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The role of catecholamines, melatonin and nitric oxide in the mechanisms of stress damage to the body

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Abstract. The mechanisms of response and adaptation of the body's regulatory systems to stress factors is an urgent fundamental task and the goal of this study. The study used four experimental models of stress: acute stress, chronic stress, and their sequences. An acute stress response was induced in a 60-minute forced swimming test model in a pool. Chronic stress was simulated by restricting mobility (hypokinesia), which was done by placing the rats into special AE1001-R1 rat retainers, made of transparent polypropylene (OOO OpenScience, Russia), in which they were kept for 10 days, 20 hours a day. The levels of biogenic amines (adrenaline, norepinephrine, serotonin), melatonin and nitric oxide in blood serum were determined by a method of enzyme-linked immunosorbent. the results obtained provide fair evidence of an increased functional activity of both stress-response (sympathoadrenal system) and stress-limiting systems (ST, MT, NO) in stress reactions of the body, the severity of which depends on the duration and sequence of stress stimuli on animals.

1. Introduction

One of the primary concerns of modern physiology and medicine is to study the body's adaptive processes to factors of various nature and intensity. Some of these factors cause the body to develop a stress response. In the modern world, psycho-emotional and physical stresses are the most common stress factors. It is the stress that triggers the growth of civilization diseases, which include pathologies of the cardiovascular, nervous, immune, digestive, and endocrine systems [1, 2]. One of the widespread stress factors is hypokinesia (HS, hypokinetic stress, or restriction of mobility), which causes the development of chronic stress and a number of specific and nonspecific changes in the functioning of almost all organs and systems of the body. HS is an important issue in medicine, because treatment of many diseases requires staying in bed, which can last weeks and even months.

At the same time in real conditions, the body is influenced by a number of factors of various nature and intensity, which can modify the body's responses to one another. If a person has experienced chronic stress for a long time, acute stress can lead to an adaptive breakdown and the development of cardiovascular and other socially significant diseases [3, 4].

Little is yet known about the etiology and the processes occurring in the body during acute stress, or when it is combined with chronic stress.

It should be noted that in the mechanisms of human and animal adaptation to stress factors, the nervous, endocrine and immune systems are of great importance. Currently, the idea is established that there is an integrated regulatory system of the body, i.e. the neuroimmunoendocrine system. The major neuroendocrine components of this system are the sympathoadrenal system (SAS) and the hypothalamic-pituitary-adrenal system (HPAS), the mediators and hormones of which have a wide range of regulatory effects on the functional activity of almost all cells of the body; the major immune components – a complex pattern of cytokines – signaling molecules produced by cells of the macrophage-mononuclear and immune systems, which can modulate the neuroendocrine and behavioral responses of the body [3, 5, 6].

Thus, to study the mechanisms of response and adaptation of the body's regulatory systems to stress factors is an urgent fundamental task and the goal of this study.

2. Materials and methods

The experimental part of the study was carried out on the basis of the Core Facilities Center "Experimental Physiology and Biophysics" of the Department of Human and Animal Physiology and Biophysics of Taurida Academy (a structural subdivision of V.I. Vernadsky Crimean Federal University).

The animals were kept in the standard vivarium conditions at a temperature of 18-22° C on Rehofix MK 2000 corncob bedding, with a natural 12-hour light-dark cycle, free access to water (the State Standard 33215-2014 "The Guidelines for the Maintenance and Care of Laboratory Animals. Rules for Equipment of Premises and Organization of Procedures") and complete granulated feed according to the State Standard R-50258-92. The study was carried out in accordance with the State Standard R-53434-2009 "The Principles of Good Laboratory Practice" and the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

2.1. Experiment design

The study used four experimental models of stress: acute stress, chronic stress, and their sequences. For this, the male rats were divided into groups: a control group (C), the animals (n=60) of which were biological control; a group of animals (n=60) exposed to acute stress (AS); a group of animals (n=60) subjected to chronic hypokinetic stress through restriction of mobility (HS). In the fourth and fifth groups, the effects of cross-adaptation were studied by determining the impact of the modifying effect of chronic hypokinetic stress on the functional changes in tissue microhemodynamics under acute stress and the modifying effect of acute stress on the functional changes in tissue microhemodynamics under chronic hypokinetic stress. The fourth group consisted of the animals (n=60) subjected to sequential chronic and acute stresses (HS-AS), when the animals were first kept in the conditions of 10-day hypokinesia, and on day 10 they were subjected to acute stress. The fifth group consisted of the animals (n=60) first subjected to acute (1st day of the experiment) and then chronic 10-day stress (AS-HS).

2.2. Acute stress simulation

An acute stress response was induced in a 60-minute forced swimming test model [7] in a pool. The animals of all the groups had been deprived of food, with having free access to water 24 h prior to the stress exposure. To increase the stress effect, 3-4 rats were simultaneously placed into the pool (water level – 30 cm, water temperature +20° C).

2.3. Chronic hypokinetic stress simulation

Chronic stress was simulated by restricting mobility (hypokinesia), which was done by placing the rats into special AE1001-R1 rat retainers, made of transparent polypropylene (OOO OpenScience, Russia), in which they were kept for 10 days, 20 hours a day. The remaining 4 hours each day the rats were used for experimental studies, animal feeding, and care.

The animals were slaughtered within the period stipulated by the experiment design, by guillotine decapitation (OOO OpenScience, Russia).

After decapitating the experimental animals, their blood was collected in BD Vacutainer® SST tubes (with serum gel separator) (BD Vacutainer® SST™ II Advance). The material for the research was the blood serum obtained by double centrifugation for 10 minutes at 1300 x g at 25° C [8].

The levels of biogenic amines (adrenaline, norepinephrine, serotonin), melatonin and nitric oxide in the blood serum were determined by a method of enzyme-linked immunosorbent assay (ELISA) on 96-well microplates, using an Anthos 2010 microplate photometer with filters (400-750 nm) and ADAP+ software (Biochrom, UK).

The serum level of serotonin was determined using an ELISA Kit for 5-Hydroxytryptamine (5-HT) (cat. no. CEA808Ge), the level of epinephrine – by an ELISA Kit for Epinephrine (EPI) (cat. no. CEA858Ge), the level of norepinephrine – by an ELISA Kit for Noradrenaline (NA) (cat. no. CEA907Ge), the level of melatonin – by an ELISA Kit for Melatonin (MT) (cat. no. CEA908Ge), the level of nitric oxide – by a Nitric Oxide (NO) Assay Kit (No. A013-2), all the kits produced by Cloud-Clone Corp. (China). To wash the microplates, an Immunochem 2600 microplate washer (HTI, USA) was used, and to incubate the samples, an Elmi ST-3 microplate incubator-shaker (Elmi, Latvia) was used.

Statistical processing of the results of the biochemical studies involved the determination of the significance of differences between the groups, using one-way analysis of variance (ANOVA) with Tukey's HSD test [9]. The data are presented in the form of the arithmetic mean and its error; their statistical analysis and graphical presentation were made using GraphPadPrism 7.0 software.

3. Results

The level of catecholamines in the rats' blood serum increased after stressful stimuli of various durations and their combinations (Figure 1).

The concentration of adrenaline (A) in the control was 0.079 ± 0.008 μg/ml (Fig. 1A), after AS it increased at a trend level by 13%, after HS – by 42.8% ($p \leq 0.01$), after the combination of HS-AS – by 17.5% ($p \leq 0.05$), and after the combination AS-HS – by 39% ($p \leq 0.01$).

The level of noradrenaline (NA) in the blood serum of the intact group was 0.112 ± 0.011 μg/ml (Fig. 1C), after AS it decreased by 14.1% ($p \geq 0.05$), after HS stress it increased by 20.4 % ($p \leq 0.05$), after the HS-AS combination, it increased by 17% ($p \leq 0.05$), and after the AS-HS combination, it increased by 8.3% at a trend level.

The level of serotonin (ST) in the control was 141.3 ± 3.66 ng/ml (Fig. 1C), and after stressful stimuli of various durations and their combinations, it increased: after AS at a trend level by 11.7%, after HS – by 34.7% ($p \leq 0.05$), after the combination of HS-AS – at a trend level by 10.6% (the concentration almost returned to the control values), and after the combination of AS-HS – by 37.0% ($p \leq 0.01$).

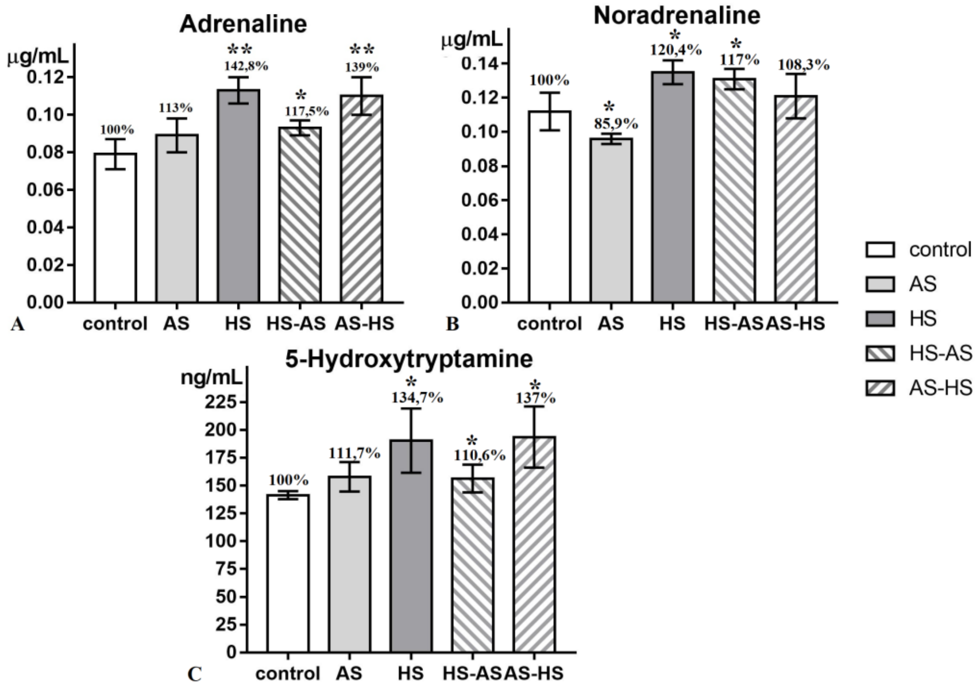

Figure 1. The level of catecholamines in the rats' blood serum in various experimental stress models.
Note: A – adrenaline, B – noradrenaline, C – serotonin.
AS – acute stress, HS – hypokinetic stress, HS-AS – sequential action of chronic and acute stress, AS-HS – sequential action of acute and chronic stress.

The concentration of melatonin (MT) in the blood of rats of the control group was 25.80±1.02 pg/ml (Figure 2A); after OS this indicator increased at a trend level by 10.4%; after HS it decreased by 30.8% ($p \leq 0.05$); after the combination of HS-AS, it decreased by 22.0% ($p \leq 0.05$), and after the combination of AS-HS, it decreased by 25.4% ($p \leq 0.05$)

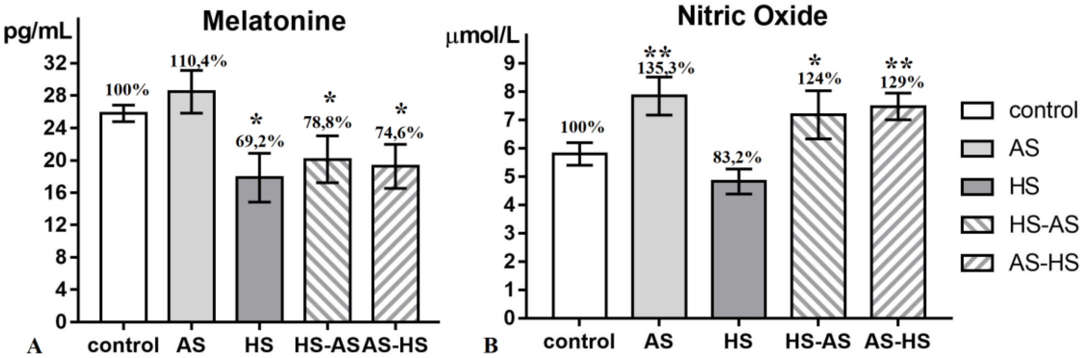

Figure 2. The level of melatonin and nitric oxide in the rats' blood serum in various experimental stress models.
Note: A – melatonin, B – nitric oxide. For abbreviations see note in Figure1.

The concentration of nitric oxide (NO) in the animals of the control group was 5.80±0.40 μmol/L (Figure 2C); after AS this indicator increased by 35.3% ($p \leq 0.01$), after HS – decreased by 16.8% ($p \leq 0.05$), after the combination of HS-AS – increased by 24% ($p \leq 0.05$), and after the combination of AS-HS – increased by 29.0% ($p \leq 0.01$).

Thus, a pronounced mediator imbalance developed in the animals under the influence of stress factors of different intensity and duration. For instance, in the animals of the HS and AS groups, there was a unidirectional increase in A and ST and a multidirectional change in the other indicators: with HS, there was an increase in the concentration of NA and a decrease in the concentration of MT and NO, whereas with AS, on the contrary, there was a decrease in NA, and an increase in MT and NO. It is likely that in AS, along with an increased activity of the stress-response system, stress-limiting systems (ST, MT, NO) are also activated, which limits the release and/or production of stress hormones (NA), thereby reducing stress damage to the body. At the same time, a long-term (for 10 days), though less intense stress stimulation in HS than AS, reduces the activity of MT and NO, which can lead to more significant damage to the body. Under the influence of the combined stress factors in the animals of the HS-AS and AS-HS groups, there was a unidirectional neurohormonal reaction: there was an increase in A, NA, ST, and NO against the background of a decrease in MT, which reflects a simultaneous increase in the activity of both stress-response and stress-limiting systems. It can mean that an increase in the activity of stress-limiting systems in case of combined stress damage limits the trigger of a cascade of pathophysiological reactions that have a negative effect on the body, which makes it possible for the body to adapt to the action of stressors.

4. Conclusion
In general, the results obtained provide a fair evidence of an increased functional activity of both stress-response (sympathoadrenal system) and stress-limiting systems (ST, MT, NO) in stress reactions of the body, the severity of which depends on the duration and sequence of stress stimuli on the animals. The observed changes in the levels of catecholamines, MT and NO in case of combinations of AS and HS may result from a general systemic restructuring of physiological and biochemical mechanisms under the sequential action of two stress factors, when one factor modifies the body's response to the other factor – so-called "cross adaptation". Our results at the biochemical level confirmed that under the influence of a sequential combination of chronic HS and AS, intensive mobilization of resources due to HS results in their depletion or decrease when the body is confronted with a new stimulus in the form of AS, which leads to regressive adaptation and is reflected in the corresponding changes in the levels of catecholamines (A, NA, ST), MT, and NO.

The experimental obtained data can explain the formation of pathological processes associated with stress and in connection with a significant role of acute and chronic stress in the pathogenesis of many somatic and psychosomatic diseases in humans, which is a pressing problem of modern therapeutic and preventive medicines.

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