CHARACTER OF EARLY RECOVERY PERIOD AND LEVEL OF CARBOHYDRATE-LIPID SUBSTRATES AFTER 24-HOUR IMMOBILIZATION HYPOTHERMIA

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Strict immobilization is known to be a powerful stressor factor, capable of inhibiting shivering thermogenesis, as a powerful response of the body to cooling [13]. The writers showed previously [2, 4] that in rats subjected to such immobilization not only can the body temperature (T_b) be lowered to 20°C without any marked exposure to cold, but deep hypothermia also can be maintained for 24 h at a below-comfortable ambient temperature (T_a), without the use of drugs.

In the investigation described below the survival of rats and their ability to rewarm themselves in the early recovery period after deep 24-h immobilization hypothermia (T_b about 20°C) were studied. Since the adequacy of temperature regulation of a warm-blooded animal correlates closely with its supply of the necessary energy substrate [8], particular attention was paid to the study of the state of carbohydrate-lipid metabolism in the blood and tissues of the rats after 24-h immobilization hypothermia and after 5 h of rewarming, as compared with the general metabolic rate.

EXPERIMENTAL METHOD

Experiments were carried out on 52 male rats weighing 201 ± 12 g. To obtain immobilization hypothermia the animals were securely fixed in spacial frames. After fixation for 1 h (at the time of maximal lowering of the catecholamine level in the peripheral mediator component of the sympathicoadrenal system [7]) the rats were put in a chamber with T_a = 5°C. After stable hypothermia was reached, with T_b = 20.4 ± 0.4°C, T_a was raised to 15°C, and the animals were left under these conditions for 24 h, then unfixed, and transferred to individual cages with T_a at 19-21°C. Changes in the rectal temperature, measured with a TPEM-1 electrothermometer at a depth of 3.5 cm, were monitored during period of creation and prolongation of hypothermia. Parameters of carbohydrate-lipid metabolism and of the intensity of metabolism were determined in intact, unfixed, normothermic animals and in the experimental rats after 24 h of hypothermia and 5 h of rewarming. The following determinations were made; glucose in the blood and liver by the ferricyanide method [5], glycogen in the liver, skeletal muscles, and myocardium, by a modified Kerr's method [1], total lipids in the liver by Folch's method [5], total blood lipids by Bio-La-Test kits (from Chemapol, Czechoslovakia), and nonesterified fatty acids (NEFA) by Novac's method [12]. The metabolic rate was estimated from the oxygen consumption (OC) in a closed system with the "Godart" gas analyzer, and expressed in ml/kg/min. The heart rate (HR) was calculated from the ECG, recorded on an EEGP-4-02 instrument.

The data were subjected to statistical analysis by computer using a standard program with verification of the homogeneity of the data, determination of mathematical expectancy and dispersion, and determination of a significance of differences by Student's test.

EXPERIMENTAL RESULTS

The observations showed that after 24 h of immobilization hypothermia, when T_b averaged 21.1 ± 0.4°C, and after 5 h of rewarming, when T_b had reached 34.5 ± 0.6°C, the survival rate of the animals was 100%. Data on the carbohydrate-lipid substrate levels at the above times are given in Fig. 1.

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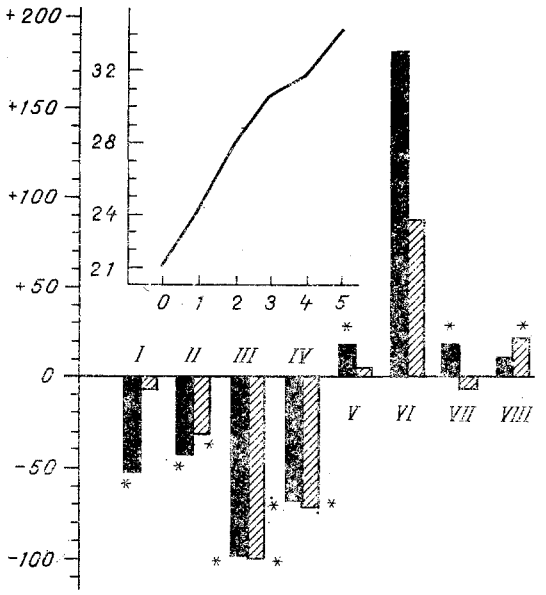

Fig. 1. Quantitative changes in energy substrates in blood and tissues of rats (in per cent of initial values during normothermia) after 24-h immobilization hypothermia and after 5 h of rewarming. I) Blood glucose (normal 4.95 ± 0.05 mmole•liter^{-1}), II) liver glucose (9.8 ± 0.2 μmoles/g tissue); liver glycogen (230.0 ± 19.6 μmoles/g tissue); IV) skeletal muscle glycogen (19.6 ± 1.7 μmoles/g tissue); V) myocardial glycogen (13.52 ± 1.69 μmoles/g tissue); VI) serum NEFA (280.6 ± 9.7 μmoles•liter^{-1}); VII) total blood lipids (2.87 ± 0.04 g•liter^{-1}); VIII) total liver lipids (0.0639 ± 0.0006 g/g tissue). Black columns show data obtained after 24 h of hypothermia, obliquely shaded columns — after 5 h of rewarming. *P < 0.05 compared with initial value. Inset — rewarming temperature chart: abscissa, time (in h); ordinate, T_b) in °C.

Prolonged and deep hypothermia with immobilization led to a fall in the blood and liver glucose levels by almost half compared with normothermia, accompanied by marked exhaustion of the liver glycogen. The glycogen level in the skeletal muscles also was considerably lowered, although by a lesser degree than in the liver, and at the same time, its concentration in the myocardium was a little higher than initially. An increase in the blood NEFA level (by more than 2.5 times) was observed, but changes in the blood and liver lipid concentrations were not statistically significant. These changes in carbohydrate-lipid metabolism took place against a background of a sharp decrease in total metabolism, characterized by a fall of OC by 4.7 times and HR by 3.4 times compared with the initial values (Table 1). The results are in agreement with existing views on positive correlation between OC and T_b [9]. Incidentally, a low metabolic rate coupled with depression of activity of catecholaminergic systems [4] could help to preserve the high blood NEFA level, which was maintained, despite the preferential utilization of these acids in hypothermia, evidence for which, in turn, is given by the absence of accumulation of total lipids in the rats' liver. Meanwhile, the increase in the myocardial glycogen concentration which was found was evidently due to an accompanying reduction of cardiac activity.

It is considered that the factor limiting the duration of exposure of the warm-blooded organism to cold is an insufficiency of carbohydrates [7, 10], which are utilized in the early stages of acute cooling during activation of shivering thermogenesis [14]. Prolongation of deep hypothermia in unimmobilized animals leads to the development of cerebral hypoglycemia, resulting in death [6, 11]. The 100% survival rate of the rats observed in the course of our experiments suggest that although the blood glucose level is reduced by half under these conditions of a marked decline in the metabolic rate, this is sufficient to satisfy the energy demands of the brain.

In the early recovery period, at room temperature, 5 h after unfixing of the animals their T_b rose by 13.4 ± 0.6°C, i.e., the mean rate of rewarming was 2.6 ± 0.4°C in 1 h. As

TABLE 1. Changes in T_b, OC, and HR in Rats after 24-h Immobilization Hypothermia and after 5 h of Rewarming (M ± m)

Parameter	Initial level	Hypothermia for 24 h	Rewarming for 5 h
T_b, °C	37,4±0,1	21,1±0,4*	34,5±0,6**
OC, $ml \cdot kg^{-1} \cdot min^{-1}$	55,3±1,4	11,3±1,3*	36,5±3,1**
HR, beats/min	505±13,4	148±8,4*	385±16,3**

Legend. *P < 0.05 compared with initial level, **P < 0.05 compared with 24-h hypothermia.

Fig. 1 shows, during the rise of T_b, the rate of rewarming gradually decreased. Corresponding to the increase in the general metabolic rate, OC and HR increased by 3.2 and 2.6 times at this time, although they remained significantly below normal (Table 1). During the period of rewarming of the animals the blood glucose concentration rose almost to the control values and the liver glucose increased by 1.2 times compared with that observed in hypothermia, but did not reach the initial value. The liver glycogen, as before, was present in trace quantities, in the skeletal muscles no significant changes were found in this parameter relative to the period of hypothermia, and in the myocardium it showed a tendency toward normalization. The blood NEFA level, despite a significant fall ($P < 0.05$) continued to remain high: The total lipid level fell to normal in the blood and showed a tendency to rise in the liver. The increase in the blood and liver glucose concentrations, together with the virtually complete absence of glycogen in the liver, may be connected with stimulation of gluconeogenesis, known to be a result of lowering of the glycogen level in the liver and accumulation of lipids in that organ. Under these circumstances fatty acids may not only participate in gluconeogenesis, but they may also stimulate it from amino acids [3]; the intensity of gluconeogenesis in this period, moreover, insufficient for raising the glycogen potential of the liver, may evidently provide a glucose supply for contractile thermogenesis, which is activated by temperatures of between 28 and 32°C, although not completely (as shown by the slowing of rewarming). This hypothesis is supported by the absence of any further decline in the glycogen reserves in the skeletal muscles, despite the fact that shivering obtains its energy supply predominantly from oxidation of carbohydrates [8].

The results thus show that the possibility of maintaining deep hypothermia for a long time when the metabolic rate is considerably reduced under conditions of immobilization is evidently linked with preservation of the glucose reserves of the body at an adequate level for supplying the energy requirements of vitally important organs. Meanwhile, in the early recovery period after 24-h immobilization hypothermia levels of energy-yielding substrates, both carbohydrate and lipid, although sufficient to maintain viability of the organism, nevertheless limit the rate of its rewarming.

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MONOAMINES AND PEPTIDERGIC GOMORI-POSITIVE NEUROSECRETORY CELLS OF THE PARAVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS DURING CHRONIC ALCOHOL CONSUMPTION

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The effect of chronic alcohol intake on metabolism and concentrations of monoamines in the hypothalamus have been studied in some investigations [1, 2, 7, 9, 12], and secretory activity of Gomori-positive neurosecretory cells (NSC) of the hypothalamus [5], producing nonapeptide neurohormones and possessing a rich monoaminergic innervation [4] in others.

The aim of this investigation was to make a parallel study, in one experiment, of the concentrations of individual monoamines, the fluorescence of monoaminergic structures, and the functional state of peptidergic Gomori-positive NSC of the paraventricular nucleus (PVN) in the hypothalamus of rats during chronic (6 months) alcoholization. Data on the functional state of the monoaminergic and peptidergic systems of the hypothalamus, the center for neuroendocrine regulation, are of great importance for the analysis of neuroendocrine disturbance during chronic alcohol exposure, more especially since the role of disturbances of the brain monoaminergic systems in the pathogenesis of chronic alcoholism has been discovered [1, 2].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats fed on a standard diet but allowed to drink only a 20% solution of ethanol for 6 months. For the next two weeks the rats had the choice of drinking ethanol or water. Experimental rats were divided into two groups: 1) preferring alcohol, 2) preferring water. Rats given only water to drink for 6.5 months served as the control. All rats weighing 350-400 g were decapitated in the fall. Concentrations of dopamine (DA), noradrenalin (NA), and serotonin (5-HT) in the hypothalamus were determined by a modified method [14]. The significance of the results was determined by Student's test. Monoaminergic structures of the hypothalamus were revealed by the fluorescence-histochemical method of Falck and Hillarp (in 5 rats in each experimental group and in the control). The intensity of fluorescence of the monoamines was estimated by counting the number of varicose thickenings of terminals of monoaminergic fibers in the hypothalamic nuclei. Some of the sections treated by the Falck–Hillarp method were stained with paraldehyde–fuchsin by the Gomori–Gabe method and counterstained with Heidenhain's azan. The quantity of Gomori-positive neurosecretory material in the bodies and axons of the NSC in PVN was then determined in the preparation. The estimation was done visually, using a five-point system, with an accuracy of 0.5 point.

EXPERIMENTAL RESULTS

The intensity of green fluorescence of noradrenalin- and dopaminergic nerve fibers in the hypothalamic nuclei of the rats of group 1 was statistically significantly lower than in the

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