Metabolism in Rats during Antiorthostatic Hypokinesia

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O_2 consumption and CO_2 release in 3 groups of awake rats were studied on a MM-100 metabolic monitor system (CWE Inc.). The animals of 2 groups were preadapted to 4-h maintenance in special boxes (2 weeks). The rats could perform rotational movements and limited movements in the rostrocaudal direction (hypokinesia). The animals of one group were daily exposed to 4-h antiorthostatic load (≤45°) for 2 weeks. After 2 weeks, the intensity of metabolism in rats with antiorthostatic hypokinesia was lower than in hypokinetic specimens (by 15-20%, $p<0.05$) and freely moving animals (by 20-25%, $p<0.05$). Interleukin-6 concentration in rats with antiorthostatic hypokinesia (0.25±0.09 pg/ml) was lower than in hypokinetic (4.01±0.57 pg/ml) and freely moving animals (3.69±0.56 pg/ml). The decrease in the concentration of a proinflammatory cytokine interleukin-6 during experimental antiorthostatic hypokinesia reflects inhibition of metabolic processes, which are activated during antiorthostatism (but not hypokinesia).

Key Words: *antiorthostatism; hypokinesia; indirect calorimetry; interleukin-6; skin temperature*

Antiorthostatic hypokinesia (ANOH) is typical of clinical practice and serves as an experimental model in studies of the regulation of functional systems under conditions of weightlessness [1]. There is no general agreement about the processes occurring in mammals during experimental microgravity. Little is known about the mechanisms of adaptation to a specific posture [3,4,11,12]. Under these conditions, the factors of volume and pressure contribute to the increase in hemodynamic load on the heart and major reflexogenic zones in blood vessels. What is the period of adaptation? It remains unclear whether adaptation concerns only functional systems for the maintenance of homeostasis [6,9] or induces a variety of structural changes in organs and tissues. Specific variations in metabolic processes are unknown. On the one hand, hypodynamia is characterized by the reduced energy consumption. On the other hand, abnormal body position is accompanied by a stress response and activates heat production. Does blood flow redistribution during ANOH involve only the processes occurring in the sympathetic nervous system [1]? The question arises: do hormonal [4] and other regulatory factors of the vascular [9] or humoral nature play a role in the complex process of hemodynamic changes? Here we studied the role of proinflammatory cytokine interleukin-6 (IL-6) in metabolic transformations in rats with ANOH.

MATERIALS AND METHODS

Experiments were performed on 24 male Wistar rats weighing 0.25±0.03 kg. The animals were maintained in a vivarium under standard conditions (21±1°C, 12/12-h artificial light-dark cycle) and had free access to food and water. The rats were preadapted

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to experimental conditions for 2 weeks. This procedure involved handling and 9-10 training sessions in special boxes. The time of training varied from 30 min to 3-4 h [10]. The rats could perform rotational movements and were restrained in the rostrocaudal direction. The animals were divided into 3 groups (8 rats per group). Group 1 rats were daily exposed to 4-h ANOH in boxes (P45°) for 2 weeks. Group 2 rats were daily subjected to 4-h hypokinesia in boxes (similarly to group 1 rats). Freely moving rats of group 3 served as the control. The intensity of metabolism in animals of 3 groups was studied on a MM-100 metabolic monitor system (CWE Inc.). Monitoring was performed before handling, 2 weeks after adaptation to experimental conditions, and 1 or 2 weeks after the start of ANOH. THE MM-100 system allowed us to measure air flow (liter/min) in the individual metabolic cell (total of 8 cells) and to estimate the concentrations of O_2 and CO_2 in the inspired and expired air (%). The respiratory exchange ratio was calculated from O_2 consumption (VO_2, ml/h) and CO_2 release (VCO_2, ml/h) as follows: $RER=VCO_2/VO_2$. The intensity of heat production was estimated taking into account the level of O_2 consumption per unit time, respiratory exchange ratio, and weight of animals (kcal/kg/h). The data were processed by MMCom software and recorded in the ASCII format.

On day 14, the intensity of metabolism in 24 rats was studied for 5 min. The animals were adapted to metabolic cages for 40-60 min. Group 1 and 2 rats were placed in the antiorthostatic position using special platforms in metabolic cages. The intensity of metabolism was studied after 40 and 80 min. Gas exchange parameters in all rats were recorded after resumption of the horizontal position. The intensity of metabolism in freely moving rats of groups 1 and 2 was studied after removal from boxes.

Copper-constatan thermocouples for recording of surface body temperature (Physitemp thermometer) were fixed on the ventral surface of tail skin in 3 rats by the 2nd week after adaptation to experimental conditions [10]. Skin temperature was monitored at 29°C for 4 h. The animals were exposed to ANOH for 3 h.

After the last recording of metabolic parameters, the rats were decapitated using a SAD 51330 guillotine. The blood was sampled and centrifuged at 1500 rpm for 15 min. Serum IL-6 concentration was measured by means of ELISA with Biosource Internat. kit for rats.

The results were analyzed by Student's *t* test and one-way analysis of variance (ANOVA). The differences were significant at $p<0.05$.

RESULTS

Parameters of heat production in rats of 3 groups on day 14 of the study are presented on (Fig. 1, *a*). The intensity of metabolism in group 1 rats decreased to 0.0026±0.0001 kcal/kg/h ($p<0.05$) and was lower compared to control animals (0.0035±0.0002 kcal/kg/h) and group 2 specimens (0.0033±0.0001 kcal/kg/h). On day 14, no differences in metabolic changes were revealed in group 1 and 2 rats during 40-min antiorthostasis in a metabolic cage (Fig. 1, *b*). After 80-min ANOH (Fig. 1, *c*) the intensity of metabolism in group 1 and 2 animals was 0.0036± 0.0002 and 0.0046± 0.0002 kcal/kg/h, respectively ($p<0.05$). Intergroup differences in the intensity of metabolism were found after the boxes were placed horizontally (0.0031±0.0001 and 0.0037±0.0002 kcal/kg/h, respectively, $p<0.05$; Fig. 1, *d*). No intergroup differences in metabolic processes were revealed in freely moving animals after removal from boxes.

On day 14, IL-6 concentration in group 1 rats decreased to 0.25±0.09 pg/ml ($p<0.01$) and was lower than in group 2 animals (4.01±0.57 pg/ml) and control specimens (3.69±0.56 pg/ml).

Tail skin temperature in rats varied from 30 to 33°C at 2-3-min intervals (atmospheric temperature 29°C). Skin temperature decreased to 29°C several seconds after the start of ANOH, remained unchanged for 10-15 min, and increased to 35-36°C over the next 2-3 min. Temperature remained unchanged for 1 h and progressively decreased to 31-32°C over 30 min. Tail skin temperature increased to 34-35°C after 5-10 min and remained unchanged for 30-40 min.

The concentration of proinflammatory cytokine IL-6 is affected by various factors, including stress, hemorrhage, trauma, infection, postural response, and endotoxin translocation from the gastrointestinal tract into the internal medium [5,7,8]. Among a variety of IL-6-producing cells in organs and tissues, particular attention is paid to vascular endothelium. ANOH is accompanied by changes in hemodynamic load on this structure [2]. It could be expected that repeated ANOH and hypokinesia for 2 weeks would increase the concentration of IL-6. However, IL-6 concentration in hypokinetic rats did not differ from that in control specimens (freely moving animals). However, serum IL-6 concentration decreased by more than 10 times during ANOH. The decrease in IL-6 concentration in the internal medium during ANOH was accompanied by the inhibition of metabolism. The increase in skin temperature during ANOH is related to prolonged dilatation of blood vessels and elevation of blood flow

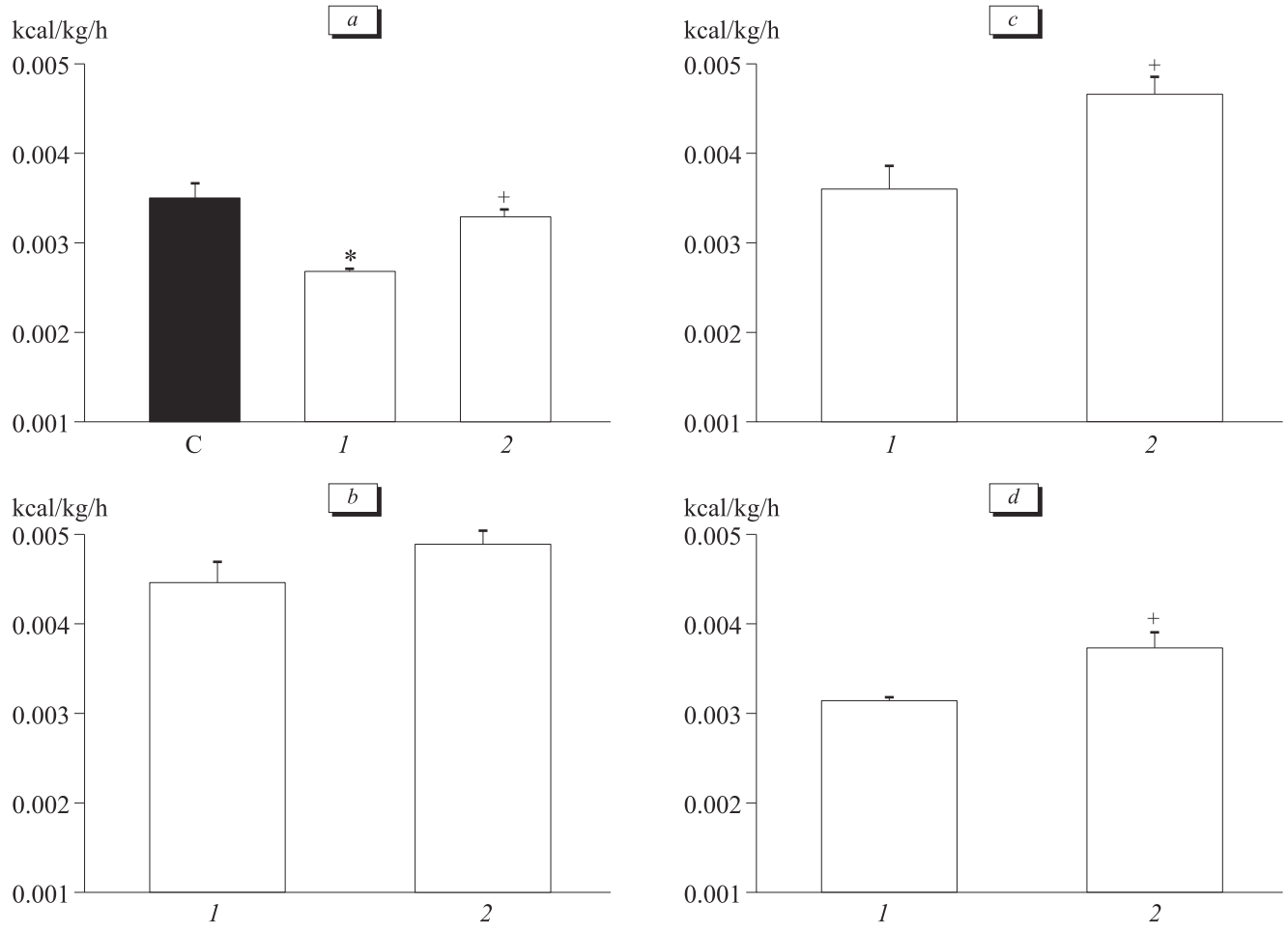

Fig 1. Heat production in rats on day 14. (*a*) Freely moving animals (*C*), ANOH (*1*), and hypokinesia (*2*). Antiorthostatic load test for 40 (*b*) and 80 min (*c*), and after the boxes were positioned horizontally after load test (*d*). $p<0.05$: *compared to the control (*C*); +compared to ANOH.

in tail tissues [10]. These changes are probably associated with the increased production of nitric oxide [7] and accompanied by intensification of heat emission [10]. These features (prolonged vasodilation, inhibition of metabolism, and decrease in serum IL-6 concentration during experimental ANOH) may serve as markers of processes, which reflect the impaired interaction between functional systems on the model of weightlessness. The observed changes are manifested in pathological disorders under natural conditions of space flight.

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