) 90th minute, 6) 120th minute of exposure; 7) after exposure. Ordinate, volume of nuclei and nucleoli (in μ^3).

16.8 ± 0.4 μ^3 (control 19.2 ± 0.3 μ^3; $P < 0.001$; Fig. 2). Considering that the size of the nucleoli is one of the most important parameters of neurosecretion synthesis by cells of the hypothalamus [5], it can be postulated that at this stage of exposure activity of SON and PVN cells was depressed. This conclusion is contradicted by the observed increase in size of the NSC nuclei at the 20th minute. However, investigations have shown that during exposure to stress, inhibition of secretion formation in certain cases may be accompanied by an increase in volume of NSC nuclei [2].

At the 70th-120th minute of exposure a tendency was found for the serum T_4 level to rise, accompanied by a decrease in the T_3 concentration that was significant ($P < 0.05$) compared with an exposure of 20 min. The TSH concentration continued to rise at these times. The hormone concentration at the 120th minute was significantly higher than that in rats of the control group ($P < 0.05$; Fig. 1).

At these times, compared with the initial period, thyroid activity was thus intensified somewhat, and the thyrotrophic function of the adenohypophysis was enhanced. The volumes of the nuclei of NSC in SON and PVN remained increased at the 50th minute of exposure, but at the 70th minute they were significantly reduced in size (Fig. 2). The trend of the change in volumes of nucleoli of SON and PVN cells compared with that observed after an exposure of 20 min was changed; their dimensions were significantly increased ($P < 0.001$; Fig. 1). Consequently, activity of SON and PVN cells was increased at these times. In the final stages of cooling (90th-120th minute) a marked decrease in size of these intracellular structures was observed. The volumes of the nuclei of NSC in SON and PVN were 567.8 ± 12.6 and 538.8 ± 9.5 μ^3 respectively; the volumes of the nucleoli were 15.5 ± 0.3 μ^3 and 16.4 ± 0.3 μ^3 ($P < 0.001$; Fig. 2). This character of change in the morphological and functional parameters in the later stage of exposure is evidence of marked depression of the functional state of the anterior hypothalamic nuclei.

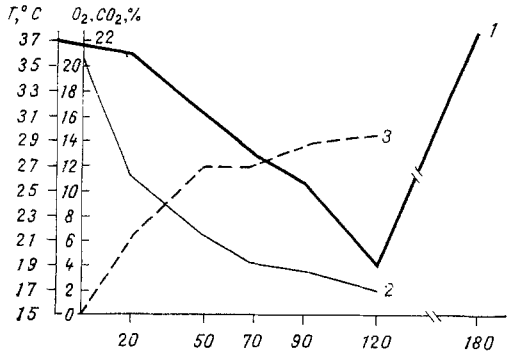

Fig. 3. Dynamics of rectal temperature (1) and change in concentrations of O_2 (2) and CO_2 (3) in pressure chamber at various stages of combined exposure and thereafter. Abscissa, time (in h); ordinate, temperature (in °C), concentrations of CO_2 and O_2 (in rel. %).

In the recovery period (3 h after cooling) the serum T_4 level was 47% less than in intact rats. The T_3 concentration did not differ significantly from the level of the hormone found in rats of the control group. The blood TSH concentration reached its highest value, namely 1.8 ± 0.21 ng/ml (Fig. 1). The volumes of the nuclei and nucleoli of NSC in SON and PVN remained significantly lower than in intact animals 48 h after exposure ($P < 0.001$; Fig. 2).

Cooling of the animals under conditions of hypoxia and hypercapnia thus caused phasic changes in the functional state of this system. If combined exposure to these factors is regarded as exposure to a single stress stimulus, the fact that the animals used in the model were in a constantly changing atmosphere must be taken into account. At each stage of exposure, different relations are thus established between the organism and its environment, and these determine the character and magnitude of the functional changes in the hypothalamus–pituitary–thyroid system. After exposure of 20 min the rats' temperature fell but not significantly (36.0 ± 0.12°C), whereas the CO_2 concentration in the pressure chamber rose to 6.4 ± 0.3 vol.% and the O_2 concentration fell to 11.0 ± 0.5 vol.%. Correspondingly, reorganization of the system at this stage evidently depends largely on the hypoxic-hypercapnic component of combined exposure. Later, increasing hypoxia and hypercapnia (by the 120th minute the CO_2 concentration in the atmosphere was 14.9 ± 0.15 vol.%, the O_2 concentration 2.5 ± 0.2 vol.%, see Fig. 3), and this affected the intensity of metabolism [8, 10], blocked temperature regulation [10], and began to have an action on the animal similar in direction to that of the cold stimulus, which led to a marked fall of body temperature (to 20.4 ± 0.03°C by the 120th minute). Development of a state of hypothermia is known to be an adequate stimulus for the system under investigation [13, 15]. At later stages of exposure (50th-120th minutes) its response was therefore largely determined by the state of the animal caused by hypothermia. However, development of the hypothermic state under conditions of gradually increasing hypoxia and hypercapnia forms an important feature in the response of the hypothalamus–pituitary–thyroid system. As the results showed, functional changes during combined exposure were confined between certain limits. In a hypoxic and hypercapnic atmosphere, although low temperature is a specific stimulus for this division of the endocrine system, no response of the thyroid or change in thyrotrophic function of the adenohypophysis was observed. The response of nuclei of NSC of the anterior hypothalamus did not develop up to the highest possible level, and in the final stages of cooling it was inhibited. This protects the system against overstrain, and against participation in reactions connected with "over-regulation." The form of response of the hypothalamus–pituitary–thyroid system discovered is of substantial importance in the endocrine regulation of adaptive processes, maintaining the vital activity of the body during exposure to these conditions and in the development of an enhanced level of resistance of the rats to the action of extremal stimuli.

The state of function of the hypothalamus–pituitary–thyroid division of the endocrine system was still changed during the recovery period 3 and 48 h after exposure. This evidently reflects two factors: the degree of damage of the system under investigation and the character of its participation in the regulation of homeostasis, disturbed by exposure.

LITERATURE CITED

1. B. V. Aleshin, Histophysiology of the Hypothalamo–Hypophyseal System [in Russian], Moscow (1971).
2. O. A. Danilova and G. N. Kamel, Probl. Endokrinol., No. 4, 42 (1980).
3. N. V. Korostovtseva, Increased Resistance to Hypoxia [in Russian], Leningrad (1976).
4. V. F. Maiorova, Arkh. Anat., No. 8, 101 (1960).
5. I. A. Okhonskaya, Tsitologiya, No. 8, 925 (1972).
6. A. L. Polenov, Usp. Fiziol. Nauk, No. 1, 28 (1979).
7. N. V. Popovichenko, Role of the Hypothalamic Neurosecretory System in Adaptive Reactions of the Organism [in Russian], Kiev (1973).
8. I. S. Repin, "Pathophysiological characteristics of the effect of hypercapnia on the CNS," Author's Abstract of Doctoral Dissertation, Leningrad (1963).
9. L. N. Simanovskii, Usp. Sovrem. Biol., 68, No. 3, 434 (1969).
10. Z. K. Sulimo-Samuilo, Hypercapnia [in Russian], Leningrad (1971).
11. N. N. Timofeev, Usp. Fiziol. Nauk, 12, No. 4, 2 (1981).
12. R. Andjus, C. R. Acad. Sci. (Paris), 232, 1591 (1951).
13. C. Fortier, A. Delgado, P. Ducommun, et al., J. Can. Med. Ass., 103, 864 (1970).
14. I. Giaja and R. Andjus, C. R. Acad. Sci. (Paris), 229, 1170 (1949).
15. H. Selye, Stress, Montreal (1950).

POTENCY TRIALS OF LOW-MOLECULAR-WEIGHT TETANUS ANTITOXIN

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KEY WORDS: tetanus antitoxin; neuronal receptor; artificial membrane.

There is much evidence in the literature to show that tetanus antitoxin can neutralize free tetanus toxin circulating in the blood stream. At the same time it has been shown that tetanus toxin, when bound with brain membrane receptors [2, 6, 11] or on artificial membranes [10], can itself be bound with antitoxin. The present writers [3, 12, 13] have put forward the idea that low-molecular-weight fragments of antitoxin, possessing specific activity, and with a reduced size of their molecule, might be more effective if they passed more easily through the blood–brain barrier and penetrated into the brain. Investigations [5] have shown that such antitoxin fragments (Fab'-fragments) neutralize more effectively than the ordinary Soviet "Diaferm-3" antitoxin, tetanus toxin bound to protagon, which is a complex of gangliosides with cerebrosides isolated from brain and specifically binding tetanus toxin [15].

The aim of the present investigation was to study the effectiveness of antitoxin with different molecular weight on various stages of tetanus intoxication in laboratory animals of different species.

EXPERIMENTAL METHOD

Pseudoglobulin, extracted with ammonium sulfate from high-affinity horse antitetanus serum, prepared at Stavropol' Research Institute of Vaccines and Sera, was subjected to proteolysis by trypsin, chymotrypsin, papain, and pronase. Better results were obtained by the use of crystalline "Difco" trypsin with an enzyme–substrate ratio of 1:50; pH 8.5; 37°C, for 24 h in the presence of 0.02M cysteine. The coagulated proteins were removed by centrifugation and the supernatant was fractionated on Sephadex G-100 (column 35 × 3.5 cm) in 0.15M NaCl solution, and the fractions collected on an XKOB-1 automatic collector, identified in the agar gel precipitation inhibition reaction between concentrated tetanus toxoid and "Diaferm-3" antitetanus serum, pooled, and concentrated by dehydration against polyethylene-

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