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ABSTRACT

of the dissertation submitted for the degree of Doctor of Philosophy

EVALUATION OF EMOTIONAL STRESS BASED ON SOME BIOCHEMICAL INDICATORS OF BLOOD AND SALIVA IN HUMANS DURING STRESS

Specialty: 2411.01 – Human and animal physiology

Field of Science: Biology

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INTRODUCTION

Stress is a phenomenon that makes its presence felt throughout a person's life. Currently, people live in a rapidly developing and changing technological and competitive environment, and in certain situations, they face difficulties. This leads to the emergence of stress in individuals. In addition, factors such as job loss, exposure to violence, and illness also contribute to stress. Since the level of stress in individuals is directly related to the type and strength of their nervous system, it does not always manifest at the same level. The variation in stress manifestations is also influenced by factors such as working conditions, upbringing, and the surrounding environment.

Stress is a significant factor that primarily negatively impacts people's family, social, and work lives, as well as their health. The stress created by an individual can lead to psychological and physical problems, even affecting the person themselves. Stress has a destructive effect on the body, leading to chronic and permanent diseases. In the work environment, stress often conflicts with the goals of individuals and organizations. This also creates difficulties in individuals' family and social lives. Thus, stress becomes a problem for the individual. Therefore, both people and responsible employees of organizations must fight against stress to minimize its impact.

Stress significantly affects success in people's professional lives. People working in various domestic, socio-economic, and industrial conditions are always surrounded by the influence of ecological, social, professional, and other factors. In such situations, stress emerges as a characteristic psychological condition. Recently, stress has become a scientific problem that has attracted the attention of researchers.

Stress, which is related to the condition of the nervous system, is considered a general reaction to the impact that disrupts the body's homeostasis. Stress refers to the body's response to tension in the surrounding environment. Calmness and stress cannot coexist. In modern times, sensitivity, nervous tension, and haste create a range of problems for people, leading to certain failures, all of which are related to stress. Stress, which plays a role in reducing and increasing the

body's resistance, manifests in various situations. Most of these situations are associated with difficult times. In this context, Hans Selye regarded stress as an indispensable component of human life and emphasized the importance of not fearing it[1].

The term "stress" is widely used in biological research. Stress is related to both physiological and psychological events. Theories and forms of stress have rapidly developed since the mid-20th century, and the number of scientific and theoretical perspectives on the nature, causes, development, regulatory mechanisms, and manifestations of stress has significantly increased.

According to most scientists today, the basis of stress involves intense arousal processes encompassing various regions of the brain, the secretion of releasing factors of different origins by the hypothalamus, and ultimately, the secretion of regulatory hormones from the pituitary gland, which controls the synthesis and secretion of hormones in all endocrine glands of the body. Among the hormones secreted by regulatory glands under the control of the pituitary gland, the hormones of adrenal origin – namely, mineralocorticoids and glucocorticoids – play an exceptional role in the onset and manifestation of stress[2].

It should be noted that certain types of work require individuals to remain calm, avoid agitation, correctly assess unexpected situations, and make adequate and prompt decisions. It has now been proven that 80% of airplane accidents are due to human factors more specifically, mistakes made by air traffic controllers[3].

Taking such matters into account, it is one of the pressing problems to determine whether individuals in high-responsibility positions are agitated or fatigued in a timely, short-term, accurate, and non-invasive manner.

In light of the points mentioned above, it should be emphasized that research on the molecular mechanisms underlying stress is still insufficient, and there is a need to study various aspects of stress from

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[1] Selye Hans. Stress. Ümumi adaptasiya sindromu // "Parni iz Baku" Nəşriyyat evi - Bakı. -2009, - s. 6

[2] Stress, Appraisal, and Coping - Richard S. Lazarus, Susan... https://books.google.com

[3] Walter Bradford Cannon. https://en.wikipedia.org

different perspectives particularly the consequences of the anxiety it induces.

Research Objectives and Tasks

The main goal of the research is to determine the role of Dihydropyrimidinase-like Protein 2 (DBZ2) in the generation of stress in humans in clinical settings and in experimental behavior models. The tasks to achieve this goal are as follows:

1. Determining the level of DBZ2 in stressed human platelets using the indirect immunoenzymatic assay method.
2. Determining the level of natural autoantibodies against DBZ2 in stressed human serum using the indirect immunoenzymatic assay method.
3. Determining the level of DBZ2 in stressed human saliva using the indirect immunoenzymatic assay method.
4. Determining the level of cortisol hormone in stressed human serum using the indirect immunoenzymatic assay method.
5. Studying the effect of intracerebral administration of DBZ2 on the behavior of rats in the elevated plus-maze test, which determines the level of stress.
6. Studying the effect of intracerebral administration of DBZ2 on the behavior of rats in the open field test, which determines the level of stress.
7. Studying the effect of intracerebral administration of DBZ2 on the behavior of rats in the T-maze test, which involves decision-making, to determine the impact of DBZ2 on behavioral responses.

Key Hypotheses for Defense

1. An increase in the level of DBZ2 in the platelets of stressed individuals is observed.
2. A noticeable increase in the level of natural autoantibodies against DBZ2 in the serum of stressed individuals is observed.
3. A decrease in the level of DBZ2 in the saliva of stressed individuals is observed.
4. The intracerebral administration of DBZ2 to rats in the elevated plus-maze leads to changes in behavioral indicators that reflect an increase in the level of stress.

5. In the open field test, intracerebral administration of DBZ2 results in changes in behavioral indicators reflecting an increase in the level of anxiety.
6. In the T-maze with barriers, intracerebral administration of DBZ2 disrupts the proper selection of the arm with more food reinforcement, reflecting the increased anxiety in the rats.
7. Under the conditions requiring correct decision-making, there is a high likelihood of making incorrect choices due to the anxiety associated with changes in the level of DBZ2 in the organism's fluid environments.

Scientific novelty of the research. Although various studies related to stress have been conducted both in several countries around the world and in Azerbaijan, the evaluation of emotional tension in humans based on certain biochemical indicators in blood and saliva during stress has not previously been the subject of research. The dedication of the dissertation to this topic highlights its relevance. For the first time in the dissertation, compared to healthy individuals, the level of dihydropyrimidinase-like protein 2 (DPYSL2) in platelets and saliva of stressed individuals, as well as the level of natural autoantibodies against DPYSL2 and cortisol hormone in blood serum, has been determined. In addition, in the elevated plus maze, compared to the control group, in test animals that were injected intracerebrally with DPYSL2, the total duration of vertical stance and grooming behavior, the number of squares crossed and rearing acts, and the total duration of freezing behavior indicating anxiety levels were clarified.

Furthermore, in the T-maze with an obstacle, the identification of correct choice behavior in the test animals injected with DPYSL2, compared to the control group, is also one of the scientific novelties of the dissertation.

For the first time, the dissertation experimentally substantiates the connection between elevated levels of DPYSL2 in the brain and anxiety, as well as the emergence of incorrect decisions as a result. It is confirmed that it is possible to measure the degree of anxiety by determining the level of DPYSL2 in human platelet and saliva samples, and the level of natural autoantibodies against DPYSL2 in blood serum using immunochemical methods.

Practical significance of the study. The main result of the studies is the change in the level of DBZ2 in the blood and saliva samples of people experiencing anxiety. In particular, an increase in the level of DBZ2 is observed in the platelets of anxious people compared to healthy people. At the same time, the level of natural autoantibodies against DBZ2 is sharply increased in the serum samples of anxious people compared to healthy people. The first result reflects an increase in the level of DBZ2 in the cerebral cortex of anxious people, and the second result indicates a significant increase in the level of DBZ2 in the subcortical structures of the brain of these people[4]. Finally, a decrease in DBZ2 levels has been reported in the saliva of anxious individuals. Reliable changes in DBZ2 levels in the fluid environments of anxious individuals can be used as an objective marker of arousal using highly sensitive immuno-enzyme assays or immunochemical biosensors. Thus, objective immunochemical measurement of DBZ2 levels in human fluid environments is of great importance for detecting the exact level of arousal in workers who perform highly responsible jobs (e.g., airport dispatchers) that require rapid decision-making. The results of studies conducted in the T-shaped maze with an obstacle course show that the arousal induced by intracerebral injection of DBZ2 allows animals to make incorrect decisions when they are given different amounts of food reinforcement in the arms of the maze. The results obtained in this behavioral model confirm the incorrect decision-making due to arousal.

The results of the study on the assessment of emotional tension based on some biochemical indicators of blood and saliva during excitement can be used in conducting a number of studies related to stress.

In addition, the theoretical generalizations obtained during the research can be used in future textbooks, monographic studies, and scientific articles.

Research approval. The main provisions of the work are stated in 16 scientific articles, conference materials and theses published by the

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[4] Quliyeva Ş., Mehdiyev A., Hüseynzadə S. İnsanlarda həyəcanlığın tənzimlənməsində dihidropirimidinaza bənzər zülal2-nin rolü //Azərbaycan fizioloqlarının VI Qurultayı – Bakı . - 2023, -s.108.

author in journals and collections included in the list of the AAK, dedicated to various problems related to the topic at the X International Scientific Conference on "Scientific Achievements and Challenges in Biology" held by Baku State University (May 6-7, 2021); at the IV International Congress on "Medical Sciences and Innovations" organized by Turkey and Azerbaijan (July 5-6, 2021); Fundamental and Applied Scientific Research; Current Issues, Achievements and Innovations, LIX International Scientific and Practical Conference (September 10, 2022); at the I International Medical Forum (June 18-21, 2022); at the VI Congress of Azerbaijani Physiologists (October 30, 2023); Scientific work was reflected in the reports he delivered at the XVII International Scientific Research Conference (March 7, 2024) and the II International Scientific Conference on Education and Research Activities in the New Era: Realities and Challenges (December 13-14, 2024, Mingachevir).

Name of the organization where the dissertation work was completed. The dissertation work was completed at the Institute of Physiology named after academician Abdulla Garayev of the Ministry of Science and Education of the Republic of Azerbaijan and the Faculty of Medicine of Nakhchivan State University.

Publishing activities. Based on the materials of the dissertation, 17 scientific works have been published, of which 5 are theses, 11 are articles, and 1 is a patent of the Eurasian Patent Agency. 4 articles and 1 thesis have been published in impact factor journals.

Structure of the Dissertation. The dissertation has been prepared based on traditional scientific principles and in accordance with the requirements set by the Higher Attestation Commission (HAC) of the Republic of Azerbaijan. The research work consists of the following main chapters: *Introduction*, *Literature Review*, *Materials and Methods*, *Research Results*, *Discussion*, *Conclusions*, and *Practical Recommendations*. Each chapter is structured systematically and aligned with the objectives and tasks of the study. The list of references used in the dissertation includes a total of 168 sources. Among them, 12 are in Azerbaijani, 25 in Turkish, 8 in Russian, 114 in English, and 9 are internet resources. The dissertation consists of the following number of characters: table of Contents – not specified,

Introduction – 11,992 characters, Literature Review – 105,269 characters, Materials and Methods – 14,865 characters, Research Results – 24,116 characters, Discussion – 35,448 characters, Conclusions – 1,550 characters, Practical Recommendations – 521 characters, total – 195,691 characters.

CHAPTER I. LITERATURE REVIEW

Chapter I of the dissertation is compiled on the basis of local and foreign literature materials and is of a review nature. The chapter reflects literature materials on stress and its physiological mechanisms, hormonal changes occurring in the body during stress, the effect of stress on the amount of biogenic monoamines in the brain, the similarity of the amount of biogenic monoamines in platelets to changes in the amount of monoamines in the brain, the presence of natural autoantibodies against all tissue proteins in blood serum. A number of scientists emphasize the multidimensional nature of stress and note that it can cause emotional difficulties[5]

CHAPTER II. MATERIALS AND METHODS OF THE STUDY

The results of the presented studies were conducted on patients of both sexes up to 40 years of age immediately before the scheduled surgical operations. The surgical operations of the patients were planned to be performed in the surgical department of the Nakhchivan Autonomous Republic Hospital (Nakhchivan city, A. Aliyev street, №1). Behavioral experiments were conducted on 3-4-month-old male Wistar rats with a body mass of 160-220 g. All experiments were conducted in accordance with the International Convention of the European Union (November 13, 1987, Strasbourg) and the principles of animal protection..

2.1. Biochemical Methods

Isolation of DBZ2 was performed from the brain of large horned cattle. The whole cow brain was homogenized in an extraction buffer containing 0.3 M sodium chloride, 5 mM ethylenediaminetetraacetic acid (EDTA), 0.05 M phosphate buffer (pH 7.4), and 0.1% Triton X-

[5] 5. Karatopçu C., Akalın H., Sağlam A., Kazancı H., Özbek Y. Eğitim kurumları ve eğitimcilerde stres ve stresle baĝa çıkma kavramının incelenmesi. İntertnational journal of sosial and Humanities Aciences Researsch (JSHSR). Uluslararası Sosyal ve Beşeri Bilimler Araştırma Dergisi, 2023, s.1130.

100, at a tissue-to-buffer volume ratio of 1:4. The brain homogenate was centrifuged at 6000 rcf for 10 minutes using a K-23 centrifuge, and the supernatant protein extract was collected. Proteins were precipitated from the extract by adding ammonium sulfate to 40% saturation for 4 hours in a refrigerator (at 4°C). After precipitation in 40% ammonium sulfate, the total protein solution was centrifuged again at 6000 rcf for 15 minutes, and the precipitated protein fraction (S40) was separated from the supernatant. The S40 protein fraction was then dialyzed against 0.01 M phosphate buffer (pH 7.4) for 14 hours in a refrigerator (at 5°C) on a magnetic stirrer. The purpose of the dialysis was to remove ammonium sulfate salt from the S40 protein fraction and to allow the proteins to transition into the 0.01 M phosphate buffer. After dialysis, the S40 protein fraction was centrifuged again, and the supernatant was loaded onto a Sephadex G-150 column. The column was connected to a container with 0.01 M phosphate buffer, and the flow rate of the buffer through the column was adjusted to 20 ml/min. The fractions eluted from the column with 0.01 M phosphate buffer were collected in a "Bio-Frac" fraction collector.

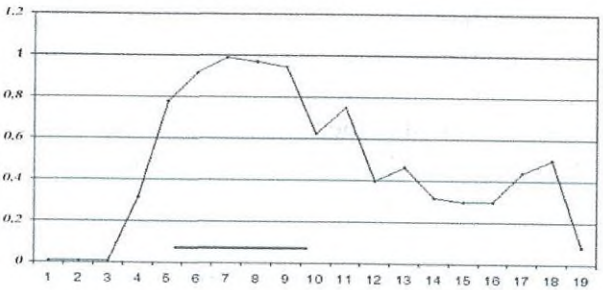

Figure 2.1. Chromatogram of the separation of the S40 fraction by gel chromatography. The immunopositive fraction is indicated by the horizontal line.

After gel chromatography, the collected protein fraction is concentrated using ultrafiltration, where the concentration is doubled under the influence of 6 atmospheres of air pressure. Exposure to a 40 mM EDTA solution is then carried out to disrupt calcium-dependent interactions and to separate DBZ2 from tubulin and actin proteins.

Isoelectric focusing is based on the effective separation of different proteins in the protein solution according to their specific isoelectric points, across a pH gradient formed by ampholytes. In the isoelectric focusing process, ampholytes with a pH range of 7–9 are used as the

catholyte, while a 1 M phosphoric acid solution is used as the anolyte. Ampholytes are applied at a final concentration of 2%. The separation of fractions is carried out by gradually increasing the voltage until the current decreases threefold and stabilizes. After this, 1 cm-thick strips are collected from the gel on the glass surface, and the pH values of the strips are measured using a pH meter. Based on the obtained values, the pH gradient formed during isoelectric focusing is determined (Fig. 2.2). The fractions near the isoelectric point of DBZ2 are collected from the gel and then eluted with distilled water. The eluted protein fractions are dialyzed for 6 seconds in a refrigerated chamber against distilled water. After dialysis, their concentration is determined using the Bradford method at a wavelength of 595 nm, and the samples are stored at 70°C.

The fractionation process and the selection of immunopositive fractions were carried out under the control of solid phase indirect immuno-enzyme analysis using polyclonal immunoglobulins against DBZ2. The origin of the protein was determined based on the mass spectroscopy method.

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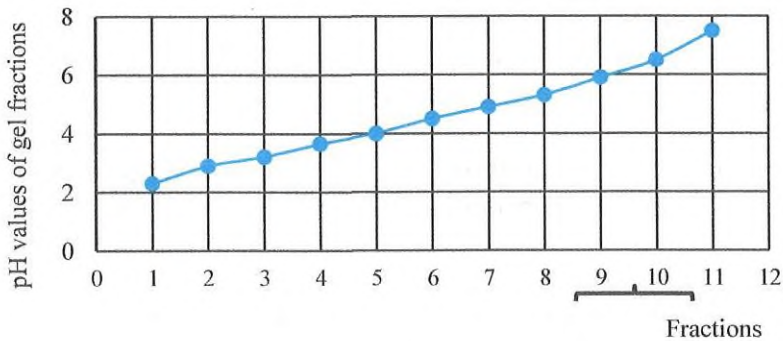

Figure 2.2. The pH gradient obtained from the pH values of the gel strips at the end of the isoelectric focusing run. The brackets enclose the fractions collected.

The fractionation process and the selection of immunopositive fractions were carried out under the control of solid-phase indirect immuno-enzyme analysis using polyclonal immunoglobulins against DBZ2. The origin of the protein was determined based on the mass spectroscopy method.

2.2. Immunochemical methods

The procedure for setting up the indirect immuno-enzyme analysis method. In the type of BDIEA, both antibodies can be identified and distinguished from a numerical point of view. In this method, the inner surfaces of the wells of the plate are coated with antigen. Serum or a sample containing antibodies is added to the well of the plate so that it binds to the antigens trapped on the surfaces of the well. The unbound primary antibodies are washed out of the well and the second antibody is added, which in turn binds to the first antibody. The unbound secondary antibodies are washed away and a specific substrate is added to the well. The enzyme substrate undergoes hydrolysis to form colored substances. The concentration of the colored substance in each well is determined by reading the 96-well plate in a spectrophotometer. Blood samples were taken from the veins of the patients on the day of surgery and before surgery, serum was separated, diluted 100 times with antibody buffer and used instead of primary antibodies in the BDIEA method placed on polystyrene plates. Dihydropyrimidinase-like protein 2, which is directly proportional to serotonin, was used instead of antigen at a concentration of 20 μg/ml in 0.1 M Tris buffer. Mouse monoclonal immunoglobulins against human immunoglobulins conjugated with horseradish peroxidase were used instead of secondary antibodies at a dilution of 1000 times with antibody buffer. 30 minutes after adding the substrate buffer, the reaction was stopped by adding 3 M NaOH and the reaction results were obtained digitally in a special photometer for performing IEA, "Molecular Devices Spectra Max 250", at a wavelength of 492 nm.

The Helena Bradford method was used to determine the amount of total protein. This method is based on measuring the intensity of color reactions formed during the interaction of aromatic amino acids contained in proteins and Coomassie brilliant blue G-250. The amounts of proteins were determined by the Bradford method at a wavelength of 595 nm on a SF-46 spectrophotometer (LOMO company, Russian Federation).

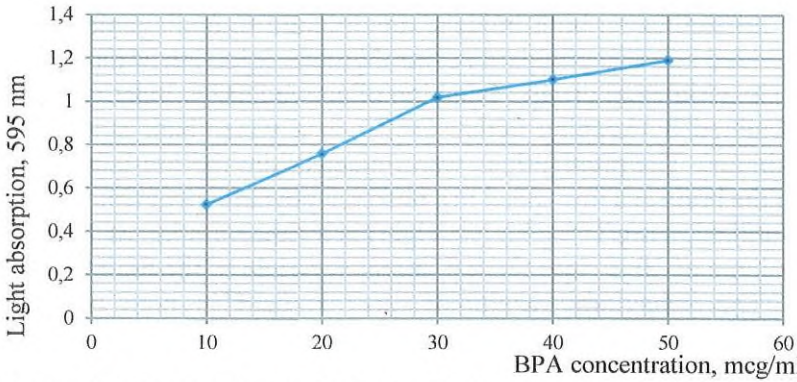

Fig. 3. Standard BPA level curve for the Bradford method.

2.3. Animal Behavior Models

The open field model and the elevated plus maze are most often used to determine the level of arousal in rats.

In the open field model, the rat is placed alone in the center of the device and the duration of various behavioral actions (acts) is recorded first for 1 minute in the dark and then for 3 minutes in light conditions (in the light of a 150-watt lamp hanging from a height of 1 meter). In particular, in this behavioral model, the total durations of vertical behavior, grooming behavior (self-washing), freezing behavior, the latency time of leaving the center and the number of squares crossed by the animal are recorded. At the end of the experiment, the durations of all behavioral types were summed up within each behavior and a statistical analysis was performed between groups. The listed behavioral types reflect the presence of a certain emotional state of the animals. In particular, staying in a vertical position reflects the search activity of the animals, grooming behavior indicates that the animal remains in a comfortable state, and the number of squares crossed indicates the locomotor activity of the animals. On the other hand, freezing behavior reflects the stress exposure of rats.

The elevated plus maze is widely used to assess the level of excitability of rats. In this model, the number of acts of vertical behavior, grooming behavior, freezing behavior, and rocking behavior is recorded within 5 minutes.

In studies on both models, rats tolerant to audiogenic stress were divided into two groups: 1) control group - injection of inactive DBZ2; 2) experimental group - injection of active DBZ2. Inactive DBZ2 was obtained by keeping active DBZ2 in a water bath at 60°C for 35 minutes. The preparations were injected into the left lateral ventricle of the brain at a low speed in a buffered physiological solution (sodium etaminal, 6 mg/100 g body weight) at a concentration of 1 mg/ml, in a volume of 10 μl, into the left lateral ventricle of the brain.

The behavioral results of rats obtained in both models were statistically analyzed using Student's t-test.

In order to determine how the excitation caused by the injection of DBZ2 in animals would affect the correct choice, we performed the next series in a T-shaped maze containing an obstacle. First, we trained rats that had been food deprived for 48 s to run to the left and right arms of the maze and find and eat barley grains there. Food reinforcement in the left arm consisted of 5 barley grains, and in the right arm only 2 barley grains. After the formation of conditioned reflex behavior, a 12 cm high plastic glass barrier was placed at the beginning of the left arm of the maze and the choice of the left and right arms was calculated as a percentage. After 24 s, the animals were divided into two groups:

1) control group – injection of inactivated DBZ2

2) experimental group – injection of active DBZ2.

The preparations were injected into the left lateral ventricle of the brain in a physiological solution, at a concentration of 1 mg/ml and a volume of 10 μl under etaminal sodium anesthesia. After 24 s, the choice of the left and right arms of the maze was determined. At the end of the experiment, based on the results of the choice of the left and right arms, their evidence was separately subjected to multifactorial statistical analysis using the Friedman $\chi 2$-criterion.

CHAPTER III. RESEARCH RESULTS

3.1. Changes in the level of dihydropyrimidinase-like protein 2 in the blood and saliva of anxious people

In most cases, instead of a marker, the level of various hormones accompanying the stress state in people's blood is used to assess the state of anxiety. For this, the level of hormones secreted by the adrenal

gland (cortisol, glucocorticoids, adrenaline) in the blood is determined. Numerous studies have been conducted by various researchers in this direction and it has been shown that the level of these hormones undergoes serious changes during stress. Changes in the levels of the aforementioned hormones in the blood can only indicate the degree of stress to the body.

To determine the adaptation or non-adaptation of the body to stress, the development of an adaptogen marker and measuring its level in body fluids is a more adequate experimental approach to achieving the goal set before us. In the first stage, blood and saliva samples were taken from patients scheduled for surgery on the day of surgery, and the levels of DBZ2 in their platelets and saliva, and the levels of natural autoantibodies against DBZ2 and cortisol in their serum were determined by solid-phase indirect immuno-enzyme analysis. The level of DBZ2 in human saliva was measured in order to obtain information about its level in the body fluids in a non-invasive and convenient way. According to the results, an increase in the level of DBZ2 was observed in the platelets of patients scheduled for surgery compared to healthy individuals. In particular, while the level of DBZ2 in the platelets of healthy individuals was 0.166±0.004 star optical units (UOV), the level of DBZ2 in the platelets of patients was 0.176±0.001 UOV ($p < 0.05$; Fig. 3.1.1).

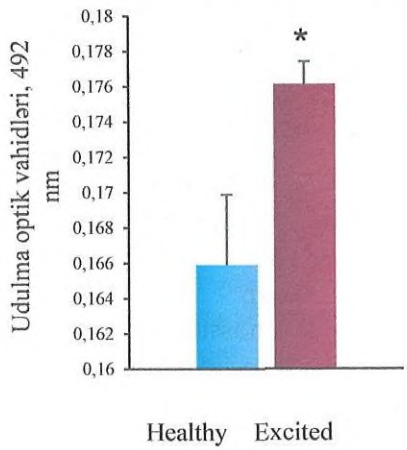

Figure 3.1.1. Changes in DBZ2 levels in human platelets in response to stress. * - $p < 0.05$.

While the level of natural autoantibodies against DBZ2 in the blood serum of healthy individuals was 0.0063±0.0005 UOV, the level of this indicator in the serum of patients increased sharply (2.5 times) and was equal to 0.0158±0.0013 UOV ($p < 0.001$; Fig. 3.1.2).

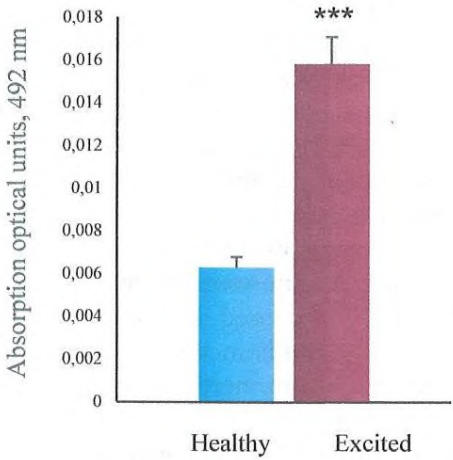

Figure 3.1.2. Changes in the level of natural autoantibodies against DBZ2 in human serum against the background of excitement. *** - $p < 0.001$.

To answer the question of how the changes in the amount of DBZ2 differ between the sexes under the influence of stress, a special series of studies was conducted on patients. Within the framework of this series, the amount of natural autoantibodies to DBZ2 in the blood serum of female and male patients was determined and it was found that this indicator did not differ sharply between men and women under the influence of excitement. In particular, the amount of natural autoantibodies to DBZ2 in the blood serum of women and men was equal to 0.017±0.002 and 0.014±0.002 ($p > 0.05$; Fig. 3.1.3) UOV, respectively. Such a result allows the possibility of analyzing the indicators of patients of both sexes within one group.

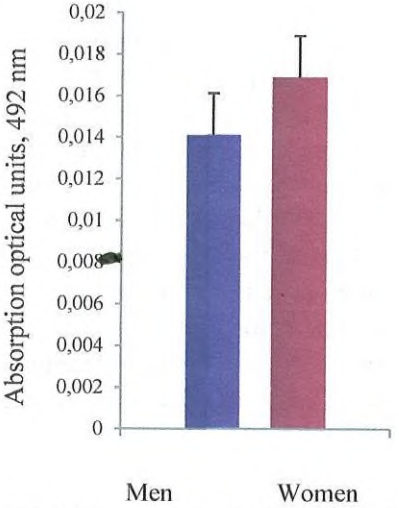

Figure 3.1.3. The amount of natural antibodies against DBZ2 in the serum of men and women against the background of excitement.

According to the literature, changes in the activity of the serotonergic system in platelets fully reflect the activity of the serotonergic system in the human cerebral cortex, including the levels of enzymes involved in serotonin synthesis and the levels of serotonin specific receptors. Taking into account such observations, the increase in the level of DBZ2 in the platelets of patients scheduled for surgical operations indicates an increase in the level of DBZ2 in their cerebral cortex against the background of an arousal state.

A sharp increase (2.5 times) in the serum of patients experiencing a state of excitement indicates an increase in the level of DBZ2 in other areas of their brain. In particular, the results of studies conducted in previous years in the laboratory "Molecular Basis of Integrative Activity" on depressed rats prove that the levels of natural autoantibodies to DBZ2 in the blood serum fully reflect the level of DBZ2 in the subcortical structures of the brain of these animals. Thus, a sharp increase in the levels of natural autoantibodies to DBZ2 detected in the serum of patients undergoing surgical operations reflects directly proportional changes in the level of this protein in the subcortical structures of their brain.

On the other hand, the level of DBZ2 in the saliva of healthy individuals was 0.199±0.005 UOV, while in surgical patients its level was significantly reduced and equal to 0.147±0.009 UOV ($p < 0.001$; Fig. 3.1.4). However, our attempt to identify natural autoantibodies to DBZ2 in the saliva was unsuccessful: no difference was detected in the analysis of the saliva samples of healthy individuals and patients from the buffer wells used to detect the non-specificity of IEA (instead of samples, the dilution buffer of the samples is added, i.e. "blank wells") using the IEA-i method with a special photometer. In our opinion, the absence of an immuno-enzyme reaction in the saliva samples of healthy individuals and patients indicates that not only immunoglobulins against DBZ2, but also immunoglobulins in general do not cross the hematosalivary barrier.

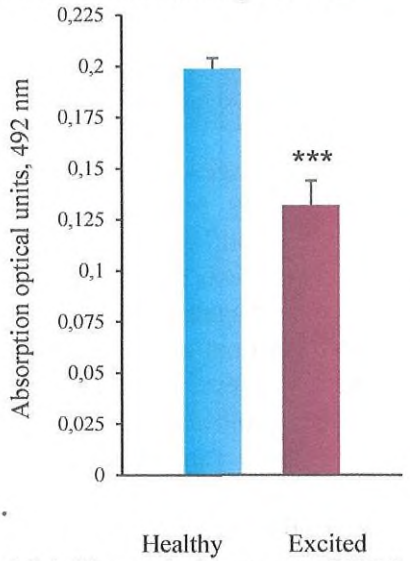

Figure 3.1.4. Changes in the amount of DBZ2 protein in human saliva against the background of excitement. *** - $p < 0.001$.

Since patients scheduled for surgery have much higher levels of natural autoantibodies to DBZ2 in their serum than in healthy individuals, and immunoglobulins from the blood do not cross the hematosalivary barrier, they recognize and bind to DBZ2 molecules in the blood, preventing DBZ2 molecules from entering the saliva, even

when DBZ2 levels are high in the blood. In addition, since immunoglobulins cannot cross the hematosalivary barrier, and since autoantibodies to DBZ2 in the blood serum combine with DBZ2 to form a high-molecular-weight antigen-antibody complex, it is clear that such a complex cannot cross the hematosalivary barrier. In our opinion, it is precisely due to this mechanism that the level of DBZ2 in the saliva of relatively anxious people, in contrast to platelets, shows a decreasing trend compared to healthy individuals.

In another series of studies, in order to more accurately assess the degree of stress in patients scheduled for surgery, serum samples were taken from them and the amount of the hormone cortisol, which reflects the level of stress, was determined.

First, the amount of cortisol in the serum of male and female patients was determined separately using the IEA method. The results obtained show that the amount of cortisol in the serum did not change significantly between men and women experiencing anxiety. In particular, the amount of cortisol in men was 511.8±21.6 nM, while in women it was 607.4±59.2 nM ($p > 0.05$; Fig. 3.1.5).

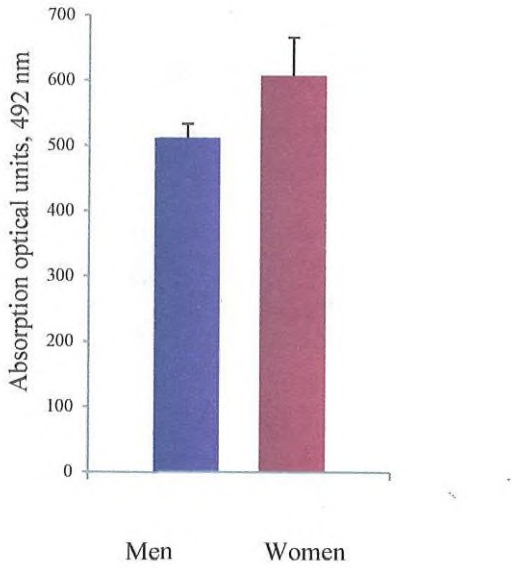

Figure 3.1.5. Serum cortisol levels in men and women under stress. $p > 0.05$.

When determining the amount of cortisol in the blood serum of anxious people, cortisol values obtained from men and women were combined into one group and analyzed based on statistical calculations. The results showed a significant increase in the amount of cortisol in the blood serum of these patients (by 31.5%) compared to healthy people. In particular, while the amount of cortisol in healthy people was 443.3±26.1 nM, in patients before surgery its amount was 582.9±43.5 nM ($p < 0.01$; Fig. 3.1.6). Thus, such a reliable change in the stress hormone cortisol in the blood serum of patients confirms that they are really exposed to a stressful situation. On the other hand, it should be noted that since there was no difference in the levels of both natural autoantibodies to DBZ2 and cortisol levels in the blood serum obtained from the two sexes of patients experiencing anxiety, it can be concluded that the biochemical processes reflecting the state of anxiety in the two sexes of people are similar in terms of the degree of manifestation.

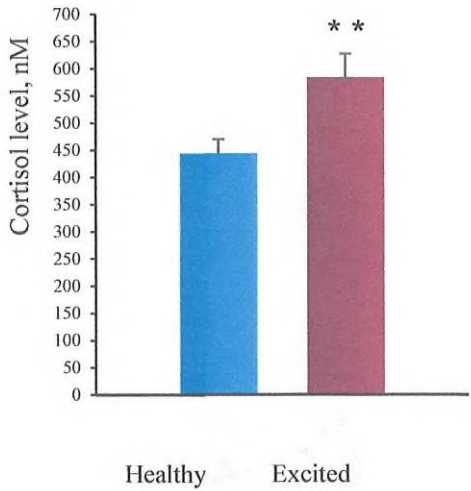

Figure 3.1.6. Serum cortisol levels in humans under stress. ** - $p < 0.01$.

3.2. Effects of intracerebral injection of dihydropyrimidinase-like protein 2 on animal behavior in models of excitability

Animal experiments were conducted on 3-4-month-old male Wistar rats weighing 160-220 g. At the initial stage, stress-resistant animals were selected from the rats in the audiogenic stress model. For this, the rats were exposed to audiogenic stress with a power of 90-120 decibels for 2 minutes. Stress-resistant rats began to have epileptic seizures or run away without stopping when exposed to strong sound, while resistant animals did not respond to strong sound. At this stage, we intended to isolate audiogenic stress-resistant rats based on the course of similar neurochemical processes in their brain regions and, on this basis, the demonstration of similar types of behavior of animals in different behavioral models and the low deviation of behavioral indicators. In total, 27 out of 35 rats were resistant to audiogenic stress. We took only stress-resistant animals for the subsequent experimental stages. We used the elevated plus maze and open field model to determine the excitability of rats. Both behavioral models belong to the second group according to the above classification. During the experiment, which lasted for 5 minutes after intracerebral injection of DBZ2 into rats, changes in behavioral indicators in the plus maze were noted. Compared to the indicators of the control group animals, a sharp decrease in the number of vertical positions reflecting exploratory behavior was observed in the experimental group animals in the closed arms of the maze ($p < 0.001$; Fig. 3.2.1). In particular, while the number of vertical positions in the control group was 13.7±0.6, this indicator was 1.3±0.5 in the experimental group. Studies show that models of arousal applied in behavioral experiments on animals are divided into two large groups: conditioned-reflex and spontaneous or natural responses of animals to

stress[6].

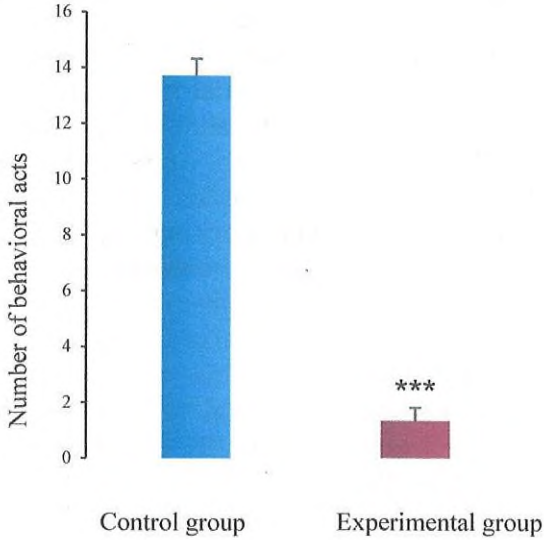

Figure 3.2.1. Effect of intracerebral injection of DBZ2 in rats on the number of vertical positions in the closed arms of the plus-maze. *** - $p < 0.001$.

Significant decreases in the experimental group's grooming behavior, which reflects the animal's comfort level, were also detected. In particular, the total time spent grooming in the closed arms of the maze in the control group was 37.3±3.5 seconds during the experiment, while after DBZ2 administration, the total time spent grooming in the experimental group was reduced by 2.5±1.7 seconds ($p < 0.001$; Fig. 3.2.2).

[6] Mekhtiev A.A., Allahverdiyeva T.N., Movsum-zadeh S.K. DNA integrity-protecting and survival-promoting activity of serotonergic system in sturgeon juveniles and sazans // Fish Physiology and Biochemistry. - 2017, V. 43, - № 4, - p. 1153-1160.

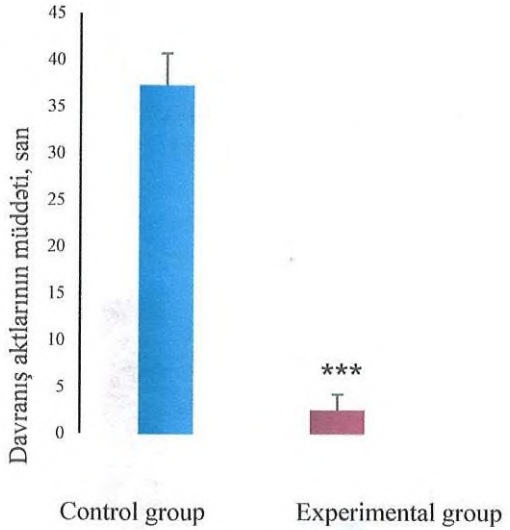

Figure 3.2.2. Effect of intracerebral DBZ2 injection in rats on the total duration of grooming behavior in the closed arms of the plus-maze. *** - $p < 0.001$.

The number of maze-dwelling acts also differed significantly in the experimental group compared to the control group. In particular, the number of maze-dwelling acts in the control group was 15.4±1.1, while the number of maze-dwelling acts in the experimental group was 3.7±1.2 ($p < 0.001$; Fig. 3.2.3).

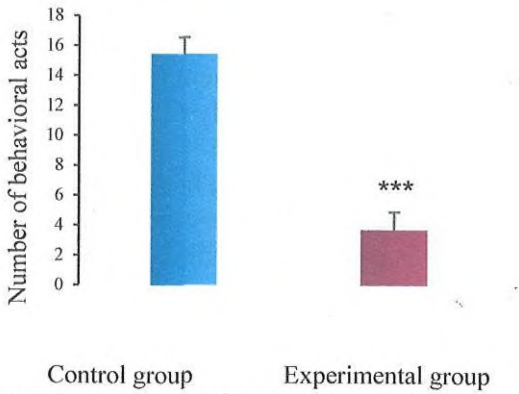

Figure 3.2.3 Effect of intracerebral injection of DBZ2 in rats on the number of rocking acts in the plus-maze test.***- $p < 0.001$.

The total time spent freezing in the closed arms of the elevated plus maze differed significantly from the above-mentioned behaviors in terms of the between-group difference in freezing behavior. In particular, while the freezing behavior in the control group was 66.3±2.8 seconds, this indicator was increased in the experimental group, and an increase of 226.7±14 seconds was recorded ($p < 0.001$; Fig. 3.2.4).

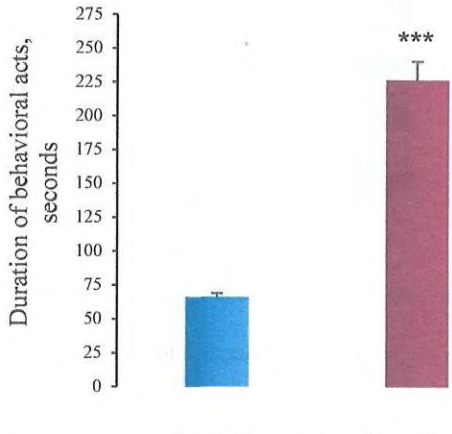

Figure 3.2.4. Effect of intracerebral DBZ2 injection in rats on the total duration of freezing behavior in the closed arms of the plus-maze. *** - $p < 0.001$.

The difference between the control and experimental groups in the number of squares passed by the animals in the closed arms of the maze was also striking. Thus, if the control group animals passed 28.7±1.0 squares during the experiment, it is noteworthy that the experimental group animals passed only 6.2±1.5 squares.

The results obtained in the open field test model show that intracerebral injection of DBZ2 in rats has an anxiogenic effect. The results obtained in this model fully coincide with the results obtained in the elevated plus maze model and allow us to say that DBZ2 has anxiogenic activity. In the open field test, rodents at the initial stage

demonstrate a deviation from the center of the device and a tendency to move towards the walls[7].

During stress, very pronounced biochemical changes are observed in the human body. Pronounced shifts in the levels of neurotransmitters in different areas of the brain lead to serious disruption of numerous types of higher nervous activity. Summarizing the above results, it can be concluded that stress on the body, through the release of catecholamines into the prefrontal cortex, leads to a disruption of working memory.

CHAPTER IV. DISCUSSION

The evidence presented in this chapter on the destructive effect of anxiety and stress on the realization of work and memory, convincing evidence, requires the emotional state of responsible and highly attentive professionals to be stable.

The main goal of our research is to determine to what extent the changes noted in the blood composition during an anxious state are accurately reflected in the composition of saliva. In addition, in anxious people, an increase in the level of DBZ2 and natural autoantibodies in platelets is observed. Increased levels of DBZ2 in platelets indicate increased levels of this protein in the cerebral cortex. The results of studies conducted on depressed rats in the "Molecular

Garayev Institute of Physiology confirm that a sharp increase in the level of natural autoantibodies against DBZ2 in the blood serum indicates significant changes in DBZ2 in the subcortical regions of the brain. Anxiety is a state of heightened alertness associated with

[7] Hart PC, Bergner CL, Smolinsky AN, Dufour BD, Egan RJ, LaPorte JL, et al. Experimental Models of Anxiety for Drug Discovery and Brain Research // In: Proetzel G, Wiles MV, eds. Mouse Models for Drug Discovery - New York, NY: Springer New York. - 2016, - p. 290.

general sensory sensitivity conditioned by uncertainty or conflict[8,9,10,11].

Our human studies show that patients with anxiety have significantly higher levels of DBZ2 in their platelets and saliva compared to those in a calm state, and that natural autoantibodies to DBZ2 are significantly higher in their serum. The increased levels of DBZ2 in the platelets of people with anxiety indicate an increase in the levels of this protein in their cerebral cortex. On the other hand, stress, which is accompanied by increased levels of cortisol in the serum of people with anxiety compared to those in a calm state, has been biochemically determined.

We studied the effect of intracerebral injection of DBZ2 on the level of excitability of animals in two behavioral models that determine the level of excitability (open field model and elevated plus maze). The results of the studies showed that in experiments conducted in the open field model, both in dark and light conditions, intracerebral injection of DBZ2 into rats significantly reduced the total duration of vertical, grooming behavior acts, and the number of squares crossed in the experimental group animals compared to both intact and control group animals, while the total duration of freezing behavior increased significantly in the experimental group animals. It can be concluded that, in general, artificially increasing the level of this protein in the brain of animals results in an increase in the level of arousal of the animals.

In general, the results obtained in both human blood and saliva samples and animal behavioral models provide a basis for reaching a

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[8] Gray, J. R. Emotional modulation of cognitive control: approach– withdrawal states double-dissociate spatial from verbal two-back task performance // *J. Exp. Psychol. Gen.*- 2001(130), - p. 437.

[9] Cornwell, B. R., Baas, J. M., Johnson, L., Holroyd, T., Carver, F. W., Lissek, S., et al. Neural responses to auditory stimulus deviance under threat of electric shock revealed by spatially-filtered magnetoencephalography // *Neuroimage* . - 2007 (37), - p. 282.

[10] Eysenck, M. W., Derakshan, N., Santos, R., and Calvo, M. G. Anxiety and cognitive performance: attentional control theory // *Emotion.* - 2007 (7), - p. 337.

[11] 11. Grupe, D. W., and Nitschke, J. B. Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective // *Nat. Rev. Neurosci.* - 2013 (14), - p. 490.

unanimous conclusion that the increase in the levels of DBZ2 in platelets and natural autoantibodies against DBZ2 in blood serum, and at the same time the decrease in its level in saliva, prove that a person lives under stressful conditions.

FINAL RESULTS

1. Indirect immuno-enzyme analysis showed an increase in dihydropyrimidinase-like protein 2 (DBZ2) in excited human platelets compared to healthy individuals, and an increase in the level of natural autoantibodies against DBZ2 and cortisol hormone in blood serum.
2. Indirect immuno-enzyme analysis showed a decrease in the level of dihydropyrimidinase-like protein 2 (DBZ2) in the saliva of excited individuals compared to healthy individuals.
3. In the closed arms of the elevated plus maze, the total duration of upright posture, grooming behavior, the number of squares and rocking acts passed by rats significantly decreased in the experimental group animals that received intracerebral injection of DBZ2 compared to the control group animals, and the total duration of freezing behavior significantly increased.
4. In the open field model, compared to the control group, the animals of the experimental group injected with intracerebral DBZ2 had a reduced duration of upright posture, total duration of grooming behavior, a decrease in the number of square and rocking acts passed by the rats, and an increase in the total duration of freezing behavior.
5. In the T-shaped maze containing an obstacle, the correct choice state was sharply impaired in the experimental group animals injected with intracerebral DBZ2 compared to the control group animals.
6. The results obtained based on the samples of excited people and animal models injected with intracerebral DBZ2 give reason to suggest that the increase in the level of DBZ2 in the brain causes excitement and, therefore, incorrect choices.

PRACTICAL RECOMMENDATIONS

1. People working in responsible, fast and correct decision-making positions may make wrong decisions when they are under high stress. Therefore, it is advisable to include regular determination of the level of dihydropyrimidanase-like protein2 in liquid environments in safety regulations.
2. It is recommended that people working in responsible, fast and correct decision-making positions temporarily be removed from their workplace if the level of dihydropyrimidanase-like protein2 in liquid environments exceeds physiological values.

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