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EFFECT OF GLYPROLINS ON WHITE BLOOD CELL PARAMETERS AND PHAGOCYTIC ACTIVITY OF NEUTROPHILS IN CONDITIONS OF EXPERIMENTAL HYPERTHYROIDISM

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ABSTRACT: This study investigated the effects of glyproline neuropeptide compounds (selank and Pro-Gly-Pro) on the white blood cell count and phagocytic activity of neutrophils in 40 nonlinear white male rats aged 6–8 months with experimental hyperthyroidism. Experimental hyperthyroidism was simulated by intragastric administration of L-thyroxine sodium salt pentahydrate at a dose of 150 µg/kg/day for 21 days. All animals were equally divided into four groups: healthy rats (control), rats treated with L-thyroxine sodium pentahydrate (hyperthyroidism), rats treated with selank, and rats treated with Pro-Gly-Pro at doses of 200 µg/kg/day intraperitoneally for 21 days, starting one day after the last administration of L-thyroxine sodium pentahydrate. After the animals were removed from the experiment, the white blood cell count and the percentage of lymphocytes, stick, and segmentonuclear neutrophils were calculated, and the phagocytic activity of neutrophils was evaluated. In the case of experimental hyperthyroidism, neutrophilic leukocytosis with a shift to the right, lymphopenia, and decreased phagocytic activity of neutrophils were observed. Glyproline neuropeptides contributed to the correction of observed changes in white blood cell indices and phagocytic processes, which indicates the immunocorrecting effect of the test compounds. Thus, glyproline neuropeptides demonstrated pronounced immunotropic activity, which manifested in the correction of changes arising from the leukocyte count and phagocytosis processes. However, further detailed study of the pharmacological effects of neuropeptide agents on experimental hyperthyroidism is necessary.

Keywords: glyprolins; neuropeptides; selank; experimental hyperthyroidism; immunotropic activity; leukocytes; phagocytic number; phagocytic index.

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ВЛИЯНИЕ ГЛИПРОЛИНОВ НА ПОКАЗАТЕЛИ БЕЛОЙ КРОВИ И ФАГОЦИТАРНУЮ АКТИВНОСТЬ НЕЙТРОФИЛОВ В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО ГИПЕРТИРЕОЗА

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Резюме. В условиях экспериментального гипертиреоза исследовано влияние глипролиновых нейропептидных соединений (селанк и Pro-Gly-Pro) на количество лейкоцитов и фагоцитарную активность нейтрофилов у 40 нелинейных белых крыс-самцов 6–8-месячного возраста. Экспериментальный гипертиреоз моделировали с помощью внутрижелудочного введения пентагидрата натриевой соли L-тироксина в дозе 150 мкг/кг/сут в течение 21 дня. Все животные были разделены на 4 равные группы: контрольную — интактные животные (контроль); животные, получавшие пентагидрат натриевой соли L-тироксина (гипертиреоз); крысы, получавшие селанк, и крысы, получавшие Pro-Gly-Pro, в дозах 200 мкг/кг/сут внутривнутрибрюшинно ежедневно в течение 21 дня, начиная через сутки после последнего введения пентагидрата натриевой соли L-тироксина. После выведения животных из эксперимента подсчитывали количество лейкоцитов и процентное содержание лимфоцитов, палочко- и сегментоядерных нейтрофилов, а также оценивали фагоцитарную активность нейтрофилов. Установлено, что в условиях экспериментального гипертиреоза наблюдалось развитие лейкоцитоза с нейтрофилизом со сдвигом вправо и лимфопении, а также снижение фагоцитарной активности нейтрофилов. Введение глипролиновых нейропептидов способствовало коррекции наблюдаемых изменений со стороны показателей белой крови и фагоцитарных процессов, что свидетельствует об иммунокорригирующем действии исследуемых соединений. Таким образом, глипролиновые нейропептиды обладают выраженной иммунотропной активностью, проявляющейся в коррекции изменений, возникших со стороны лейкоцитарной формулы и процессов фагоцитоза, что актуализирует дальнейшее детальное изучение фармакологических эффектов нейропептидных средств в условиях экспериментального гипертиреоза.

Ключевые слова: глипролины; нейропептиды; селанк; экспериментальный гипертиреоз; иммунотропная активность; лейкоциты; фагоцитарное число; фагоцитарный индекс.

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BACKGROUND

To date, a developed concept confirms the causal relationship of thyroid diseases with pathological changes in the neuroimmunoendocrine system as a whole [1–3]. Changes in thyroid gland functions are accompanied not only by hormonal imbalance and neuropsychiatric disorders but also by pathological changes in the immune system. Thyroid hormones were found to have a modulating effect on immune cells, which is accompanied by various changes in reactivity indicators. Thus, mild hyperthyroidism is accompanied by the stimulation of the immune response, an increase in the total leukocyte count, and a decrease in the phagocytic activity of neutrophils. In turn, pronounced hyperthyroidism is characterized by suppressive changes in immune processes and increased phagocytosis [4–6]. Thyroid hormones implement immune effects through two main mechanisms, namely, interaction with nuclear receptors, manifesting the so-called genomic effect, and with glucose transporters, mitochondrial proteins, calcium adenosine triphosphatase, adenylate cyclase, etc. [6–8].

The hormonal imbalance resulting from thyroid diseases is a major pathogenetic factor in the development of immune pathology. Thus, secondary immune disorders that occur in thyroid dysfunction must be treated. Neuropeptide compounds of glyproline nature [9], which are characterized by the presence of versatile pharmacological effects and high safety, are of particular interest in correcting many dysregulatory pathological conditions.

One of the well-known registered glyproline drugs is Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro), an analog of the regulatory peptide tuftsin (Thr-Lys-Pro-Arg), whose main function is immunomodulation. Tuftsin stimulates neutrophil and macrophage functions, activating phagocytosis. Along with the immunotropic action, the neuromodulatory activity of this peptide has also been proven, which manifests anxiolytic and nootropic effects [10–12]. The functions of the tripeptide Pro-Gly-Pro—inhibiting thrombogenesis and degranulation of mast cells and exhibiting antiulcer properties, etc.—were revealed.

Despite knowledge of the biological action of glyproline neuropeptides, no studies have confirmed the immunotropic activity of Selank and its new derivatives under experimental hyperthyroidism [13, 14].

The study aimed to analyze the effect of glyproline neuropeptide compounds Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) and Pro-Gly-Pro on the leukocyte count and phagocytic activity of neutrophils under experimental hyperthyroidism.

MATERIALS AND METHODS

The effect of glyproline compounds on the leukocyte count and phagocytic activity of neutrophils was evaluated on 40 white male rats aged 6–8 months in accordance with the Order of the Ministry of Health of the Russian

Federation No. 199n of 04/01/2016 “On Approval of the Rules of Laboratory Practice” and GOST 33215–2014 “Guidelines for the maintenance and care of laboratory animals.” Experimental hyperthyroidism was induced by the intragastric administration of L-thyroxine sodium salt pentahydrate from Sigma (MO, USA) at a dose of 150 µg/kg for 21 days. All animals were divided into four equal groups, namely, control group (intact animals), L-thyroxine sodium salt pentahydrate (hyperthyroidism)-treated group, Selank-treated group, and Pro-Gly-Pro-treated group, at doses of 200 µg/kg/day intraperitoneally daily for 21 days, starting on the day after the last injection of L-thyroxine sodium salt pentahydrate.

The leukocyte count and percentages of lymphocytes, stabbed and segmented neutrophils, and eosinophils were counted in the Goryaev chamber, and hemogram was then assessed in Romanovsky-Giemsa-stained smears. The phagocytic activity of neutrophils in the blood serum was assessed by the latex test, and the phagocytic number (PN) and phagocytic index (PI) were determined.

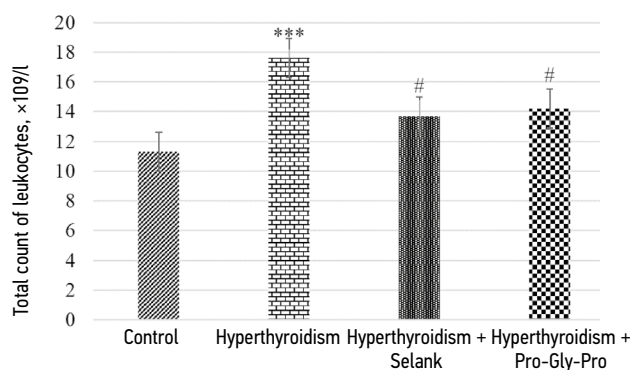
Statistically, the arithmetic mean (M) and error of the arithmetic mean (m) were calculated and presented as $M \pm m$. Group differences were assessed using Student's t -test, followed by multiple comparisons using the Bonferroni method. Differences were considered statistically significant at $p < 0.05$. The relationship between various attributes in the study sample was determined using correlation analysis by the value of the Spearman correlation coefficient (r).

RESULTS AND DISCUSSION

To confirm the development of hyperthyroidism, animal behavior, body weight, heart rate, and rectal temperature were evaluated. In the group with thyroid pathology, changes were noted, namely, the animals became aggressive, which manifested as the formation of inter-male confrontations, the body weight decreased by 30% ($p < 0.05$), heart rate increased by 42% ($p < 0.01$), and the rectal temperature reached $39.3 \pm 0.2 \text{ }^{\circ}\text{C}$ in relation to intact rats.

The leukocyte count increased significantly ($p < 0.001$) by 56% in the experimental hyperthyroidism group compared with the control group. The administration of Selank and the Pro-Gly-Pro compound was accompanied by a significant ($p < 0.05$) decrease in the leukocyte count by 22% and 20%, respectively, in relation to the hyperthyroidism group (Fig. 1).

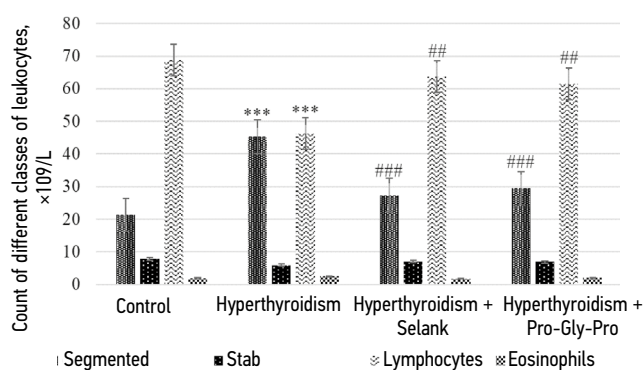
In the experimental hyperthyroidism group, the count of segmented neutrophils increased by 2.1 times ($p < 0.001$) and that of stab neutrophils increased by 24% ($p < 0.05$), and the lymphocyte count decreased by 33% ($p < 0.01$) compared with the control group. Selank contributed to the restoration of quantitative indicators of segmented and stab neutrophils, reducing them by 40% ($p < 0.001$) and 18% ($p < 0.05$), respectively, and an increase in the lymphocyte count by 38% ($p < 0.01$) compared with the hyperthyroidism group. The Pro-Gly-Pro compound led to a decrease in



*** — $p < 0,001$ relative to control; # — $p < 0,05$ relative to hyperthyroidism

Fig. 1. Effect of neuropeptide compounds on the total leukocyte count in experimental hyperthyroidism

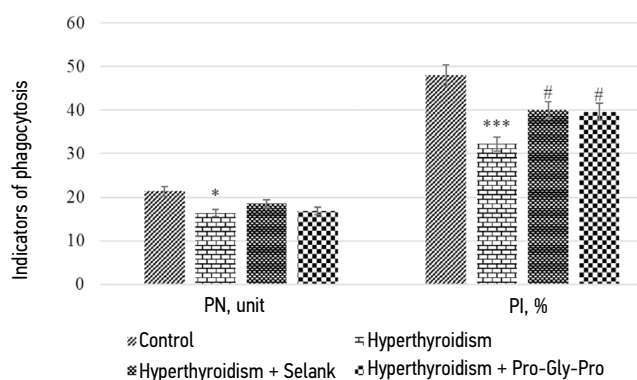
Рис. 1. Влияние нейропептидных соединений на общее количество лейкоцитов в условиях экспериментального гипертиреоза



*** — $p < 0,001$ relative to control; # — $p < 0,01$; ## — $p < 0,001$ relative to hyperthyroidism

Fig. 2. Effect of neuropeptide compounds on the percentage of leukocyte classes in experimental hyperthyroidism

Рис. 2. Влияние нейропептидных соединений на процентное содержание различных классов лейкоцитов в условиях экспериментального гипертиреоза



* — $p < 0,05$; *** — $p < 0,001$ relative to control; # — $p < 0,05$ relative to hyperthyroidism

Fig. 3. Effect of neuropeptide compounds on the phagocytic activity of neutrophils in experimental hyperthyroidism

Рис. 3. Влияние нейропептидных соединений на фагоцитарную активность нейтрофилов в условиях экспериментального гипертиреоза

segmented neutrophils by 35% ($p < 0.001$), stab neutrophils by 15% ($p < 0.05$), and lymphocyte count by 33% ($p < 0.01$) in relation to rats with hyperthyroidism (Fig. 2).

A decrease in the PN by 23% ($p < 0.05$) and the PI by 33% ($p < 0.001$) was found in the experimental hyperthyroidism group compared with the control group. Following the administration of Selank and Pro-Gly-Pro, the PN increased; however, these changes were not statistically significant. Selank and Pro-Gly-Pro caused an increase in the PI of approximately 25% ($p < 0.05$) compared with the control group (Fig. 3).

Our results were comparable with the findings of Zenkov [8] and Shcherba and Korda [14]. They have indicated that hyperthyroidism is accompanied by neutrophilic leukocytosis with a shift to the right and lymphopenia, which is typical for thyroid pathology that occurs with thyrotoxicosis syndrome. Moreover, Chenchak [7] and Zenkov [8] also state that

the administration of glyproline neuropeptide compounds contributes to the correction of the changes registered in white blood parameters and phagocytic processes, which may be associated with the ability of neuropeptides to activate cyclooxygenase, which inhibits the functional activity of leukocytes by increasing the levels of cyclic adenosine monophosphate in the cells.

CONCLUSION

The study established the presence of pronounced immunotropic activity in glyproline neuropeptides, which manifests as the correction of changes arising from the differential leukocyte count and phagocytosis processes. Thus, further detailed studies of the pharmacological effects of neuropeptide agents in experimental hyperthyroidism are needed.

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