

Effect Of Oligopeptides - Homologues of the Fragment of ACTH₁₅₋₁₈ on Morphogenetic Markers of Stress in the Adrenal Glands on the Model of Acute Cold Injury in Rats

Short Title in English: Stress-Protective Properties of Oligopeptides-Homologues of ACTH₁₅₋₁₈

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ABSTRACT

Objectives: The study's aim was to evaluate stress-protective effect of oligopeptides-homologues of the ACTH fragment 15-18 on morphogenetic signs of stress reaction of the adrenal glands under acute cold exposure in rats.

Materials and Methods: The acute cold stress was reproduced by placing random-bred male rats in a freezer at a temperature of -18 ° C for 2 hours. The peptides-homologues of the ACTH₁₅₋₁₈ Acetyl-(D-Lys)-Lys-Arg-Arg-amide (KK-1) and Acetyl-(D-Lys)-Lys-(D-Arg)-Arg-amide (KK-5) and the reference medicine Semax were administered intranasally in a dose of 20 mcg/kg 30 minutes before and after cold exposure. Rectal temperature was measured before and 10 minutes after cold exposure. Zona glomeruloza, zona fasciculata, zona reticularis, and the area of cells and nuclei of adrenocorticocytes of zona fasciculata were measured.

Results: KK-1 significantly prevented structural changes in the adrenal cortex and medulla, stabilized the secretory activity of glucocorticoid-producing cells. However, the congestion of capillaries of zona fasciculata and zona reticularis remained in some locations. Zona fasciculata cells had a marked tendency to decrease, and the area of nuclei significantly decreased ($p < 0.05$) recovering the width to control animals' markers. KK-5 had more marked recovery of the adrenal glands (a greater saturation of cytoplasm of adrenocorticocytes of zona glomerulosa and zona fasciculata). The number of chromaffin cells at rest was increased in the adrenal medulla. KK-5 statistically significantly normalized both the area of cells ($p < 0.05$) and the area of nuclei ($p < 0.05$) of zona fasciculata, unlike KK-1, which reliably restored only the marker of the nuclei area. Some morphometric parameters of acute stress hypertrophy remained in the adrenal glands of rats receiving Semax.

Conclusion: KK-1 and KK-5 prevented the manifestation of acute stress reactions in the adrenal cortex of rats. KK-5 had a more marked stress-protective effect compared to the peptide KK-1. Both studied substances exceeded the reference medicine Semax. KK-5 is a promising stressprotector and frigoprotector.

Key words: stressprotectors, acute cold stress, cold trauma, adrenal glands morphology, neuropeptides, oligopeptides - homologues of the fragment of ACTH₁₅₋₁₈.

INTRODUCTION

Stress underlies the pathogenesis of many diseases of the cardiovascular, immune, central nervous and other systems [1]. Prolonged stress exposure reduces the adaptive capabilities of the body and leads to the development of adaptation disease [2]. One of the leading links in the in the deleterious effects of stress is the disruption of the peptidergic system [3, 4], which makes it relevant to search for stress detectors among these substances. The range of peptidergic drugs having stress-protective activity is narrow. It is known that stress activates the release of adrenocorticotrophic hormone (ACTH) which led to glucocorticoids hormones release by adrenal glands. Taking into account the leading role of the hypothalamus-pituitary-adrenal axis in regulating the body's response to stress [5, 6], it seems promising to search for stress detectors among oligopeptides - homologues of the fragment of adrenocorticotrophic hormone. Peptides-homologues of the fragment of ACTH 15-18 (Lys-Lys-Arg-Arg) were obtained at the Institute of Highly Pure Biopreparations (St. Petersburg). In previous studies, we have established the stress-protective effect of peptides on models of acute immobilization [7, 8] and acute cold stress on the effect on behavioral reactions, anxiety and physical endurance of animals [9], markers of carbohydrate metabolism [10], blood systems [11]. The issue of the morphogenesis changes of the adrenal response to acute cold stress and the influence of frigoprotectors on these processes has been insufficiently studied, but such data are important for optimizing the frigoprotective effect. Taking into account that adrenal glands have one of the main role in the body-response to stress, the purpose of the current study is to evaluate the stress-protective effect of oligopeptides - homologues of the ACTH fragment 15-18 on morphogenetic signs of stress reaction of the adrenal glands under acute cold exposure in rats.

MATERIALS AND METHODS

Experimental animals and ethical clearance

30 adult (3 months old) random-bred male rats (249±5 g) aged were taken from the vivarium of the National University of Pharmacy (Kharkiv, Ukraine). The animals were housed in standard polypropylene cages, at 22-24°C and 50% humidity in the well-ventilated room with a 12-hour light/dark cycle and free access to food and water. The work was carried out in the Educational and Scientific Institute of Applied pharmacy of National University of Pharmacy in compliance with Directive 2010/63/EU of the European Parliament and the Council "On the protection of animals used for scientific purposes" (Brussels, 2010). All experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy (№ 6, 8 June, 2021).

Drugs and chemicals

Peptide homologues of the fragment of ACTH 15-18 (Lys-Lys-Arg-Arg) under the laboratory codes KK-1 (Acetyl-(D-Lys)-Lys-Arg-Arg-amide) and KK-5 (Acetyl-(D-Lys)-Lys-(D-Arg)-Arg-amide) were synthesized at the Institute of Highly Pure Biopreparations by A. A. Kolobov. The peptides were obtained by solid-phase synthesis methods using Boc-technology and purified by preparative reverse-phase chromatography, their purity is at least 98%. In these compounds, one (KK-1) or two (KK-5) natural amino acids are replaced by the corresponding D-stereomer. Peptides don't relieve any hormonal activity and due to the

presence of D-amino acids have increased resistance to human serum proteases, are practically non-toxic substances [12].

Study design

The model of acute cold stress was reproduced by placing animals in a freezer "NordInter-300" at a temperature of -18°C for 2 hours in individual plastic pencil cases with a volume of 5 dm^3 without limiting airflow and motor activity [13, 14]. This experimental model was chosen according to the aim study and the results of previous investigation that showed positive influence of peptide homologues of the fragment of ACTH 15-18 on behavioral reactions, anxiety, and physical endurance of animals [9], markers of carbohydrate metabolism [10], blood systems [11]. The peptides-homologous of the fragment of ACTH₁₅₋₁₈ were administered intranasally (i/n) in the form of a solution in an effective stress-protective dose of 20 mcg/kg 30 minutes before and after cold exposure. This dosage was chosen to take into account the cerebroprotective and stressprotective properties relieved in previous investigations [7-11, 44]. The reference medicine Semax (Peptogen, RF) was administered i/n at a dose of 20 mcg/kg in a similar treatment. Heptapeptide Semax (Met-Glu-His-Phe-Pro-Gly-Pro) is a synthetic analogue of ACTH fragment 4-7 without hormonal activity. It is used to increase the adaptive capacity of the body under stress [15].

The animals were divided into 5 groups (6 rats in each group). Group 1 - control; group 2 – cold exposure (CE) (rats were exposed to acute cold stress); groups 3-5 - animals with an acute cold stress model CE who received experimental therapy: group 3 – CE+KK-1 peptide, group 4 - CE+KK-5 peptide, group 5 - CE+ reference medicine Semax. Rectal temperature was measured with a WSD-10 thermometer before and 10 minutes after cold exposure.

Adrenal glands histopathology

Animals were removed from the experiment 2 hours after cold stress exposure by decapitation under thiopental anesthesia (40 mg/kg). The adrenal glands of the animals were fixed in a 10% formalin solution, dehydrated in alcohols of increasing concentration, poured into paraffin. The sections were stained with hematoxylin and eosin [16].

The photographs were processed on a Pentium 2.4 GHz computer using the Toup View program. In the photographs, using the Toupcam Granum program, the width of zones of adrenal glands cortex (microns, μm) was measured: the zona glomerulosa (WZG), the zona fasciculata (WZF), the zona reticularis (WZR); the area of cells and nuclei (microns, μm^2) of adrenocorticocytes of zona fasciculata (AcZF, AnZF).

Statistical analysis

Statistical processing was carried out by methods of variational statistics using a standard package of statistical programs "Statistica", V. 6.0" [17-19]. The results are expressed as mean \pm standard error of mean (the level of statistical significance was considered as $p < 0.05$) or Me (LQ;UQ). Statistical differences between groups were analyzed using the parametric Student's t-test in cases of normal distribution as well as non-parametric Mann-Whitney U-test and Kruskal-Wallis test in cases of its absence.

RESULTS

After the 2-hour acute cold exposure in rats of the ~~control pathology~~ group with CE, the rectal temperature significantly decreased by an average of 3°C ($p < 0.05$), or 8% relative to the initial level (Table 1). All three peptides decreased the severity of hypothermia compared to CE group: body temperature decreased by 1.0°C , or 2.7% (KK-1); by 0.3°C , or 0.8% (KK-5) and 0.4°C , or 1.1% (Semax). This decrease was statistically significant for all groups respectively ($p < 0,05$). The temperature of rats treated with the studied oligopeptides did not differ from that of intact animals.

The histostructure of the adrenal cortex and medulla of the rats of the ~~intact~~-control group fully corresponds to the status of a physiologically functioning organ (Fig. 1).

According to morphometry, the functional state of the adrenal glands of intact control rats is characterized by the following indicators: WZG was 44.64 microns, WZF – 174.1 microns, WZR – 78.4 microns; AcZF – 168.8 microns, AnZF – 34.1 microns (Table 2).

In the majority of rats of the control pathology CE group, focal disorientation of connective tissue fibers in the capsule, their plasma impregnation, and tearing were observed. (Figure 2 A, B).

In the zona fasciculata, the linearity of corticocyte strands is broken in places, there is a decrease in their vacuolization, appearance of areas of complete cell defatting, which sometimes extends to the outer zone up to the zona glomerulosa. Some nuclei had not a rounded, but a more elongated, "lobed" shape. The boundary between the zona glomerulosa and the zona fasciculata is not always clear, the capillary net is expanded and congested (Figure 2 C, D, E). The cells of the zona reticularis have not been visually changed. The expansion and congestion of the venous-capillary net of the zona reticularis were observed (Figure 3 F). In the adrenal medulla, the number of cells with basophilic cytoplasm increased markedly, with a decrease in the number or absence of vacuoles (Figure 2 G).

According to morphometric measurements, the WZF in the rats of the control pathology CE group had significantly increased. There was a clear tendency towards an increase in AcZF and a significant increase in AnZF; WZG and WZR did not change in comparison with the markers of intact control (Table 2).

Changes in the connective tissue fibers of the adrenal capsule of rats that received the KK-1 peptide under acute general hypothermia were expressed insignificantly. There is only a weakly pronounced focal tearing of the capsule. The cells of the zona glomerulosa retain their location, their cytoplasm contains a different number of vacuoles. Cytoplasm vacuolization was increased in corticocytes of the zona fasciculata, there were no marks of defatting (Figure 3 A). The congestion of the capillaries of the zona fasciculata and the zona reticularis is preserved in some locations. The number of cells with basophilic cytoplasm and the presence of vacuoles in the medulla is increased (Figure 3 B).

The results of morphometric analysis confirmed the positive effect of the KK-1 peptide on the prevention of the adrenal glands hypertrophy under acute cold stress. Thus, AcZF had a marked tendency to decrease, and AnZF significantly decreased compared to rats of the control pathology CE group. All this contributed to the recovery of WZF to the markers of control animals (Table 2).

The peptide KK-5 had a more marked positive effect on the histostructure of the adrenal glands. This was expressed in a greater and evenly saturation of the cytoplasm of adrenocorticocytes both of the zona glomerulosa and the zona fasciculata (Figure 4 A). More chromaffin cells of the medulla were in a state of functional rest (Figure 4 B).

Morphometric markers of the adrenal cortex confirmed the marked positive effect of KK-5 peptide on the adrenal glands under acute stress hypertrophy. WZF, AcZF, and AnZF were significantly reduced in comparison with the markers of the control pathology CE group and practically reached the level of intact control (Table 2). Normalization of AcZF and AnZF significantly exceeded such markers of the reference drug.

Small focal segments with disorientation, plasma impregnation, and fibrillation of connective tissue fibers are observed in the adrenal capsule of some rats having received Semax. The structure of the zona glomerulosa is mostly restored, although there were small areas with indistinctness of arcade-like cell formations. There was not always clear linearity of the location of corticocytes in the zona fasciculata, there were still enough cells with a decrease/absence of cytoplasm vacuolization. Foci of cell defatting with different severity were also observed in the middle and outer sections of the zona fasciculata (Figure 5 A, 5 B). In some locations, extension and congestions of capillaries are observed both in the zona fasciculata and the zona reticularis. The state of chromaffin cells of the medulla varied in

different animals from the condition of rest to functional tension (Figure 5 C, D).

Morphometric parameters of the adrenal glands of rats after administration of the reference medicine Semax indicate that some markers of acute stress hypertrophy remained (Table 2). Thus, AcZF and AnZF had no statistically significant differences compared to animals of the control pathology CE group.

DISCUSSION

Stress is essential in the body's adaptation to adverse environmental factors, which, with prolonged exposure, turns into a pathogenic process [2, 20]. One of the most common stressful factors is cold stress, which negatively affects health and productivity [21]. Due to complexity of pathogenesis of cold injury associated with cardiovascular, endocrine, central nervous, respiratory, immune, and other systems, the treatment is challenging problem [1]. Considering that stress leads to disruption of the functioning of the peptidergic system [4], it is advisable to study the stress-protective properties of neuropeptides. Since the hypothalamus-pituitary-adrenal system participates in the mechanism of stress [22], neuropeptides-homologues of ACTH deserve special attention in this aspect. Among such medicines, synthetic ACTH 1-24 Tetracosactide (Synacthen Depot®) are known, which is used for the treatment of disseminated sclerosis, a number of allergic diseases [23]; Semax is a synthetic analogue of ACTH 4-10, with nootropic and neuroprotective activity [24, 25]. Stress-protective properties of these peptides are being studied currently [26-29].

In our previous studies on the model of acute immobilization stress in rats, stress-protective properties of oligopeptides homologous to the primary amino acid sequence of ACTH 15-18 were revealed [7, 8]. Under acute cold stress, a positive effect of peptides on behavioral reactions, physical endurance, and carbohydrate metabolism was shown [9, 10]. In the current study, using a model of acute cold injury, we confirmed the frigoprotective properties of these peptides to reduce hypothermia and found their marked protective effect on the histostructure of the adrenal glands of rats under cold stress.

Exposure to acute cold stress caused marked morphological changes in all areas of the adrenal glands of rats that did not receive experimental therapy. Thus, there was disorientation of connective tissue fibers in the capsule, a violation of the arcade-like arrangement of cells in the zona glomerulosa was observed, in the zona fasciculata part of the nuclei had not a rounded, but an elongated shape. All these morphological changes indicate functional tension of the adrenal glands. It is believed that this is a compensatory mechanism that aims to intensify metabolic processes by increasing the surface of the junction of the nuclear and cytoplasmic parts [30]. Hypertrophy of the adrenal cortex was characterized by an increase in the area of adrenocorticocytes of the zona fasciculata of the adrenal cortex and their nuclei, which led to the expansion of this zone. An increase in the zone of adrenocorticocytes is also typically for models of chronic immobilization stress [31, 32], chronic variable/unpredictable stress in rats [33]. However, the level of corticosterone did not change in these stress models. Under acute cold stress in our study, an increase in cell size is accompanied by defatting of the adrenal cortex, which indicates functional stress associated with stimulation of glucocorticoid hormones production. An increase in corticosterone production under stress is typically for both acute and chronic heat stress in rats [34, 35]. Activation of the adrenal cortex under acute cold stress is accompanied by changes in its vascular system: expansion and congestion of capillaries, which coincides with the data of other scientists [36].

The KK-1 peptide significantly prevented structural changes in the adrenal cortex and medulla under acute cold stress, stabilized the secretory activity of glucocorticoid-producing cells, and also contributed to the maintaining of lipid saturation. However, the congestion of capillaries of the zona fasciculata and the zona reticularis remained in some locations. Much more complete recovery of the adrenal glands of rats receiving the KK-5 peptide was observed, which was reflected in a greater and uniform saturation of the cytoplasm of

adrenocorticocytes of the zona glomerulosa and the zona fasciculata. The number of chromaffin cells in the state of rest was increased in the adrenal medulla. The peptide KK-5 statistically significantly normalized both AcZF and AnZF, unlike the peptide KK-1, which reliably restored only the marker of AnZF.

The results of our study demonstrate a marked frigo- and stress-protective effects of peptides, which might be connected with a reduction of the effect of ACTH on the synthesis of glucocorticoids in the zona fasciculata and the release of epinephrine in the adrenal medulla. It is known that ACTH belongs to the melanocortin family [37, 38]. The ACTH receptor, known as MC2R, is located in the zona fasciculata and the zona reticularis of the adrenal cortex [22, 39] and selectively binds only to ACTH [40]. Stimulation of MC2R promotes the production of glucocorticoids and mineralocorticoids by the adrenal cortex under stress [41]. The sequence of amino acids of ACTH at positions 15-19 is responsible for its binding to MC2R [42]. Also, it is supposed that ACTH participates in the regulation of its own secretion through negative feedback via MC2R mRNA detected in the pituitary gland [43]. We assume that the stress-protective effect of the studied peptides-homologues of the fragment of ACTH15-18 is realized due to affinity to MC2R receptors, which leads to the prevention of the effect of ACTH on the development of histostructure changes in both the cortex and the adrenal medulla under stress.

The reduction range of morphogenetic marks of a stress reaction in the adrenal glands does not directly depend on the severity of the antihypothermic effect of the studied peptides. Thus, despite almost the same indicators of body temperature in the groups of animals receiving the peptide KK-5 and Semax under cold exposure, morphological changes in the adrenal glands are almost absent in animals treated with the peptide KK-5 but moderately expressed in rats treated with Semax.

Thus, both studied peptides, especially KK-5, reduce morphogenetic signs of a stress reaction in the adrenal glands of rats under acute cold injury, surpassing the reference medicine Semax. The KK-5 peptide can be considered the most promising frigoprotector.

CONCLUSION

1. On the model of acute cold injury in rats, peptides-homologues of the fragment of ACTH 15-18(Acetyl-(D-Lys)-Lys-Arg-Arg-amide and Acetyl-(D-Lys)-Lys-(D-Arg)-Arg-amide) and the reference medicine Semax (Met-Glu-His-Phe-Pro-Gly-Pro) prevent hypothermia.
2. Acute cold exposure causes acute stress hypertrophy of the adrenal cortex in rats, which is characterized by an increase in the area of adrenocorticocytes of the zona fasciculata and the zona reticularis of the cortex. These changes are accompanied by defatting of the adrenal cortex. This indicates functional tension associated with stimulation of glucocorticoid hormones production.
3. Peptides-homologues of the ACTH fragment 15-18(Acetyl-(D-Lys)-Lys-Arg-Arg-amide and Acetyl-(D-Lys)-Lys-(D-Arg)-Arg-amide) prevent the manifestation of acute stress reactions in the adrenal cortex of rats, which is proved by the absence of structural changes in the cortex. Also, the peptides stabilized the secretory activity of the glucocorticoid-producing cells, and also contributed to the maintaining of their lipid saturation. Thus, preserving the reserve capabilities of secretory cells, Acetyl-(D-Lys)-Lys-Arg-Arg-amide and Acetyl-(D-Lys)-Lys-(D-Arg)-Arg-amide peptides increase the resistance of the adrenal cortex to the action of a cold factor having a stress-protective effect.
4. According to the severity of the stress-protective effect, Acetyl-(D-Lys)-Lys-(D-Arg)-Arg-amide has a more marked stress-protective effect compared to the peptide Acetyl-(D-Lys)-Lys-Arg-Arg-amide, and both studied substances exceed the reference medicine Semax.

STUDY LIMITATIONS

The absence of discussion of hormonal status is the limitation of our study.

CONFLICT OF INTEREST:

No conflict of interest was declared by the authors. The authors are solely responsible for the content and writing of this paper.

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Table 1. The effect of the peptides KK-1, KK-5, and Semax on the body temperature of rats before and after a 2-hour cold exposure (CE) at -18°C

Observation period	Group of animals, body temperature (°C)				
	Control	CE	CE + KK-1	CE + KK-5	CE + Semax
Before CE	36,8±0,21	37,4±0,38	37,5±0,5	36,6±0,35	37,1±0,45

10 minutes after CE	–	34,4±0,21*	36,5±0,4#	36,3±0,4#	36,7±0,57#
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Notes:: * - $p < 0.05$ when compared with intact, # - $p < 0.05$ when compared with CE group (Student's criterion).

Uncorrected proof

Table 2. The effect of acute cold stress on the morphometric markers of the adrenal glands of rats (Me (LQ;UQ))

Group of animals	Markers				
	WZG, μm	WZF, μm	WZR, μm	AcZF, μm^2	AnZF, μm^2
Control	44.64 (38.03;45.15)	174.1 (163.6;181.3)	78.4 (72.4;86.9)	168.8 (159.7;187.6)	34.1 (32.9;35.1)
Cold exposure	43.90 (43.09;45.10)	187.5 (179.1;191.6)*	86.2 (74.3;88.4)	186.4 (175.3;199.1)	39.0 (38.8;39.4)*
Cold exposure + KK-1	41.56 (33.34;50.63)	179.8 (169.3;190.1)	75.7 (61.3;88.4)	177.6 (156.3;191.6)	33.3 (30.3;38.8) ^
Cold exposure + KK-5	41.08 (39.85;42.28)	173.2 (166.7;182.8) ^	77.4 (75.4;80.3)	157.0 (149.1;161.1) ^	32.9 (32.4;36.3) ^
Cold exposure + Semax	40.47 (39.07;47.84)	181.7 (180.3;186.8)	81.6 (68.1;85.5)	205.2 (188.2;219.0)*#	36.1 (34.9;39.1)*#
P	0.9235	0.2468	0.8303	0.0075	0.0011

Notes: p – the level of statistical significance when comparing samples (Kruskal-Wallis criterion); * – $p < 0.05$ when compared with the control group (Mann-Whitney criterion); ^ – $p < 0.05$ when compared with the cold exposure group (Mann-Whitney criterion); # – $p < 0.05$ when compared with the group of animals treated with the KK-5 peptide (Mann-Whitney criterion).

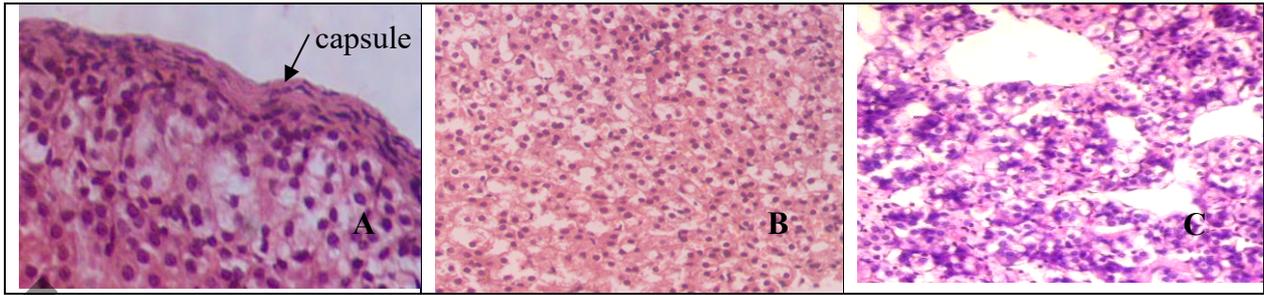


Figure 1. The adrenal gland of an **control** rat. A - capsule and cells of zona glomerulosa (arrow), hematoxylin-eosin, x400; B - zona fasciculata, hematoxylin-eosin, x400; C - chromaffin cells of the adrenal medulla, hematoxylin-eosin, x 250.

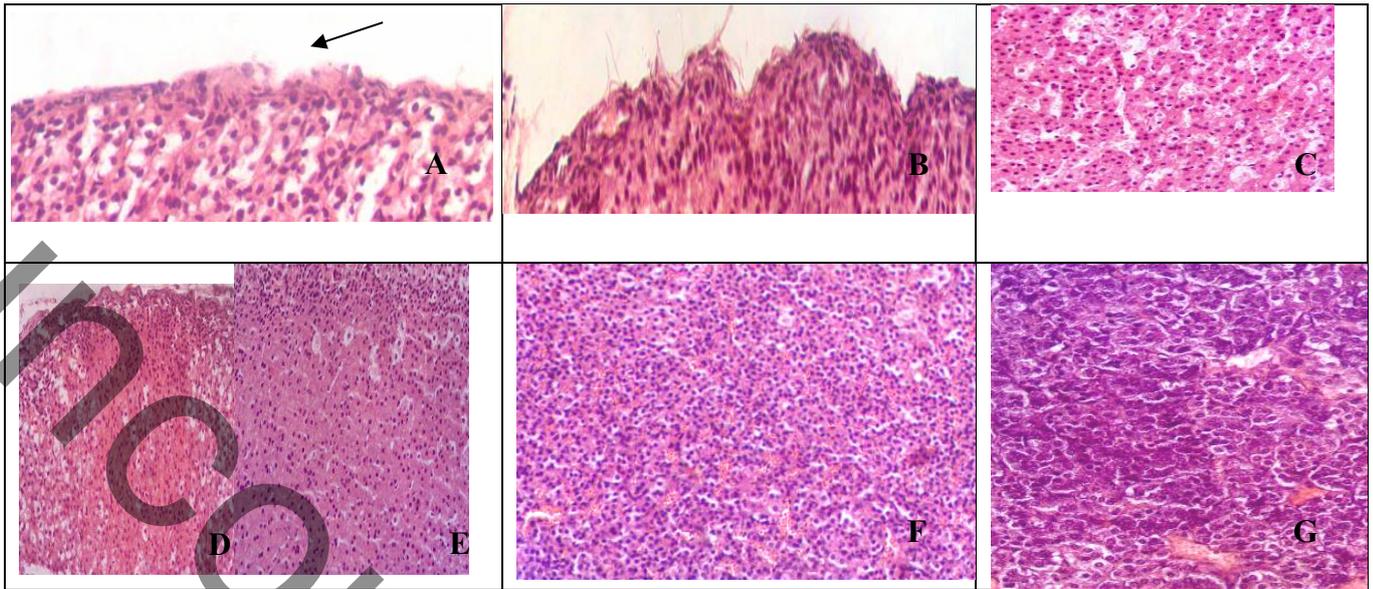


Figure 2. The adrenal gland of the animals of the CE group (acute cold stress): A - the disorientation of fibers, plasmatic impregnation in capsule (arrow); B – disruption of arcade-like arrangement of corticocytes, the absence of vacuolization of the cytoplasm of cells; hematoxylin-eosin, x 250; C – reduction of vacuolization of the cytoplasm of corticocytes of the zona fasciculata, expansion and congestion of the capillaries; hematoxylin-eosin, x 250; D, E – different size plots of complete defatting of corticocytes; hematoxylin-eosin, x 250; F – expansion and congestion of the venous-capillary net of the zona reticularis; hematoxylin-eosin, x 200; G – an increase in endocrine cells with basophilic cytoplasm of the medulla, decrease or absence of vacuolization of the cytoplasm; hematoxylin-eosin, × 250.

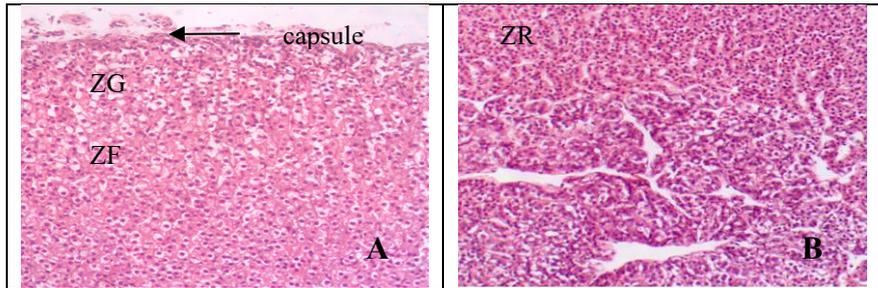


Figure 3. The adrenal gland of a rat received the KK-1 peptide under acute cold stress. A - recovering of the functional state of the zona glomerulosa (ZG) and the zona fasciculata (ZF); hematoxylin-eosin, x200; B - the normal state of cells and capillary net of the zona reticularis (ZR), an increase in chromaffin cells of the adrenal medulla, that is relevant to the state of rest; hematoxylin-eosin, x200.

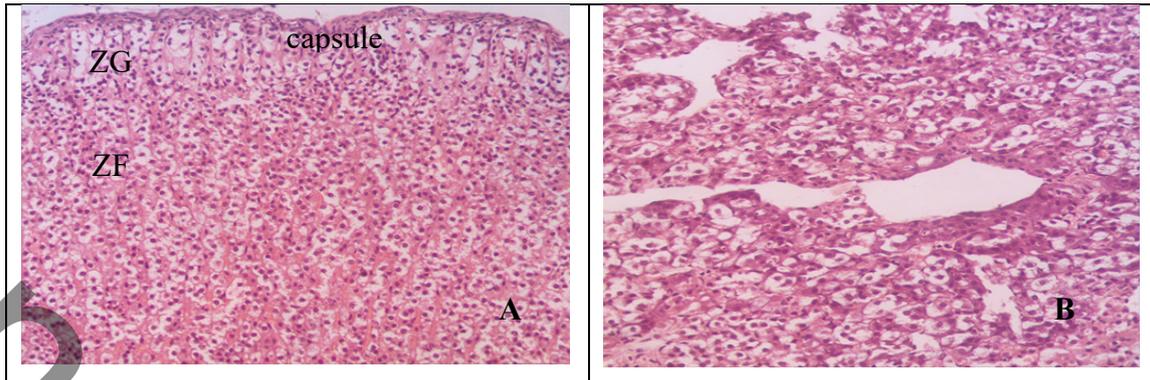


Figure 4. The adrenal gland of the rat received the peptide KK-5 under acute general hypothermia. A - complete recovering of the structural and functional state of the zona glomerulosa and the zona fasciculata; hematoxylin-eosin, x200; B - a significant number of chromaffin cells of the adrenal medulla in a state of functional rest; hematoxylin-eosin, x250.

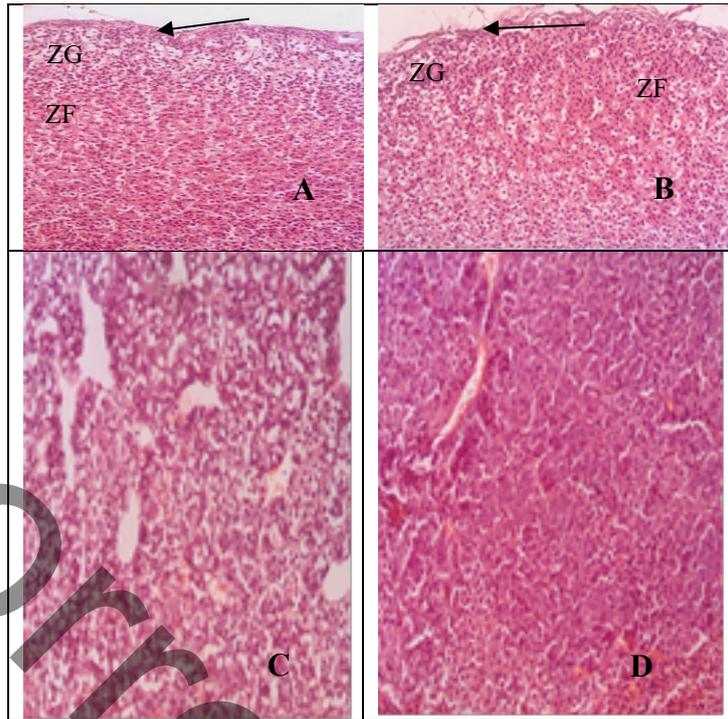


Figure 5. The adrenal gland of a rat that received Semax under acute cold stress. A - a small area of disorientation of fibers in the capsule, incomplete recovering of the structure of the zona glomerulosa (ZG), lack of linearity of the location of corticocytes of the zona fasciculata, a moderate increase in vacuolization of the cytoplasm of cells of the zona fasciculata (ZF); hematoxylin-eosin, x200. B - tearing of the capsule fibers, a site of a violation of the structure of the zona glomerulosa (ZG), a site of complete defatting of the middle and outer sections of the zona fasciculata (ZF); hematoxylin-eosin, x200. C, D - different functional states of chromaffin cells of the adrenal medulla: from a condition of rest (C) to tension (D). Hematoxylin-eosin. x200.