BIOPHYSICS AND BIOCHEMISTRY

Nitric Oxide in Paraventricular Nuclei of Rat Hypothalamus under Extreme Conditions

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The activity of nitroxide synthetase (NOS) was studied histochemically and levels of oxytocin and vasopressin immunocytochemically in rat hypothalamus paraventricular nuclei in on-earth experiments simulating space flight conditions with landing. Colonization of oxytocin and NOS was found in large-cell neurons of paraventricular nuclei. After 15 days under conditions of simulated microgravitation followed by 1-day double gravitation and a short-term (1 day) macrogravitation, activities of NOS and content of neuropeptides increased in large-cell neurons of paraventricular nuclei of experimental animals.

Key Words: *hypothalamic paraventricular nuclei; NADPH-diaphorase; nitroxide synthetase; oxytocin; vasopressin; micro- and macrogravitation*

In neurobiology nitric oxide (NO) is regarded as a nontraditional neurotransmitter participating in transsynaptic transfer of a wide range of regulatory signals involved in the mechanisms of adaptation and in some cases in neuronal death [1,4,9]. Paraventricular nuclei (PVN) of the hypothalamus belong to brain areas with NO-producing neurons [1,4,5,9]. These nuclei play an important role in the universal reaction of adaptation to extreme conditions [10-12], denoted as stress reaction. This reaction is observed at the critical stage of space flight: during landing, when microgravitation is replaced by macrogravitation of the Earth. Therefore, we investigated the behavior of nontraditional PVN neurotransmitter NO and of traditional mediators of neuronal regulation oxytocin (OT) and vasopressin (VP) in land experiments simulating micro- and macrogravitation.

MATERIALS AND METHODS

Male rat brain PVN were studied. Microgravitation effect was simulated by suspending rats by the tail for 15 days in the head-down position permitting free movements of the front paws (group 1); part of these animals after 15 days were exposed to double gravitation by permanent rotation in a centrifuge. The duration of rotation was determined by attaining the peak stress (1 day) (group 2). Group 3 animals were exposed to double gravitation for 1 day without microgravitation. Intact animals served as control (group 4). After experiment the animals were guillotined. The brain was removed, the hypothalamic area was excised, fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), exposed in 10% sucrose for cryoprotection, and frozen in liquid nitrogen. Cryostat sections (-20°C) of the hypothalamic area (12 μm) were mounted on gelatin-coated slides and assayed for NO, OT, and VP activities. NO activity was assessed from the activity of NO synthetase (NOS) that catalyzes NO production during oxidation of L-arginine. For this purpose, the histochemical marker of NOS NADPH-diaphorase was measured [6]. For measuring NADPH-diaphorase, the sections were incubated in medium with 1 mM NADPH, 0.2 mM nitroblue tetrasolium, 0.1 M Tris buffer (pH 7.4) for 1 h at 37°C [3]. VP and OT were detected immunocytochemically [2] using second

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antibodies labeled with fluorochrome (for subsequent detection on similarly stained NADPH-diaphorase sections) or by the avidin-biotin-peroxidase complex. Co-localization of neuropeptides and NADPH-diaphorase was assessed by examining the adjacent sections stained for OT, VP, and NADPH-diaphorase or slices stained for OT, VP, and NADPH-diaphorase in succession.

Immunocytochemical and histochemical reactions were estimated semiquantitatively by counting the cells exhibiting high, moderate, and low intensity of reaction in every fifth serial section, i. e., at a 48 μm interval, and subsequent calculation of the percent ratio of detected cells.

RESULTS

Analysis of the hypothalamic area of male rat brain showed that the majority of neurons containing NADPH-diaphorase were localized in the large-cell part of the hypothalamus. In the PVN such cells were in abundance in the medial large-cell subnucleus and in the anterior, periventricular, and lateral large-cell subnuclei, where they formed just a rim. Comparison of neighboring sections and sections stained for OT or VP and NADPH-diaphorase in succession showed co-localization of OT and NADPH-diaphorase in PVN large-cell neurons. The topography of OT-containing nerve cells largely coincided with the topography of neurons with NADPH-diaphorase activity. VP-producing neurons were localized mainly in the lateral large-cell subnucleus, OT-containing neurons in the medial, anterior, periventricular, and at the periphery of lateral large-cell subnuclei as a rim. It is noteworthy that large-cell neurons possessing NADPH-diaphorase activity were detected in that part of PVN where OT-containing neurons were localized, according to our findings and results of other scientists [8]. Our data are in line with a recent report [7] showing that up to 96% oxytocin-containing neurons in the PVN exhibit NADPH-diaphorase activity.

Comparative histoenzymological study of NADPH-diaphorase in the medial large-cell nucleus of control and experimental animals showed that in comparison with intact control (43%), the number of neurons with moderate and high histoenzymologic reaction (Fig. 1) in experimental animals is 1.5 times higher (67 and 66% in groups 1 and 2, respectively) and 1.7 times (72% in group 3). This increase was the greatest in group 3, particularly for cells with high enzymatic activity. Their number increased 8 (24% — group 1), 12 (36% — group 2), and 14 (42% — group 3) times in comparison with intact animals (3%). Indicating that NOS activity detected in large-cell subnuclei of hypothalamic PVN by the histochemical marker NADPH-diaphorase depended on the

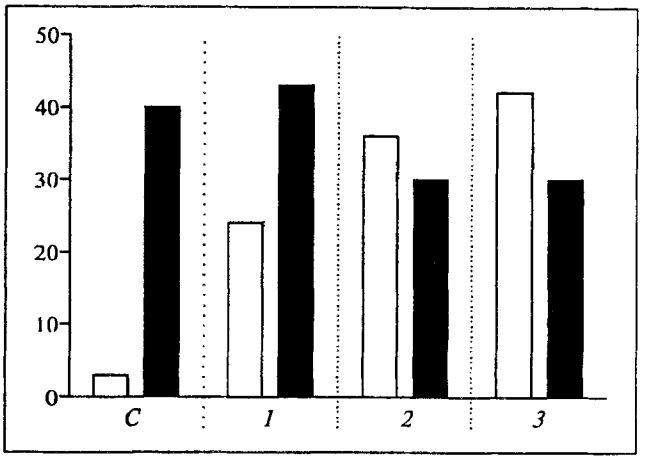

Fig. 1. Percentage of cells with high (light bars) and moderate (dark bars) activities of NADPH-diaphorase in the median large-cell subnucleus of rat hypothalamus paraventriculer nucleus. *C*) intact control; *1*) microgravitation; *2*) microgravitation+macrogravitation; *3*) macrogravitation.

conditions simulating space mission factors, and therefore, NO, a nontraditional neurotransmitter of nerve tissue produced by NOS, can be involved in the hypothalamic mechanisms of adaptation to space flight.

Immunocytochemical study revealed no appreciable intergroup differences in the reaction of OT-containing nerve cells of the median large-cell subnucleus. Simulated microgravitation, short macrogravitation, or their combination led to a similar increase (1.5-1.7 times) in the number of cells with high content of OT, although it was obvious that exposure to double gravitation (group 3) exerted the maximum effect on the content of the neurohormone. The time course of immunocytochemical reaction to OT and of diaphorase reaction in the lateral large-cell subnucleus virtually did not differ from that in the neurons of the median large-cell subnucleus. Similar changes were observed in VP-containing nerve cells of the lateral large-cell subnucleus.

Thus, comparative histochemical and immunocytochemical study of PVN of rats under conditions simulating space mission and landing showed that 15-day exposure to head-down tilt resulted in NOS activation and increase in the content of OT and VP detected by immunocytochemical method in the rat PVN. One-day exposure of intact animals to macrogravitation resulted in the greatest changes of metabolic activity of PVN large-cell neurons. The effect was weaker in rats exposed to macrogravitation after 15-day microgravitation. Activity of NADPH-diaphorase and immunocytochemically detected OT and VP levels in PVN large-cell subnuclei were higher in group 2 than in group 1 and lower than in group 3. It is obvious that the effects produced by each exposure are not summed up.

Our aim was to investigate the hypothalamic nerve structures of the brain involved in adaptation mechanisms during the most critical period of space mission when microgravitation is replaced by macrogravitation (landing). The results obtained can be regarded as preliminary, requiring confirmation and further studies of readaptation processes. Study of the effect of a longer macrogravitation (15 days) on metabolic parameters of PVN in intact animals and in animals exposed to 15-day microgravitation is of special interest.

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