STATE OF ADRENAL FUNCTION IN RATS DURING HYPOKINESIA

A. S. Kaplanskii and E. V. Vorotnikova

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Limitation of motor activity in animals is accompanied by the development of a general adaptation syndrome (GAS [4]). However, information on the state of the adrenal function during long-term hypokinesia is conflicting. Whereas some workers consider that long-term hypokinesia leads to exhaustion of the adrenals [3, 6, 7], others do not accept this [2, 9]. These differences of opinion can in all probability be explained both by differences in the experimental conditions used (in particular, the "strictness" of the hypokinesia) and also by the fact that the state of adrenal function was judged on the basis of analysis of separate biochemical or morphological parameters.

This paper describes a combined morphological and biochemical investigation of the state of function of the adrenal cortex in rats at different stages of adaptation to moderately strict hypokinesia. For a more compete evaluation of the state of adrenal function and the reserve capacity of the glands, extra functional loads were used. At the same time, a morphological investigation was made of the thymus, the target organ for corticosteroids.

EXPERIMENTAL METHOD

Experiments were carried out on 250 female albino rats (initial body weight about 170 g), which were divided into four groups; group 1 consisted of intact rats (vivarium), group 2 of intact animals in which acute stress was induced, group 3 of rats kept under conditions of hypokinesia, and group 4 of rats which were subjected to acute stress at different stages of adaptation to hypokinesia. In addition, another 10 rats, killed before the experiment began, were used as the basal control. Hypokinesia was induced by placing the animals in constricting cages which limited their motor activity, and an acute stress reaction was induced by immobilizing the rats for 5 h in an extended state, in the prone position, on special movable tables [12]. The animals (8-10 rats in each group) were decapitated 7, 14, 30, 60, and 90 days after the beginning of hypokinesia, and also 1 month after its end. Blood plasma, adrenals, and thymus was studied. The corticosterone level in the blood plasma was determined by radioimmunoassay [1, 10]. The adrenals were weighed, fixed in neutral formalin and embedded in histoplast, and were also frozen in solid carbon dioxide. In the latter case, 4 adrenal glands (one from a rat of each group) were mounted on the same carrier block, so that in the future the changes could be compared because sections of the adrenals obtained in a cryostat were identical in thickness and were stained with Oil red O and Sudan black B under identical conditions. Sections through the adrenals embedded in histoplast were stained with hematoxylin and eosin and with "astrin" [8, 11]. By the last method, it was possible to differentiate functionally active and inactive cells in the adrenal cortex on the basis of differences in staining of their nuclei. The thymus was weighed, fixed in Carnoy's fluid and neutral formalin, and embedded in histoplast. Sections through the thymus were stained with hematoxylin-eosin and methyl green-pyronine. The numerical results subjected to statistical analysis by Student's test with a $P < 0.05$ level of significance of differences between compared values, and also by the method of confidence intervals.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the corticosterone concentration in the rats' blood was increased on the 7th day of hypokinesia, returned to the normal level after 14 days, and was below normal on the 60th and 90th days of hypokinesia. The parallel histologic investigation of the adrenals showed that the structural transformation [5, 13] of the cortex

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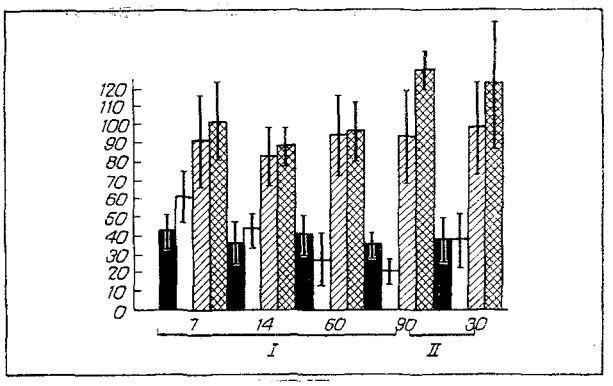

Fig. 1. Plasma corticosterone level in rats. Abscissa, duration of experiment (in days); ordinate, corticosterone concentration (in μg%). Black columns — control, unshaded — hypokinesia, obliquely shaded — control + stress, cross-hatched — hypokinesia + stress. I) Hypokinesia, II) readaptation.

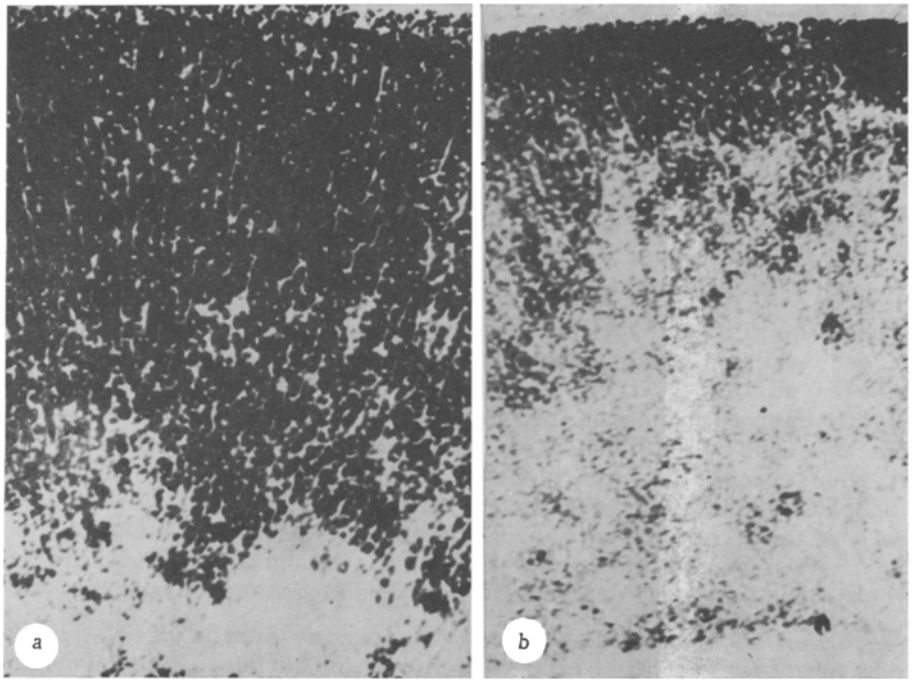

Fig. 2. Reaction of adrenal cortex of rats to 5-h exposure to stress: a) absence of marked delipoidization in adrenal cortex of a rat after 90 days of hypokinesia; b) delipoidization of adrenal cortex of a control rat. Stained with Sudan black B. 35x.

commencing in the early stages of hypokinesia led to hypertrophy of the steroidogenic tissue and to an increase in the number of functionally active cells which it contained. Hypertrophy of the zona fasciculata was preserved throughout the period of hypokinesia, i.e., during the period also when the blood corticosterone level was depressed. Delipoidization of the cortex, observed at the beginning of hypokinesia, was replaced after one month by accumulation, and after two and three months by normalization, of the lipid content in the cortex.

The thymus of rats kept under condtions of hypokinesia, to judge by the decrease in its

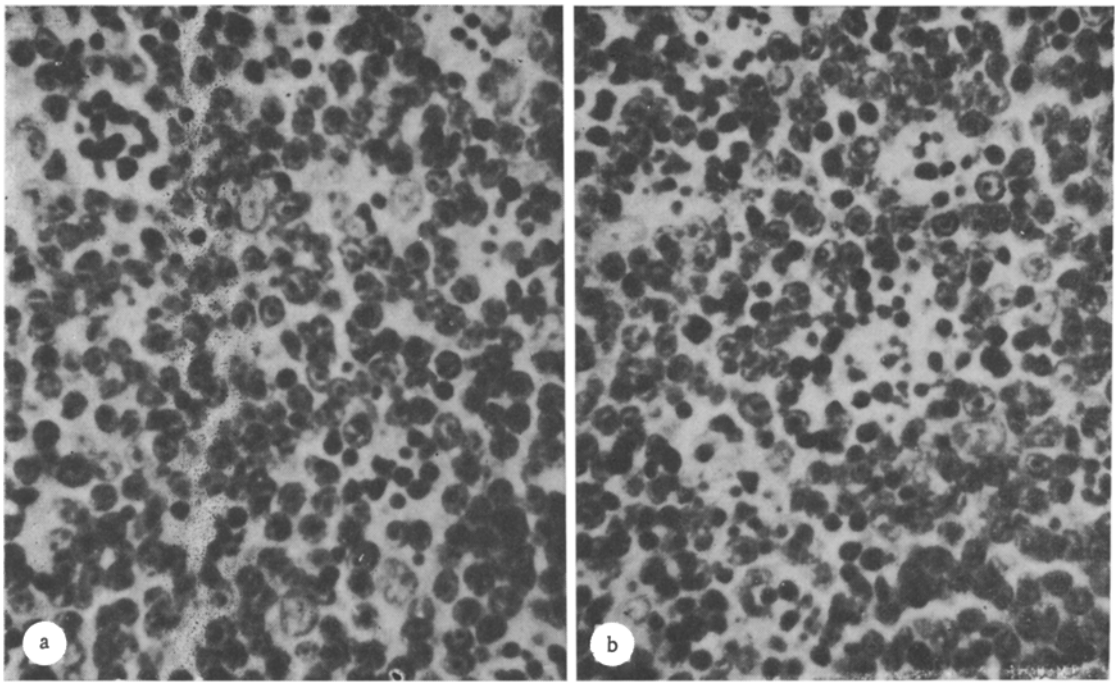

Fig. 3. Response of thymus of control rat and rat adapted to hypokinesia to exposure to stress for 5 h. a) nuclear debris in thymus of control rat, b) increased quantity of nuclear debris in thymus of rat kept for 60 days under conditions of hypokinesia. Hematoxylin-eosin, 280x.

weight and the histologic picture, underwent accidental involution, which persisted throughout the period of hypokinesia, although its rate decreased appreciably starting with the 30th day of the experiment. The blood corticosterone level and the structure of the adrenals and thymus of the rats were fully restored to normal 1 month after the end of hypokinesia.

The study of the function and reserve capacity of the adrenals during hypokinesia showed that additional stress loading led to a greater increase in the blood corticosterone concentration in rats exposed to long-term hypokinesia (60 and 90 days) than in intact animals (Fig. 1). During the first month of hypokinesia, the adrenals responded to stress by delipoidization, although this was less marked than in the intact animals, but on the 60th and 90th days delipoidization of the adrenals was not observed in response to additional stress (Fig. 2). Increased corticosterone production in rats adapted to hypokinesia, in response to acute stress, but with the absence of any morphological features of increased adrenocortical function at that time, indicated a marked increase in the reserve capacity of the steroidogenic tissue and its ability to cope easily with the increased demands presented to it.

Other evidence of an increase in the reserve capacity of the rats' adrenals after hypokinesia was given by the results of investigation of the thymus. They showed that as a result of exposure of the same duration to equally strong stress greater destruction of lymphocytes was observed in the experimental animals than in the control rats (Fig. 3). Counting the number of lymphocytes with pycnotic nuclei in the cortex of the thymus of experimental and control rats killed 5 h after the beginning of acute stress showed that in the control rats and in rats kept for 1 month under conditions of hypokinesia there were 282.51±51 and 426±45 dying lymphocytes on average per 1000 cells respectively ($P < 0.05$).

After 90 days of moderately strict hypokinesia, exhaustion of the adrenals thus does not take place. The phase of resistance of hypokinetic stress is characterized by dissociation between lowering of the blood corticosterone level and an increase in the reserve capacity of the adrenal cortex, which responds to the additional stress-inducing stimulus without any visible morphological features of structural reorganization or delipoidization of the cortex, whereas the increased destruction of lymphocytes in the thymus is the only morphological sign of increased corticosterone production by the adrenals.

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THE MICROCIRCULATORY SYSTEM OF HAMSTERS UNDER STRESS AND AFTER PROPHYLACTIC INJECTION OF IONOL

M. P. Gorizontova

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Disturbances of the terminal blood flow, of vascular permeability, and of the morphological and functional state of the mast cells were found in experiments on rats subjected to immobilization or to electrical stimulation [1, 2]. The degree of generalization of these disturbances and their organ specificity, however, remained unexplained, which necessitated the study of the state of the microcirculatory system in the mucosa of the retrobuccal pouch (MRP) of hamsters after exposure to stress. In consideration of data showing that stress-induced injuries can be effectively prevented by the antioxidant ionol [4-6, 9], and experimental study of prevention of disturbances to the microcirculation with the aid of this compound was carried out under conditions of stress.

EXPERIMENTAL METHOD

Experiments were carried out on 96 Syrian hamsters weighing 120-150 g. Immobilization of the animals for 1 and 24 h and graded whole-body electrical stimulation for 3 h were used as extraordinary stimuli.

For the biomicroscopic study of the microcirculation in the hamster MRP, an apparatus based on the "Docuval" microscope (Carl Zeiss, East Germany) was used.

Quantitative estimation of vascular permeability was carried out by luminescence contact biomicroscopy on an apparatus based on the Soviet LYUMAM KF-1 microscope, using bovine globulin, labeled with fluorescein isothiocyanate (FITC) as the marker. Ionol was injected intraperitoneally in a dose of 100 mg/kg three times in the course of three days. In the case of immobilization for 24 h, the 4th injection of ionol was given 8 h after the beginning of immobilization. The results were subjected to statistical analysis by Peters' method, using Moldenhauer's factor [7].

Laboratory of General Pathology of the Microcirculation, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 12, pp. 673-675, December, 1985. Original article submitted February 4, 1985.

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