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## DALARGIN ANTISTRESS EFFECTS IN EXPERIMENTAL CHRONIC STRESS MODEL

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#### Abstract

The problem of acute and chronic stress and the associated majority organisms' physiological systems functional disorders has recently attracted the attention of both practical and general theoretical specialists. Stress and its consequences are the largest cause of morbidity and mortality in developed countries. We supposed it should be important to study the antistress efficacy of one of the regulatory peptides - dalargin - in rats on conditions of chronic stress. The purpose of the work was to investigate the stress protective effects of peptide compound dalargin in conditions of experimental chronic stress model. In rats under conditions of chronic stress, the severity of aggressive-defensive behavior was determined, morphometric studies were performed, and the effect of dalargin on these indicators was studied. The data obtained showed the increase of aggressive-defensive behaviour severity in rats under chronic stress. The weight of both stress-competent and lymphopoietic organs continue to increased in rats with chronic stress experimental conditions which is accompanied by adrenal glands both cortical and medulla layers width increase. The use of dalargin in rats with chronic stress contributed to above mentioned behavioural and

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morphological changes normalization. Author suppose that dalargin stress-protective effects in rats in conditions of chronic stress model are the experimental background for this compound antistress efficacy testing reasonability for human body stress-induced changes complex pharmacological correction performing.

# Key words: chronic stress; rats; dalargin; adrenal glands; spleen; thymus; pathogenetic mechanisms; pharmacological correction

The problem of diseases incidence growth with stress or stress-related common pathogenetic factor and in general the issue of developing a comprehensive pharmacological, pathogenetically justified correction of these pathological conditions determines the constant relevance and significance of studying the common pathogenetic factors of stress-induced diseases to develop general measures for their treatment. The problem of acute and chronic stress and the associated majority organisms' physiological systems functional disorders has recently attracted the attention of both practical and general theoretical specialists [12, 14].

Stress and its consequences are the largest cause of morbidity and mortality in developed countries [8, 9, 16]. Under these conditions, the problem of complex pharmacological correction of chronic stress manifestations and its consequences becomes highly relevant. We supposed it should be important to study the antistress efficacy of one of the regulatory peptides - dalargin - in rats on conditions of chronic stress. The choice of dalargin is not accidental - the use of neuropeptide in conditions of experimental chronic stress is advisable due to their efficacy in conditions of peptic ulcer disease [1] and in adaptative disorders correction under such types of stress-generating pathology as experimental peritonitis and acute experimental pancreatitis [2, 6, 7].

The aim of the work is to investigate the stress protective effects of peptide compound dalargin in conditions of experimental chronic stress model.

Material and methods. The experiments were conducted under chronic experimental conditions on 62 male Wistar rats weighing 180-250 g, which were kept in vivarium conditions. The maintenance, handling and manipulation of animals were carried out in accordance with the "General Ethical Principles of Animal Experiments" approved by the Fifth National Congress on Bioethics (Kyiv, 2013), while being guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985), the methodological recommendations of the State Research Center of the Ministry of Health of Ukraine

"Preclinical Studies of Drugs" (2001) and the rules for the humane treatment of experimental animals.

Chronic stress in rats was reproduced through their daily (during 1.5 hrs) immobilization and single electric shocks (voltage 50 V, duration 5 s) to the tail root area. This procedure duration was 6 days [2].

Rats were started to be cared for 30 min after the end of their last immobilization. The experiments groups were randomized as follows: group 1 - intact rats (control, n=12); group 2 - animals with chronic stress (n=13); group 3 - animals with chronic stress which were administered dalargin (DAL; n=10). Dalargin was administered immediately after the end of the last immobilization and electric shock i.p. at a dose of 50  $\mu$ g/kg.

The expression of rats aggressive-defensive behavior (ADB) was determined via their reaction in response to an attempt to grab with the hand. The accepted scale for behavioral reactions severity assessing was used: 0 points - no reaction when grabbed with the hand; 1 point - the animal runs away when the hand approaches it; 2 points - the animal does not run away, but turns away and does not resist the grab with the hand; 3 points - the animal actively attacks the researcher's hand, breaks away when grabbed with the hand, bites in an attempt to free itself [11].

After animals' euthanasia with sodium pentobarbital overdose (85 mg/kg) the adrenal glands, spleen and thymus were removed and weighed. In addition, the width of the different layers of the adrenal glands was measured morphometrically in rats.

The data obtained were statistically analyzed using the Kruskal-Wallis test. P<0.05 was chosen as the significance level.

#### **Results.**

The ADB expression in rats with chronic stress significantly changed (Fig. 1). Thus, when trying to take the rats by hand, in response to the approach of the palm, all rats persistently tried to bite the glove and pounced on it. The animals' ADB severity under these conditions exceeded this index by 5 times in the control group (p<0.001). At the same time, dalargin contributed to a significant decrease in the ADB severity in relation to the corresponding index in rats with chronic stress (p<0.05).



Fig. 1. The influence of dalargin (DAl) on aggressive-defensive behaviour expression changes in rats with chronic stress.

Nores: \*\*\* - p<0.001 - significant differences of investigated indexes vs the same data in control observations;

# -  $p{<}0.05$  – significant differences of investigated indexes vs the same data in rats with chronic stress.

The weight of the left and right adrenal glands in rats with chronic stress significantly increased (respectively, by 28% [p<0.05] and by 32% [p<0.01]) compared with the same indexes in rats of the control group (Fig. 2).

The weight of the spleen in rats under the specified conditions decreased by 29% (p<0.01), and the weight of the thymus - by 22% (p<0.05). Dalargin use in rats with chronic stress also proved effective effects in terms of stress-responsive organs weight normalization.



Fig. 2. The influence of dalargin (DAl) on stress-competent organs weight changes in rats with chronic stress.

Notes: \* - p<0.05, \*\* - p<0.01 - significant differences of investigated indexes vs the same data in control observations;

# -  $p{<}0.05$  - significant differences of investigated indexes vs the same data in rats with chronic stress.

Morphometric studies revealed that rats with chronic stress showed a significant increase in the width of both cortical and medulla layers of the adrenal glands. These changes in adrenal glands both layers thickness were eliminated by dalargin (p<0.05; Fig. 3).



Fig. 3. The influence of dalargin (DAl) on adrenal gland layers width changes in rats with chronic stress.

Notes: \* - p<0.05, \*\* - p<0.01 significant differences of investigated indexes vs the same data in control observations;

# -  $p{<}0.05$  - significant differences of investigated indexes vs the same data in rats with chronic stress.

#### Discussion

Thus, the results we obtained indicate significant stress-induced changes that occur in stress-competent and lymphopoietic organs in rats under immobilization-electropain stress. These findings are expressed in adrenal glands weight decrease and in spleen and thymus weight increase as well as an adrenal glands cortical and medulla layers width increase. In addition, the behaviour of rats changed significantly, as evidenced by the increase in their aggressiveness within the experimental conditions.

Opioid peptide dalargin use in rats with chronic stress was effective in normalizing all of the above behavioral and morphological changes. Considering the data [5] it should be

remembered that adrenal hypertrophy and lymphopoiesis organs atrophy are one of the most important manifestations of stress response (or general nonspecific adaptation syndrome, according to G. Selye). These indexes both can be used to assess the severity of stress-related changes in the body development, and to determine the certain pharmacological compounds stress-protective effect efficacy. That is, the dalargin stress-protective efficacy which we revealed in chronic stress conditions can be estimated as experimental basis for the feasibility of conducting further studies to increase the effectiveness of complex pharmacological correction of stress-related manifestations [3].

Interestingly, the data obtained are somewhat consistent with those when studying the efficacy of another neuropeptide compound – sandostatin – in the same model of chronic stress [7]. These experimental trials, among other things, also showed the liposomal form of sandostatin high efficacy in the aspect of lipoperoxidation stress-induced disorders correction that are consistent with some other data [13, 15].

The data obtained, in our opinion, appear significant and promising for further conducting a number of experimental trials due to the general pathophysiological ideas about the stress mandatory involvement in the pathogenetic links of most diseases including surgical ones. Interesting in this aspect are the studies which proved the effectiveness of including dalargin in the complex pathogenetic treatment of acute inflammatory diseases of the genital organs and uterine appendages precisely for the purpose of correcting the stress component of the pathological condition [4, 9, 10]. Regarding surgical diseases, it should be noted that in addition to traditional treatment, improving the specific correction of the stress component of a particular disease, the patient's condition before and after surgery is a promising direction in these diseases treatment efficacy increasing.

#### Conclusions

1. The severity of aggressive-defensive behaviour increases in rats under chronic stress.

2. The weight of both stress-competent and lymphopoietic organs continue to increased in rats with chronic stress experimental conditions which is accompanied by adrenal glands both cortical and medulla layers width increase. We consider these morphological changes as a manifestation of a compensatory-adaptive reaction in response to stress factor action.

3. The use of dalargin in rats with chronic stress contributed to above mentioned behavioural and morphological changes normalization.

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4. Dalargin stress-protective effects in rats in conditions of chronic stress model are the experimental background for this compound antistress efficacy testing reasonability for human body stress-induced changes complex pharmacological correction performing.

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#### **Institutional Review Board Statement**

The experimental studies were carried out in the conditions of a chronic experiment in accordance with international standards of humane treatment of vertebrate animals and approved by the Ethics Committee of Odesa National Medical University (N7/21, 11 October 2021)

### **Informed Consent Statement**

The data of experimental studies are given. Written informed consent from the patients was not necessary to publish this paper.

#### **Data Availability Statement**

The data presented in this study are available on request from the author.