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# MODERN DIAGNOSTIC CAPABILITIES OF ADRENERGIC SYSTEM DYSFUNCTION IN ISCHEMIC STROKE

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**Abstract**

*One of the main pathogenic mechanisms of ischemic stroke is the hyperreactivity of the sympathetic nervous system, which provokes the release of various biogenic monoamines. The degree of its expression depends on the functional state of the membrane-receptor cell complex.*

*The purpose of the study was to investigate the dielectric properties of peripheral blood erythrocytes in patients with ischemic stroke in vitro effect of adrenergic drugs in treatment dynamics depending on the severity of the disease.*

*To measure the complex dielectric constant of medium, the extremely high-frequency dielectrometry method, realized in the millimeter area of radio waves, was used. The criterion was the adrenergic reactivity of erythrocyte membranes in patients in the acute period of ischemic stroke. Adrenaline and propranolol were used as influence factors*

*During the experiment, in all cases there was a significant increase in the absolute values of complex dielectric constant after the treatment course, which indicates a change in the level of erythrocyte hydration. This significantly affects the rate of metabolic processes in the cell environment, changing the mode of cell functioning. Thus, taking into account free water fraction increase, we can conclude that positive changes in the metabolism of biological objects are confirmed by physical and biological methods.*

*The research has shown the possibility of using the waveguide extremely high-frequency dielectrometry in a medical-biological experiment. It was proved that the dynamics of the electrophysical characteristics of peripheral blood directly depends on the physiological state of a donor. Thus, in an experiment using clinical material, it was established that significant changes in complex dielectric constant of erythrocytes, when they were influenced by adrenergic agents, were observed mainly in the control group and were poorly expressed in the erythrocytes of patients in the acute period of ischemic stroke, indicating a significantly reduced β-adrenergic reactivity.*

*Obtained results of the dielectric study made it possible to differentiate the functional states of the adrenergic system under normal conditions and ischemic stroke at the cellular level.*

***Keywords:*** *adrenergic reactivity, ischemic stroke, adrenaline, receptor.*

## Introduction

Within the framework of the conceptual scheme of the pathogenesis of the ischemic stroke, the dysfunction of the membrane-receptor complex of the cell as a primary reacting system is given a special place. Changes in the functions of membrane-receptor complexes are considered as a central defect, which is the basis of the pathogenesis of most biological reactions in response to hypoxia. This is quite natural, because each cell is under constant influence of a stream of various information signals – biologically active substances, hormones, allergens, microorganisms, etc. And the earliest

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events unfold exactly at the level of cell membranes and their receptors [*Belous O et al., 2001*].

It's not by chance that membrane-receptor complex is an effector target for the therapeutic effects of most pharmacological drugs that already exist and are being developed. And, finally, the signal systems responsible for the recognition and carrying out of the information signal play a key role in coordinating the work of multicellular ensembles, including intercellular interactions that occur during hypoxia of the brain substance [*Zhadobov M et al., 2006; Beneduci A et al., 2014*].

A special place in the pathogenesis of ischemic stroke belongs to hyperreactivity of the centers of the sympathetic nervous system with subsequent stimulation of subordinate neurohumoral factors. Among them, the most important are biogenic monoamines, such as noradrenaline, adrenaline, dopamine, serotonin, etc. [*Mechetny Y, Dzyba A, 2002*].

The hypertonic effect of the above catecholamines is primarily associated with an increase in the intracellular concentration of electrolytes, which contribute to an increase in the sensitivity of the vascular wall to direct constrictor effects. As it is known, in the stabilization of the activity of the sympathoadrenal system, adrenergic receptors play a significant role. The number of adrenergic receptors always increase with stress, atherosclerosis, arterial hypertension, endothelial dysfunction and should decrease with adequate therapy [*Tylicki L et al., 2001; Stryk R, Dlusskaya I, 2003*].

Hypersympathicotonia is a generally accepted and recognized factor linking the physiological regulation of vascular tone, cardiac activity and sustained pressor response. It is generally known that there is a pronounced correlation between diurnal variability and the level of arterial pressure on one side and the plasma content of the catecholamines on the other side. In this case, the elevation of the catecholamines' pool in blood plasma is an independent marker of the high risk of acute cardio- and cerebrovascular events. At the same time, the importance of local (or tissue) sympathoadrenal system in the pathogenetic mechanisms of the ischemic stroke development has not been fully established [*Stryk R, Dlusskaya I, 2003*].

Moreover, the studies of the last decade found a stable relationship between the degree of stenosis of the cerebral arteries and the content of a number of biologically active substances in the blood flowing from the brain. At the same time, the highest elevation of the plasma pool of endogenous catecholamines was recorded [*Nosatov A, 2015*].

In patients with ischemic stroke, the relationship between concentrations of the catecholamines in the plasma, their affinity for adrenergic receptors, their expression on the erythrocyte surface, and the intracellular accumulation of biogenic amines are disrupted. It is likely that these stable relationships are determined by the relatively stable preservation of membrane-dependent transport mechanisms of biologically active substances. It is known that membrane disorders of erythrocytes, characteristic of acute cerebrovascular pathology, can manifest themselves both in changing the functional properties of membranes and in damaging their protein-lipid matrix [*Watterson J, 1987*].

With the progression of membrane-receptor complex dysfunction, there is a disruption, and then a complete disconnection of the mechanisms of production, transport and storage of hormones, which leads to the complete failure of the regulatory axis "hormone-receptor". This is quite likely, since the disruption of the relationship between adrenergic receptors expression, their affinity and catecholamines' concentration over the receptor field develops up- and down-regulation, and are typical pathological mechanisms in arterial hypertension and ischemic stroke [*Tylicki L et al., 2001; Beneduci A et al., 2014*].

Thus, presented data indicate a combination of excessive production of endogenous catecholamines and serious disorders of transmembrane transportation, and allow in a new aspect to assess the contribution of various components of sympathoadrenal system in the formation of structural remodeling of cerebral arteries and the progression of endothelial dysfunction in patients with ischemic stroke.

## MATERIAL AND METHODS

Currently, there is still no single approach to determining the activity of sympathoadrenal system, therefore, there is no unambiguous answer to the correct choice of the method, so researchers use both direct and indirect methods [*Artis F et al., 2015*].

The first ones include methods of quantitative determination of spacecraft, their precursors and metabolites (fluorometry, histochemistry, chromatography-mass spectrometry etc.). However, the determination of the catecholamine concentration in the blood plasma without taking into account the daily profile only relatively characterizes the activity of sympathoadrenal system, and besides it is an extremely laborious process. With indirect methods they investigate the activity of enzymes for the synthesis and deactivation of catecholamines, record the physiological indices of the body (arterial pressure, heart rate, electrocardiograms changes) under conditions of sympathoadrenal system stimulation (for example, bicycle ergometric test), and also assess the effect of catecholamines on membrane structures of cells [*Shandala M, 1979*].

A separate direction is the use of radioligand methods, which, using radioisotope labels, allow us to determine the number of receptors in a particular cellular structure. These methods are extremely powerful, but have two significant drawbacks – high research cost and invasiveness (modification of the object by adding radioligand or fluorescent probes that violate the native properties of the tested bioobjects).

As for the known possibilities for assessing the influence of catecholamines on the functions of the membrane-receptor complex, such several methods are known today. Among them, a special place is taken by the method of measuring the osmotic resistance of erythrocytesunder under the influence of biologically active substances, which allows obtaining valuable information about the β-adrenoreactivity state, but also completely does not solve the problem of cell modification [*Arkhypova K et al., 2015a; Nosatov A, 2015*]. Therefore, relying on the possibilities of other non-invasive research methods, which include extremely high-frequency dielectrometry, it is perspective to apply this technology.

In addition, modern concepts of signaling transmembrane and intracellular systems will allow developing new strategies for pharmacological correction of hypoxic conditions based on the revealed molecular disorders. In this sense, the concept of investigating cerebrovascular diseases as a membrane-receptor pathology can become a basis for future studies in vascular neurology.

Extremely high-frequency dielectrometry is one of the methods for measuring complex dielectric permeability (CDP) of various media, which uses a millimeter range of radio waves (frequency range from 30 to 300 *GHz*, which corresponds to a wavelength range of 10 to 1 *mm*). CDP is a complex value (denoted as ε*) and consists of two components: the real part ε' responsible for the degree of polarization of matter and the imaginary (ε'') responsible for heat losses. Both parts are inextricably linked [*Arkhypova K et al., 2015b*].

The informative nature of the method is determined by the specificity of the dispersion properties of water, the main element of bioobjects, which is the only participant in the "object-field" interaction in the extremely high-frequency range.

The measurements were carried out by the waveguide method at a fixed frequency of 39.5×109 *Hz*, which is in the region of the γ-dispersion. The technical feature of the waveguide method is that the complex reflection coefficient from the waveguide measuring structure (cuvette with a sample volume of 5-6 μl) is measured. Then the value of the reflection coefficient is recalculated in ε* – CDP using specially developed software of the measuring complex, which is its own development of the O. Ya. Usikov Institute for Radiophysics and Electronics of the National Academy of Sciences of Ukraine [*Krasov P et al., 2015*]. This method, as well as its advanced technical implementation, makes it possible to perform CDP measurements with high accuracy (ε±1%, ε±0.5%) in automated mode during the viability of blood samples (all blood manipulations were performed during 3 hours from the moment of fence).

The evaluation criterion was membranes AR of erythrocytes of patients in the acute period of ischemic stroke. As an influence factor, biologically active substances is adrenaline and its antagonist propranolol, which is a β-adrenoblocker, was used.

The measurements were carried out using erythrocytes of 30 people (17 men and 13 women, average age is 60.4±0.8 years) with orthopedic pathology without affecting the central nervous system, systemic diseases and thermoregulatory disorders, homogeneous by sex and age with a group of patients with ischemic stroke, which were assigned to the control group, as well as patients with ischemic stroke aged 46-79 years of both sexes (n=350) with an average (n=183) and severe (n=167) degree of stroke on the stroke scale of the National

Institutes of Health Stroke Scale of the USA. Blood sampling was carried out at hospitalization of patients in a hospital before the beginning of medicamentous treatment.

All studies that were conducted with the participation of people were carried out in accordance with the Helsinki Declaration (1964, the last view of the 64th WMA General Assembly, Brazil, 2013). The study protocol was approved by the Ethical Committee on Bioethics of the Medical Institute of the Sumy State University. Patients or their relatives gave information consent to participate in the study.

## Results

For the control group, the values of the average β-adrenoreactivity, which were expressed in conventional units (CU), were also obtained using the calorimetric method. This indicator was given for the validation of adrenergic state of membranes of donors' erythrocytes, and also for the possibility of assigning them to the control group.

Accoring to our results, the β-adrenoreactivity indices of the control group were within the normal range (the mean group score was 15.3±4.4 CU, n=30), which according to the procedure [*Stryk R, Dlusskaya I, 2003*] corresponds to normal β-adrenoreactivity (2-20 CU). For patients with an average severity of ischemic stroke (30.2±4.9 CU, n=183), this index exceeded the normal values in 1.97 times, which corresponds to an average degree of β-adrenoreactivity (21-40 CU), that is, indicates a decreased ability of APz to bind the blocker due to the effect of desensitization. A similar situation was observed in the case of erythrocytes in patients with severe ischemic stroke (42.4±5.6 CUn n=167), where β-adrenoreactivity exceeded normal values in 2.8 times and was characterized by a low degree of β-adrenoreactivity (> 41 CU).

Also, measurements of CDP under the adrenaline influence on erythrocytes of different groups of donors were carried out in order to reveal their reaction to the stress factor. The relative values of the real part of the permittivity (Δε'), which is the difference between the measured CDP before and after the impact of the above spacecraft.

The relative values of Δε' were clearly differentiated in groups. In the control group (-1.21±0.15), it was observed a significant decrease (in some cases the magnitude of the effect exceeded 10%) of the Δε' dielectric permittivity after adrenaline exposure. In the group of patients with ischemic stroke, the reaction to adrenaline was practically absent (within the margin of error), which can be explained by a decrease in the sensitivity of β-adrenergic receptors erythrocytes due to their desensitization. However, because of the revision in the Δε' index in the group of patients with moderate severity (0.2±0.08), it can be stated that their erythrocytes are more reactive than in the group of patients with a more severe variant of ischemic stroke (-0.48±0.18).

A similar effect was also observed when comparing erythrocyte reactions during exposure β-adrenoblocker with propranolol. Effects in the control group in 95% of cases had a negative sign, the magnitude of the effects in 44% of cases exceeded 10% regarding to samples with an equivalent addition of 0.9% NaCl solution. In the group of patients with severe course of ischemic stroke all the changes were within the margin of error, and in the group of patients with moderate disease severity a reaction to β-adrenoblocker was observed, however, the effect sign was opposite to the control value, as in the case with adrenaline.

As for the joint effect of adrenaline and propranolol (adrenaline was added after pre-incubation of cells with β-adrenoblocker, then reactions were observed only in 46.3% of cases in the group of patients with an average degree of ischemic stroke. The lack of response in the control group was expected in connection with the pre-blockade of β-adrenergic receptors.

Comparing the β-adrenoreactivity and the results obtained by the extremely high-frequency dielectrometry, it can be concluded that they have some relationship, since the expressed reactions were observed on the erythrocytes of the control group, for which the AR level was determined to be high. And on the contrary – the overwhelming absence of reactions to the action of adrenoreactive drugs was observed in groups of patients whose red blood cells had a low AR. The pronounced discrepancy of the obtained results indicates a connection between the electrophysical properties and the functional state of the erythrocyte membrane-receptor complexes under conditions of an acute cerebral catastrophe, and is of great interest for further study.

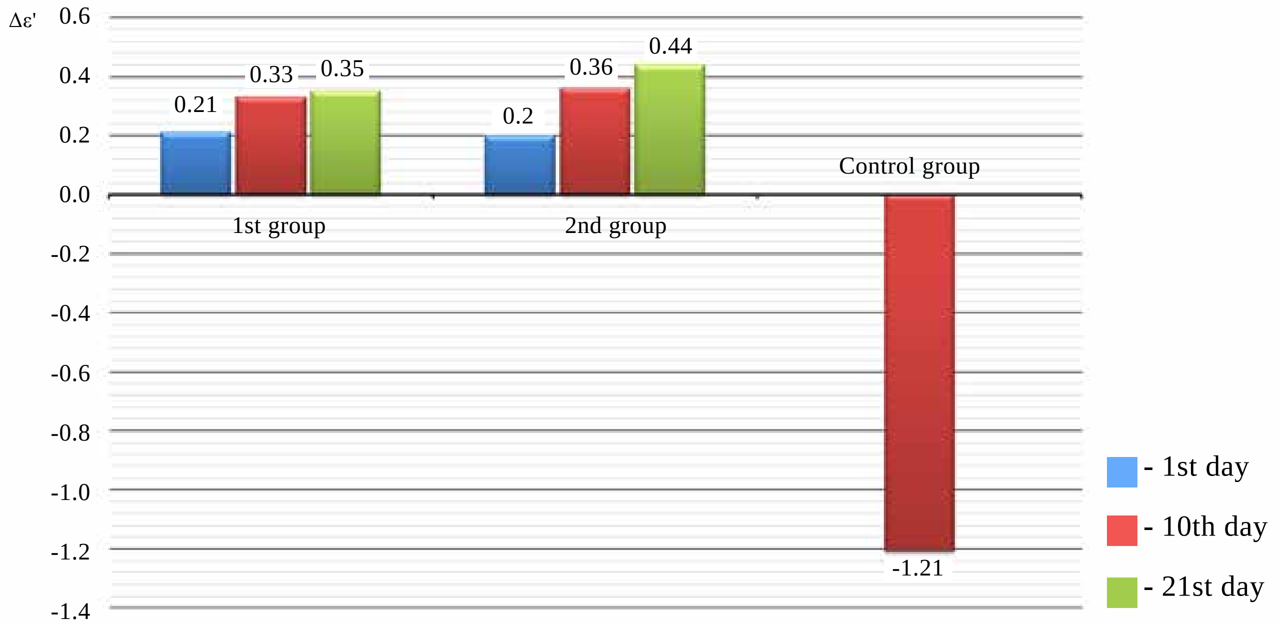


***FIGURE 1.** Reactions of erythrocytes to stimulation with adrenaline depending on the severity of ischemic stroke in dynamic of investigtion*

The next stage of the study was the analysis of repeated measurements of the samples in the dynamics of the disease (on the 21st day), that is after complex treatment in the hospital. The results are shown in figures 1.

## DISCUSSION

In the course of the experiment, an unambiguous trend in the change of β-adrenoreactivity erythrocytes was not detected, only in some cases there was a slight decrease in erythrocyte among patients with severe ischemic stroke. However, while analyzing the data, it was possible to find a rather interesting effect – an increase in the absolute values of ε' and ε'' of the erythrocytes of patients after treatment (pure blood samples without addition of biologically active substances).

In all cases, there was a significant increase in the absolute values of CDP after the course of treatment, which indicates a change in the level of erythrocyte hydration. All this indicates an increase in the amount of free water in the samples and a decrease in the amount of bound water, which is most likely caused by the stabilization of the patient's condition. This assumption can be made on the basis of the effective thickness of the membrane layer decreased to 20% in cells. This significantly affects the rate of metabolic processes in the "cell-intercellular environment" system, thereby changing the mode of cell functioning. In addition, various authors found a reduced water content in erythrocytes in comparison with the control for hypertension, which is an extremely important factor in the pathogenesis of ischemic stroke.

Thus, when observing an increase in the free water fraction, one can conclude that positive changes in the metabolism of bioobjects are confirmed by physico-biological methods at the level of membrane-receptor complex cells.

The conducted research has shown the possibility of using the waveguide extremely high-frequency dielectrometry method (39.5 *GHz*) in a biomedical experiment. It was shown that the dynamics of the electrophysical blood characteristics directly depends on the physiological state of the donor. Thus, in an experiment using clinical material, it was established that significant changes in CDP of erythrocytes when they were exposed to adrenergic agents were observed predominantly in the control group and were poorly expressed in red blood cells of patients in the acute period of ischemic stroke, which indicated a significantly reduced β-adrenoreactivity of their membrane-receptor complex.

Obtained results of the dielectric study made it possible to differentiate functional states under normal conditions and pathology at the cellular level, and are of interest for further development of diagnostic tests for evaluating the characteristics of the adrenergic system of patients with cerebrovascular pathology.

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