LEVEL OF METABOLISM AND STATE OF PERIPHERAL CATECHOLAMINERGIC SYSTEMS DURING IMMOBILIZATION HYPOTHERMIA IN RATS

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Among the experimental models of hypothermia one which deserves attention is that known as "immobilization" hypothermia, in which the body temperature is lowered without the drastic application of cold or inhibition of temperature-regulating responses by means of drugs [9, 10]. Previously the writers showed that it is possible not only to obtain deep hypothermia in immobilized rats, but also to maintain it for a long time at a below comfortable ambient temperature [1].

The aim of this investigation was to study dependence of the level of metabolism and the catecholamine (CA) concentration on the degree and duration of immobilization hypothermia. Considering the character of reactive changes in the body under conditions of immobilization associated with normothermia [6], particular importance was attached to the study of somatic manifestations of the response to stress and the state of the neurotransmitter and hormonal components of the peripheral division of the sympathoadrenal system.

TABLE 1. CA Concentration in Adrenals of Rats and Somatic Manifestations of Response to Stress during Immobilization and Immobilization Hypothermia (M ± m)

Experimental conditions	CA, μg/g tissue		Weight of organs, %			Change in body weight, %	State of gastric mucosa
	adrenalin	NA	thymus	spleen	adrenals		
Control	643±116	837±130	100±16,6	100±17,5	100±8,5	0	No change
Immobilization							
1 h	625±105	715±128	70,8±12,0*	61,4±9,2*	87,4±12,3	3,6±0,5	No change
4 h	556±86	378±59*	100,1±16,5	102,0±13,1	95,7±8,8	6,4±0,8	Hyperemia, solitary hemorrhages
24h	239±33*	207±31*	82,5±10,3	85,5±14,7	157,9±16,1*	12,2±2,0*	Edema, hyperemia, multiple hemorrhages
Immobilization + hypothermia							
1 h	402±62*,**	394±55*,**	77,0±9,3	119,1±18,7**	100,0±13,4	0,4±0,1	No change
4 h	308±47*	220±31*,**	102,5±10,4	62,8±8,2*,**	102,1±9,2	3,2±0,5**	Ulcers (2; 3.2± 1.4 mm²)
24h	151±19*,**	49±11*,**	66,4±7,3*	48,0±7,5*,**	80,0±8,9**	5,3±0,8**	Ulcers (13; 14.0 ± 3.9 mm²)

Legend. Values taken as 100% for weight of organs were: thymus 137.6 ± 15.5 mg, spleen 199.8 ± 25.6 mg, and adrenals 4.7 ± 0.4 mg. *P < 0.05 compared with control, **P < 0.05 compared with immobilization.

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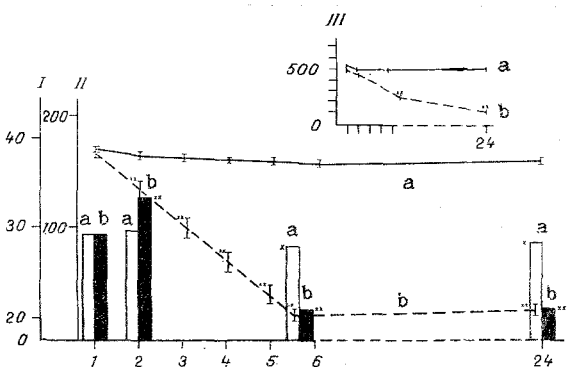

Fig. 1. T_b, OC, and HR during the period of control immobilization and immobilization hypothermia. Abscissa, time (in h); ordinate: I) T_b (in °C); II) OC (in % of normal); III) HR (beats/min); a) immobilization; b) immobilization + hypothermia. Arrows indicate period of cooling at 5°C. OC for unimmobilized rats (55.3 ± 1.4 ml/kg/min) taken as 100%. *) $P < 0.05$ compared with original level, **) $P < 0.05$ compared with control immobilization.

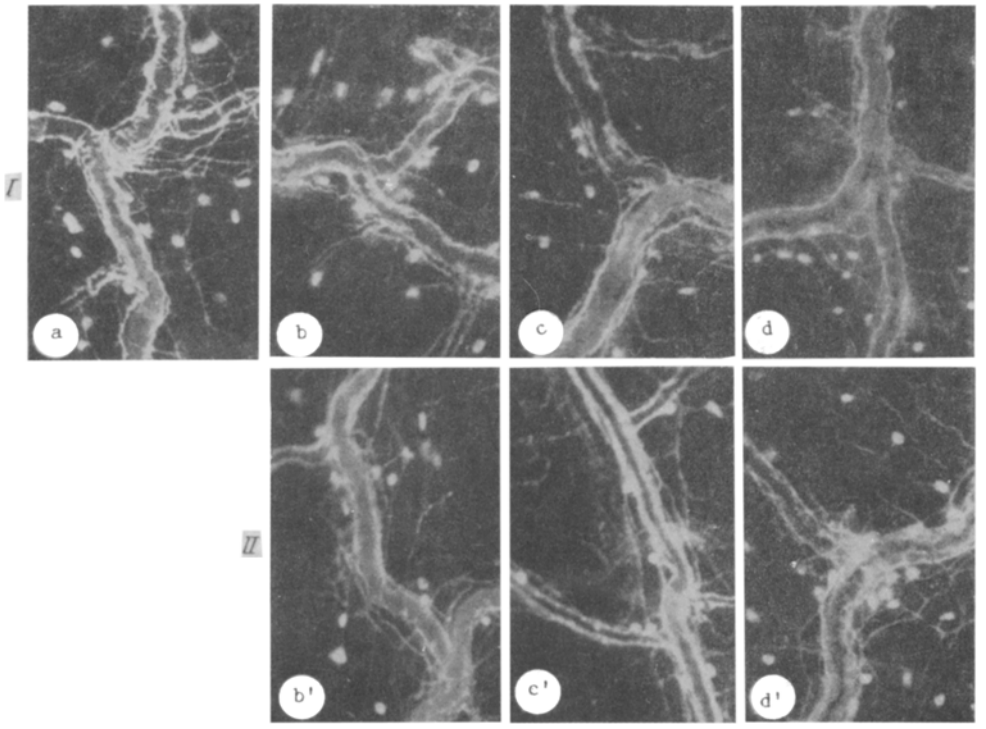

Fig. 2. Adrenergic innervation of dura mater of rats during immobilization hypothermia (I) and control immobilization (II). a) Normal state; b) 1 h, c) 4 h, d) 24 h. Falck–Hillarp method. 250×.

EXPERIMENTAL METHODS

Experiments were carried out on 105 male rats weighing 180-220 g. The animals were fixed by the neck and lumbar region in special frames. To obtain immobilization hypothermia, after fixation for 1 h the rats were placed in a chamber with an ambient temperature (T_{amb}) of 5°C, cooled to a body temperature (T_b) of 20°C, and left for 24 h at T_{amb} = 15°C. Control animals were immobilized at T_{amb} = 19-21°C.

Until stable hypothermia was obtained, T_b was measured every 30 min with a TPÉM-1 electrothermometer, placed in the rectum at a depth of 3.5 cm. The rate of cooling was judged by the value of the ratio ΔT_b/time of maximal cooling, where ΔT_b is the difference between the original T_b and T_b at maximal cooling.

The level of metabolism was calculated from the oxygen consumption (OC), determined in a closed system by the gas analyzer of the "Godart" artificial respiration apparatus, and expressed in ml/kg body weight/min.

The heart rate (HR) was calculated from the ECG, recorded on the ÉÉGP-02 apparatus. OC and HR were determined after fixation for 1 h, after cooling for 1 h, at the time of maximal fall of T_b (4-5 h), and after maintenance of hypothermia for 24 h. Some animals were decapitated at these same times, the relative weight of their lymphoid organs (thymus and spleen) and adrenals was measured, and the presence of pathological changes in the gastric mucosa noted.

The state of the peripheral neurotransmitter system was studied in the dura mater, the blood vessels and tissues of which possessed a well developed adrenergic innervation [3, 6]. The Falck–Hillarp fluorescence-histochemical method, with the writers' modification [5], was used. Concentrations of adrenalin and noradrenalin (NA) in the adrenal medulla were determined by the method in [8].

EXPERIMENTAL RESULTS

In rats subjected to immobilization only, no significant changes in T_b were found throughout the period of observation (Fig. 1). After 1 h of fixation OC and HR were reduced, and OC remained 15% below its initial level for 24 h. Immobilization for 1 h plus cooling at 5°C led to a fairly rapid fall of T_b: in the majority (60%) of animals T_b after 4.7 ± 0.5 h was 20.4 ± 0.4°C, and the remaining rats were similarly cooled after 5.5 ± 0.6 h. If hypothermia was prolonged, in the overwhelming majority (80%) of animals T_b stabilized at 21.1 ± 0.4°C. The metabolic rate was increased by 10-20% after cooling for 1 h, then it gradually fell to reach 30% of its initial level at the time of maximal lowering of T_b, and thereafter it was unchanged for 24 h. Under conditions of immobilization hypothermia, unlike immobilization in a close to thermoneutral environment, the successive stages of somatic manifestations of the stressor response [2], characteristic of the general adaptation syndrome, were not observed (Table 1). In the early stages of cooling (1 h) the decrease in weight of the thymus, spleen, and adrenals, characteristic of the stage of anxiety, was not observed. After 4 h, corresponding to the beginning of adaptation to immobilization stress, opposite changes in weight of the thymus and spleen were observed during hypothermia, whereas the weight of the adrenals was unchanged. In the gastric mucosa ulcers were found at this time. At the end of 24 h the weight of the lymphoid organs in the hypothermic rats was reduced by a greater degree than in the control. Instead of the increase in weight of the adrenals characteristic of the stage of adaptation, they showed atrophy. The number of ulcers in the gastric mucosa at this stage of immobilization hypothermia was considerably increased. At all stages of the experiment the reduction in body weight of the hypothermic rats was less than that of the control animals.

The study of the CA concentration in the adrenals showed that during immobilization hypothermia, just as during control immobilization, there was a gradual fall in adrenalin and NA concentrations in the tissues of this gland, which depended on the duration of exposure to stress. However, under hypothermic conditions the reserves of CA and, in particular, of NA, were exhausted more rapidly than during normothermia.

Comparative fluorescence-histochemical analysis of sections of the dura mater showed that the initial period of immobilization hypothermia (1 h) is accompanied by a smaller decrease of intensity of luminescence of the adrenergic nerves than at the same times of control immoblization (Fig. 2). Later (4 and 24 h) the intensity of fluorescence of adrenergic nerves of rats in a state of hypothermic continued to fall (to 30-40% of normal) and most of them were on the borderline of visibility in the luminescence microscope. Meanwhile, in the control group the corresponding parameters of neurotransmitter activity showed a trend toward normal, and reached 80% of the initial values.

The results of this investigation thus show that during development and prolongation of immobilization hypothermia correlation is found between the metabolic rate and the state of the peripheral catecholaminergic systems. This is exhibited most demonstratively in the

early stages of cooling (1 h), when activation of metabolism, aimed at maintaining temperature homeostasis, is accompanied by increased release of CA from the adrenal medulla, on the one hand, and preservation of a high proportion of the NA deposited in adrenergic terminals, on the other hand. This massive release of CA from the adrenals is not usually observed at the anxiety stage of immobilization stress, and it is evidently due to increased utilization of these hormones in their thermogenic role [4, 7]. In the later stages of hypothermia of the immobilized rats the rapid fall of T_b (at the rate of 3.9 ± 0.5°C/h) is associated not only with the considerable reduction of OC and HR, reflecting the degree of inhibition of heat production, but also with exhaustion of the CA reserves (especially NA). In this connection attention is drawn to the level of neurotransmitter activity of the adrenergic nerves. By contrast with control immobilization, characterized by restoration of the NA reserves at the stage of adaptation (4-24 h), the neurotransmitter concentration at these same times of hypothermia was sharply reduced. These findings, and also the manifestations of the response of the animals to immobilization stress noted above during hypothermia, lead to the conclusion that the response to stress under these conditions does not develop in the same way as in the general adaptation syndrome. It has important distinguishing features, thanks to which heat metabolism is stabilized at a new temperature level and immobilization hypothermia can be prolonged for a long time at below comfortable ambient temperatures.

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EFFECT OF EMOTIONAL STRESS ON LACTATE DEHYDROGENASE ISOZYME SPECTRUM IN THE RAT RETICULAR FORMATION

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Lactate dehydrogenase (LDH), the key enzyme of carbohydrate metabolism, is present in the tissues in the form of several isozymes (LDH_1-LDH_5), which differ in their physicochemical properties [7]. It has been shown [6] that the LDH isozyme spectrum is linked with the character of tissue oxidative metabolism: a high proportion of LDH_1 is characteristic of tissues with a particularly high intensity of biological oxidation, coupled with phosphorylation. In tissues with predominantly anaerobic carbohydrate breakdown the rate of LDH_5 is more important.

Changes in tissue metabolism due to different causes are reflected in changes in the LDH isozyme spectrum. The LDH isozyme spectrum can thus be used as an indicate of the direction of carbohydrate metabolism in the tissues.

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