











# The Level of Individual Biochemical Constants of the Brain of in the Krushinsky-Molodkina Inbred Rat Strain against the Background of Radon Inhalation During Epilepsy

Marina Nikolaishvili<sup>1</sup> , Zakaria Nanobashvili<sup>2</sup> , Nodar Mitagvaria<sup>3</sup> , Gvantsa Chkadua<sup>4</sup> , Tea Museliani<sup>1,5</sup> , Gogi Jikia<sup>1</sup> , Irine Bilanishvili<sup>2</sup> , Khatuna Dondoladze<sup>1,5\*</sup> 

<sup>1</sup>Laboratory of Radiobiology, Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia; <sup>2</sup>Laboratory of Neuropysiology, Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia; <sup>3</sup>Laboratory of Cerebral Circulation and Metabolism, Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia; <sup>4</sup>Laboratory of Membranology, Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia; <sup>5</sup>European University, Tbilisi, Georgia

## Abstract

**BACKGROUND:** The elucidation of the mechanism of action of radon on antioxidant processes needs further research; however, based on the results of the experiment, it can be concluded that studies on experimental animals have shown that, while inhaling Tskhaltubo water, the phenomenon of hormesis develops.

**AIM:** We decided to study the effect of inhalation of radon on OS, namely, the changes in oxidative markers in both serum and in the brain of rats (SH group), the activity of Na/K ATPase, GABA, which performs the opposite role of an inhibitory neurotransmitter.

**METHODS:** We placed ten experimental animals (Krushinsky-Molodkina rats) in Radon contained mineral water spa's sauna. Inhalation of radon-water was administered through the nose, for 10 min, once a day, in conditions of high humidity (about 90%) for 10 days.

**RESULTS:** Hormesis regulates oxidative processes in the brain due to the activation of antioxidants expressed in a decrease in existing epileptic seizures and is expressed in the activation of Na/K-adenosine triphosphatase and specific glutaminergic neurons of the "attack center" of the hypothalamus but also with the activation of the entire adaptive-compensatory system.

**CONCLUSION:** Inhalation of radon contained water can be considered as a method of treatment with an anticonvulsant effect confirmed by experimental studies.

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\***Correspondence:** Khatuna Dondoladze, Beritashvili Center of Experimental Biomedicine, Laboratory of Radiobiology, Tbilisi, Georgia. E-mail: khatuna.dondoladze003@med.tsu.edu.ge

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## Introduction

Numerous experimental observations have revealed the pathogenetic role of oxidative stress (OS) in epilepsy. An epileptic seizure occurs against the background of OS and a hypermetabolic state, which is accompanied by a sharp change in energy metabolism, and intense generation of reactive oxygen species (ROS) [1], [2], [3], [4]. During convulsions, the rate of cerebral blood flow increases by 2–3 times, oxygen and glucose consumption increases, blood pressure rises, and vasodilation is noted, which is associated with the local formation of nitric oxide and adenosine. Despite a sharp increase in the influx of glucose and oxygen into the brain, energy consumption during a seizure is so high that the brain's own energy resources are depleted quite quickly. The hypermetabolic state during a seizure is replaced by a hypometabolic state between

seizures. Disorder of the functioning of mitochondria during epileptic seizures is inevitably accompanied by an increased generation of ROS [5], [6]. The OS markers in the brain after seizures were observed both in animal models of epilepsy and in patients with epilepsy [7]. The most important consequence of the accumulation of ROS in epilepsy is the excessive and uncontrolled activation of lipid peroxidation (LPO) processes under these conditions. Intensification of LPO processes leads to changes in the structural organization of membranes (phospholipid composition, microviscosity, and ion permeability), disruption of the functions of membrane-bound enzymes and receptors, damage to mitochondrial proteins, and, as a result, to cellular energy deficiency [8], [9], [10]. Disorder of the ionic permeability of the lipid layer, including for H<sup>+</sup> and Ca<sup>++</sup> ions, causes "electrical breakdowns" by its own membrane potential, or action potential [1], [3]. "Electrical breakdown" in the form of an epileptic seizure

leads to a complete loss of the membrane of its barrier functions. Consequently, as a result of excessive and prolonged generation of LPO in epilepsy, pathological processes leading to the death of neurons are aggravated, the functioning of membrane receptors for neurotransmitters is disrupted, and membrane and ion channels are destroyed.

An important role in the theory of epileptogenesis is also assigned to the membrane theory: The trigger factor [11]. Neuronal epilepsy is caused by structural changes in the neuronal membrane, including synapses, leading to the inactivation of ion pumps and abnormal over-activity of ion channels. Changes in glucose metabolism with a deficiency in the action of Na<sup>+</sup>/K<sup>+</sup> - adenosine triphosphate (ATP)ase, which, as mentioned above, are associated with neuronal hyperactivity. This is one of the leading mechanisms for reducing the concentration of extracellular K<sup>+</sup> accumulated after seizure activity. Low activity of Na<sup>+</sup>/K<sup>+</sup> ± ATPase is associated with the development of epileptic seizures [3], [5], [12]. In addition, the activity of Na<sup>+</sup>/K<sup>+</sup> ± ATPase decreases within a few minutes after transient focal ischemia in the rat cortex and hippocampus, as well as in an experimental model of brain injury. Altered ion homeostasis may also partly explain the interaction between seizure activity and hypoglycemia. In this regard, one of the reasons for the development of epilepsy can be considered a change in the deficiency of glucose metabolism during OS and ongoing processes involving Na<sup>+</sup> ± K<sup>+</sup> ATPase [1], [5], [12], [13].

A critical feature of all audiogenic seizure (AGS) models is the reduction in neural activity in the auditory pathways due to deafness during development. The initiation and intensification of AGS activity depend on increased excitability of the auditory system, especially the inferior colliculi, where bilateral lesions eliminate AGS. Glutamate-gamma-aminobutyric acid (GABAergic) and glutaminergic mechanisms play a critical role in AGS, as in temporal models of epilepsy, and are involved in the modulatory and efferent systems, including the superior colliculus, substantia nigra, basal ganglia, and reticular formation structures. The catecholamine and indolamine systems also affect the severity of AGS. AGS models are useful for elucidating the underlying mechanisms of formation and manifestation of generalized epileptic behavior and evaluating the effectiveness of modern treatment strategies such as anticonvulsants [3], [14], [15], [16].

Our modern strategy, which is shown in the study, is applying the radon-containing waters of Tskhaltubo against epileptic seizures. We believe that the action of low doses of radon can have a significant and positive effect on patients with epilepsy (hormesis). As is known [17], glutamate and GABA are key neurotransmitters in the pathophysiology of epileptic seizures, which are potent modulators of these classical neurotransmitters, or by changing their release, or regulating their effects at the receptor

level, and, therefore, can affect the balance between inhibition and excitation. Neuropeptides also modulate monoaminergic transmissions, such as dopamine and serotonin, and thus, can alter excitability as well [4], [8], [15].

Regarding biogenic amines, dopamine is an amine formed by the precursor chemical L-DOPA, which is synthesized in the brain and kidneys [15], [18], [19]. Although the dopamine receptor is a type of G-protein coupled receptor, it can also act through mechanisms such as ion channel interactions. The released NE then binds to the appropriate receptor, enabling synaptic transmission. These receptors are classified as G protein-coupled receptors with inhibitory or stimulatory effects. NE plays an anticonvulsant role in epilepsy. In addition, a decrease in NE has been associated with increased susceptibility to the absence of epileptic seizures and/or AGSs and neuronal damage to limbic regions [6], [19], [20], [21]. The fact that NE mediates synaptically mediated excitability through modulation of ion channel conductance or mediated GABAergic and glutamatergic transmission may help explain its role in epilepsy.

5-HT<sub>2A/2C</sub> activation. It is believed that the 5-HT receptor improves memory functions by increasing the release of glutamate and acetylcholine in the prefrontal cortex and hippocampus [16], [21]. The ability of 5-HT receptors to modulate these neurotransmitters and related ion channels that 5-HT can activate various pathways in the underlying mechanism of epilepsy. As is known, β-Endorphin is a neuropeptide derived from the same precursor as ACTH. β-Endorphins are abundant in the hypothalamus and pituitary gland and are released when the body is faced with stress or pain. During pain, they have an analgesic effect. During times of stress, they are released into the limbic system and reduce anxiety, and β-Endorphin also induces non-convulsive limbic epileptiform activity when intraventricularly administered to rats. Repeated microinjections of β-endorphin into the amygdala or hippocampus lead to the development of generalized seizures, an effect that can be neutralized by a specific μ-receptor antagonist [4], [16], [22], [23].

To assess the antioxidant status in epilepsy, measurement (of the sulfhydryl group – SH in the brain tissue) and markers of OS, which prevent the development of brain disorders associated with peroxidation reactions, are used [21]. As part of a translational pilot clinical study, the aim of this study is to study the positive effects of radon therapy on epileptic seizures in the Krushinsky-Molodkina (KM) rat strain, namely, the use of low doses of radon (inhalation technique) to obtain an anticonvulsant effect in experimental animals.

The uniqueness and novelty of the study lie in the study of the effect of radon inhalation on experimental models of epilepsy in the localization of epileptic foci in the hippocampus (audiogenic staining of rats according

to the KM. We chose inhalation because it is a more direct method of using radon. However, this is rather an advantage, because we were able to measure a dose of radon that was within the therapeutic range of 1 NC, 37 bq [1], [2], [3], [18]. We decided to study the effect of inhalation of radon on OS, namely, the changes in oxidative markers in both serum and in the brain of rats (SH group), the activity of NA/K ATPase, GABA, which performs the opposite role of an inhibitory neurotransmitter. Moreover, quantitative changes were found in biogenic amines and beta endorphins, before the radon therapy during epilepsy and after 3 months with the radon inhalations.

## Materials and Methods

Rats with body mass of 200–250 g were housed under the standard laboratory conditions with a “12 h light–12 h dark” cycle, constant temperature of  $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , and water and food ad libitum. Animal care and handling throughout the experimental procedures were in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). Use of the animals in the experiments, animal care, and post-experimental euthanasia was performed in a strict adherence to the officially adopted rules for animal use and care in biological laboratories [24].

Animals: for our experiment, we used 24-month KM male rats. They are predisposed to audiogenic epilepsy (seizures in response to a strong sound). Rapid (5–7 s) development of clonic-tonic seizures and the development of postictal catalepsy are characteristic of KM rats [7], [12], [16], [25].

### ***Epileptic seizures***

Genetically seizure-determined KM rats were placed in an audiogenic stimulation chamber. The chamber represented  $60 \times 60 \times 60$  cm plexiglass box, in the upper part of which a standard wall bell was attached. The animal was in the chamber and an audiogenic stimulus was delivered to it. A high pitch sound stimulus was presented to rats (bell intensity 110 dB, time 60 s), in response to which they developed seizure reactions. Motor components of seizure activity were estimated by a slightly modified Jobe [4] scale: 0 — fear reaction; 1 — facial muscle clonus; 2 — head tremble, jaw myoclonus; 3 — wild run, forepaw myoclonus; 4 — myoclonus of fore and hindpaws and fall on a side; and 5 — clonus of the fourpaws, skeletal muscle rigidity, ataxia, and asphyxia. The mentioned KM line rats fall into two sublines: (a) Animals, which, in response to a high pitch sound stimulus, develop fear reaction and facial muscle clonus — conventionally referred to as the first subline and (b) animals developing fear and wild run to sound

stimulus followed by clonic-tonic behavioral seizures — conventionally referred to as the second subline. For induction of epileptic seizures, we used an audiogenic signal before the study to which the experimental animal responded with cramps. In particular, the trigger caused the development of myoclonic seizures with “limbic” localization. Long-term (15 min) exposure of KM rats to the action of sound according to a special scheme with alternating 10 s periods of strong and weak sound causes cerebral circulation disorders in them, externally manifested in the form of paresis and paralysis of the limbs. On the 10<sup>th</sup> days and 3 months assessment of epileptic seizure with trigger – sound in BK rats was performed [7], [12], [16], [18], [20], [21], [22], [23], [25].

### ***Amino acids and biogenic amines were determined using ELISA kits***

Brain synaptic membrane fraction obtained from the adult albino rats of both sex is served as an investigation material. The synaptic membrane fraction is obtained by means of differential centrifugation, at 0.9–1.2 M concentration gradients of sucrose, according to De Robertis and Wittaker’s recommendations. Na, K-ATPase activity is measured as a sensitive part of a total ATPase activity. The total ATPase incubation medium contained 140 mM NaCl, 5 mM KCl, and 50 mM Tris-HCl buffer at pH 7.7. Control was carried out under the following conditions: 1 mM ouabain, 145 mM KCl, and 50 mM Tris-HCl buffer at pH 7.7. Na, K-ATPase activity is calculated by the difference between these two assays. Samples are incubated at  $37^{\circ}\text{C}$  for 15 min. The ATPase activity is calculated according to the inorganic phosphorus (Pi) amount (per mg protein and per hour) resulting from the enzyme-induced ATP hydrolysis. Inorganic phosphorus is evaluated calorimetrically using the modified Fiske-Subbarow and Kazanov-Maslova method. Protein concentration is assessed by the Lowry method [3], [9], [12].

### ***Sulf-groups [10], [25]***

Cysteine thiols and their oxidized disulfide analogs are carefully balanced to maintain redox homeostasis in various cellular compartments. In this review, we discuss the role of protein thiols as scavengers of hydrogen peroxide in antioxidant enzymes, using thiol peroxidases to illustrate how thiols of the protein non-protein thiol group contribute to the transmission of redox signals, we will provide an overview of a diverse set of small molecular weight thiols [10], [25]. Determination using the ELISA kit [10], [13], [19], [20].

### ***Radon measurement***

In the Tskhaltubo spa center, where natural mineral water is used, we measured Radon radioactivity

in water. The radioactivity of Radon was 37 Becquerel (bk) in 1 m<sup>3</sup> (37 bk/m<sup>3</sup>) [5], [6], [10], [13], [23].

### Radon inhalations procedure

We placed ten experimental animals (KM rats) in Tskaltubo mineral water spa's sauna (experimental group). Mineral water temperature was 36°C, and humidity 90%. A control group of 10 KM rats was placed in another spa center's sauna, where 36°C mineral water (without radon) was delivered through inhalation. Humidity in this spa center's experimental room was 90%. None (experimental and control group of rats) of the animals took a bath, they were just in two different saunas and living in the same conditions. Inhalation was administered through the nose, for 10 min, once a day, in conditions of high humidity (about 90%) for 10 days. After each procedure of inhalation, the rats were placed in a vivarium and given food and water [2], [16], [21].

### Laboratory examination

For the study of the physiological changes, caused by inhalation of Tskhaltubo water on an oxidative level, which prevents the development of brain disorders associated with peroxidation reactions, we measured the concentrations of free radicals reactive oxygen metabolites (d-ROMs) - in the blood plasma of rats, using a photometric test and measured the concentration of hydroperoxides (ROOH) in the brain tissue, which gives us a pro-oxidant status of the tissue. Hydroperoxides, also called ROM, are formed during an oxidative attack when ROS react with various organic substrates (e.g., carbohydrates, lipids, amino acids, proteins, nucleotides, etc.).

To assess the antioxidant capacity of plasma, we used the PAT (Antioxidant Concentration Test) by measuring ferric reduction ability, and to evaluate the effectiveness of antioxidants, we determined the OS Index (OSI) and the oxidation balance status (OBRI).

All named measurements were provided by means of Photometric Analytical System FRAS 5 (H&D, Parma, Italy) [2], [7], [16], [21], [23].

## Results

On the 10<sup>th</sup> day after inhalation of low doses (37 Bq/m<sup>3</sup>) (Table 1 and Figure 1), the latent period before attacks and the pauses between attacks

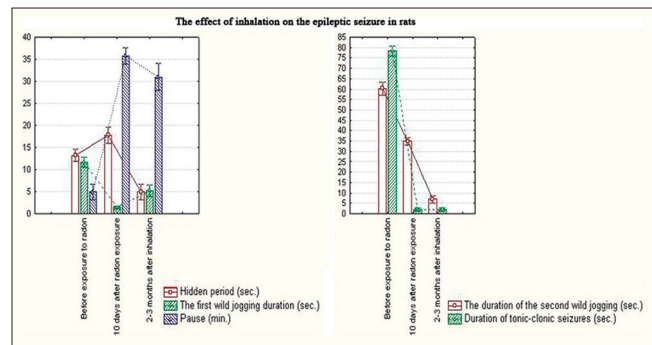


Figure 1: The effect of radon inhalation on the epileptic seizure in rats on the 10<sup>th</sup> day and 3 months after inhalation

significantly increased ( $p < 0.05$ ) in the group of radon irradiation, compared to control. The latency period before inhalation of radon in rats with epilepsy was ( $13 \pm 1.1$ ), and on the 10<sup>th</sup> day after inhalation of Tskhaltubo randomized water, the latency period increased up to ( $18 \pm 1.3$ ) s, and 3 months after inhalations, it increased up to ( $10 \pm 1.4$ ), respectively.

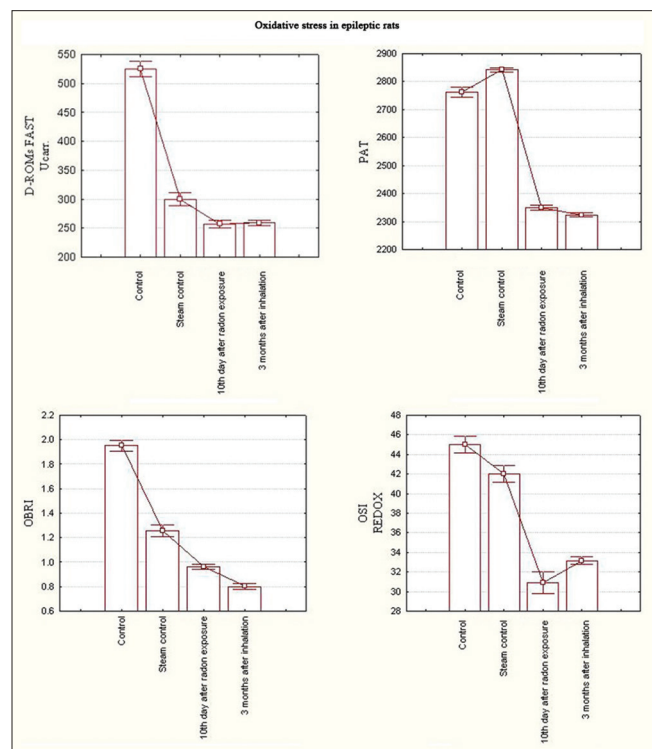


Figure 2: Oxidative stress in epileptic rats, on the 10<sup>th</sup> day and 2-3 months after inhalation

The duration of the first and second jumps after the trigger reduced in the group of rats receiving radon inhalation on the 10<sup>th</sup> day and was ( $1.5 \pm 0.1$ ), and 3 months after inhalations - ( $1.1 \pm 0.1$ ) ( $p < 0.05$ ). In all groups, the second wild jogging started later and continued in the control group ( $60 \pm 1.8$ ), on the 10<sup>th</sup> day after radon exposure - ( $35 \pm 1.1$ ), and 3 months after

Table 1: The effect of Radon inhalation on the epileptic seizure in rats on the 10<sup>th</sup> day and 3 months after inhalation

Period	Hidden period (sec.)	The first wild jogging duration (sec.)	Pause (min)	The duration of the second wild jogging (sec)	Duration of tonic-clonic seizures (sec)
Before exposure to radon ( $p < 0.05$ )	$13 \pm 1.1$	$11 \pm 1.1$	$5 \pm 0.2$	$60 \pm 1.8$	$79 \pm 1.9$
10 days after radon exposure ( $p < 0.05$ )	$18 \pm 1.3$	$1.5 \pm 0.1$	$36 \pm 2.2$	$35 \pm 1.1$	$2 \pm 0.1$
2-3 months after inhalation ( $p < 0.05$ )	$5 \pm 1.4$	$5 \pm 0.1$	$30 \pm 1.1$	$7 \pm 1.1$	$1 \pm 0.1$

radon inhalation, it was only ( $20 \pm 1.1$ ). As for tonic-clonic seizures in control, they lasted - ( $79 \pm 1.9$ ), on the 10<sup>th</sup> day after radon inhalation - ( $2 \pm 0.1$ ), and on the 3<sup>rd</sup> month after radon inhalation, 90% of seizures disappeared [9], [14], [16], [25].

From the data presented in Table 2, we can see the following. The study of dROM in genetically epileptic Molotkin's Krushinsky rats before exposure to radon showed that 10 days and 3 months after inhalation, dROM, PAT, OBRI, and OSI were within the normal range, but it should be noted that after 3 months the above data decreased even more and turned out to be exactly within the normal range, which was reflected in the behavior rats, and 80% of the rats did not have tonic-clonic seizures (Figure 2).

### Definition of sulfhydryl groups

It is known from the literature that protein cysteine thiols respond to the cellular redox state. They can oxidize and inhibit thiol-proteins and enzymes and therefore have antioxidant action. In particular, when oxidants increase in the cell, thiol-disulfide is involved in redox regulation. These redox-sensitive mechanisms are involved in redox various changes including cell hypoxia. Under hypoxic conditions, the concentration of thiols decreases. This is due to the association of metabolites produced during the recovery of hypoxia with glutathione (GSH), a cellular non-protein thiol (NPSH). That is, the metabolites react with GSH instead of oxygen. When cellular thiols are depleted, peroxide is produced [8], [17], [22] and excessive OS leads to cell death. Within the frames of our study, we examined the quantitative variation of non-protein and total sulfhydryl groups [5], [13], [19]. On the 10<sup>th</sup> day and 3 months after radon inhalations, we determined the concentration of non-protein and total SH groups in the rat brain.

In a study of non-protein SH-groups, we found that on the 10<sup>th</sup> day, it became  $1.72 \pm 0.107^{**}$ . As for the total sulfhydryl groups, on the 10<sup>th</sup> day, compared with the control, on the 3<sup>rd</sup> month after radon inhalation, it increased statistically and became ( $40.16 \pm 1.44^{**}$ ) and (control  $35.01 \pm 1.23$ ), respectively, also had higher concentrations compared to the control group ( $39.46 \pm 1.43$ ) and ( $35.01 \pm 1.23$ ), respectively), which indicates an increase in protein concentration and the number of sulfhydryl groups, on the 3<sup>rd</sup> month after radon inhalation, compared to the control group performing inhalations with ordinary mineral water

(Figure 3). We see the effect of radon inhalation on physiological processes, which acts as an activator or inhibitor of certain neurotransmitters [8], [11], [12]. In view of the foregoing, it can be said that exposure to radon regulates OS, the clinical manifestation of which may be a decrease in epileptic seizures, which is confirmed by studies (Tables 3). Considering that audiogenic epileptic seizures begin immediately after the bell and last several minutes before the Tskhaltubo water inhalation in experimental rats, as shown in Figure 3, the duration of epileptic seizures does not exceed 2 s after inhalation with Tskhaltubo water on the 10<sup>th</sup> day, and 3 months after inhalation, no audiogenic convulsions were also manifested. Na-K ATPase, which is active in animals, is known to consume large amounts of ATP. At present, there is no doubt that the energy and transmitter processes in brain tissues are interconnected. Therefore, ATP is a powerful source of energy, along with the fact that it interacts with the glutamine system, the links of which, in turn, are glutamate (excitatory transmitter) and GABA (inhibitory). Naturally, the recovery processes are disrupted in terms of consumption. In accordance with the previously discovered concepts of the occurrence of paroxysmal shift depolarization, disturbances in neurophysiological events are associated with disturbances in ionic and transient energy processes. Thus, the concept of the emergence of a neuron in the membrane allows for a primary violation in the membrane or the possibility that, as a result, is associated with insufficiency of the potassium-sodium pump, increased membrane permeability and increased expansion to depolarization, and, consequently, excessive excitability of the neuron. Development of changes in the environment, neurons, increase in MPD, dysregulation of the concentration of electrolytes or transmitters, or both. Therefore, we decided to study the activity of Na/K-ATP 3 months after radon inhalation. Changes in glucose metabolism deficiency under the action of Na<sup>+</sup>/K<sup>+</sup> ± ATPase are associated with neuronal hyperactivity. This is one of the leading mechanisms for reducing the concentration of extracellular K<sup>+</sup> accumulated after seizure activity. Low activity of Na<sup>+</sup>/K<sup>+</sup> ± ATPase is associated with the development of epileptic seizures. In addition, the activity of Na<sup>+</sup>/K<sup>+</sup> ± ATPase decreased within a few minutes after transient focal ischemia in the cerebral cortex and hippocampus of rats, as well as in an experimental model of brain injury. Altered ion homeostasis may also partly explain the interaction between seizure activity and hypoglycemia [17], [24]. As can be seen from

**Table 2: Oxidative stress in epileptic rats**

Epileptic rats	D-ROMs fast Ucorr.	PAT	OBRI	OSI Redox
Control	525 ± 3.67 Free radicals, very high	2765 ± 5.85 Antioxidants there is a deficit	1.953 ± 0.3 Oxidative status is at a dangerous level in relation to cholesterol	45 ± 2.3 Oxidative status index is on the critical edge
Steam control	301 ± 2.27 Normal range	2844 ± 5.75 Slight deficiency	1.25 ± 0.2 Normal	42 ± 2.2 Normal
10 <sup>th</sup> day after radon exposure	257 ± 1.12 Normal range	2350 ± 2.92 Normal value	0.96 ± 0.001 Normal	31 ± 2.2 Normal
3 months after inhalation	259 ± 1.13 Normal range	2324 ± 2.82 Normal value	0.8 ± 0.001 Normal	33.2 ± 2.1 Normal

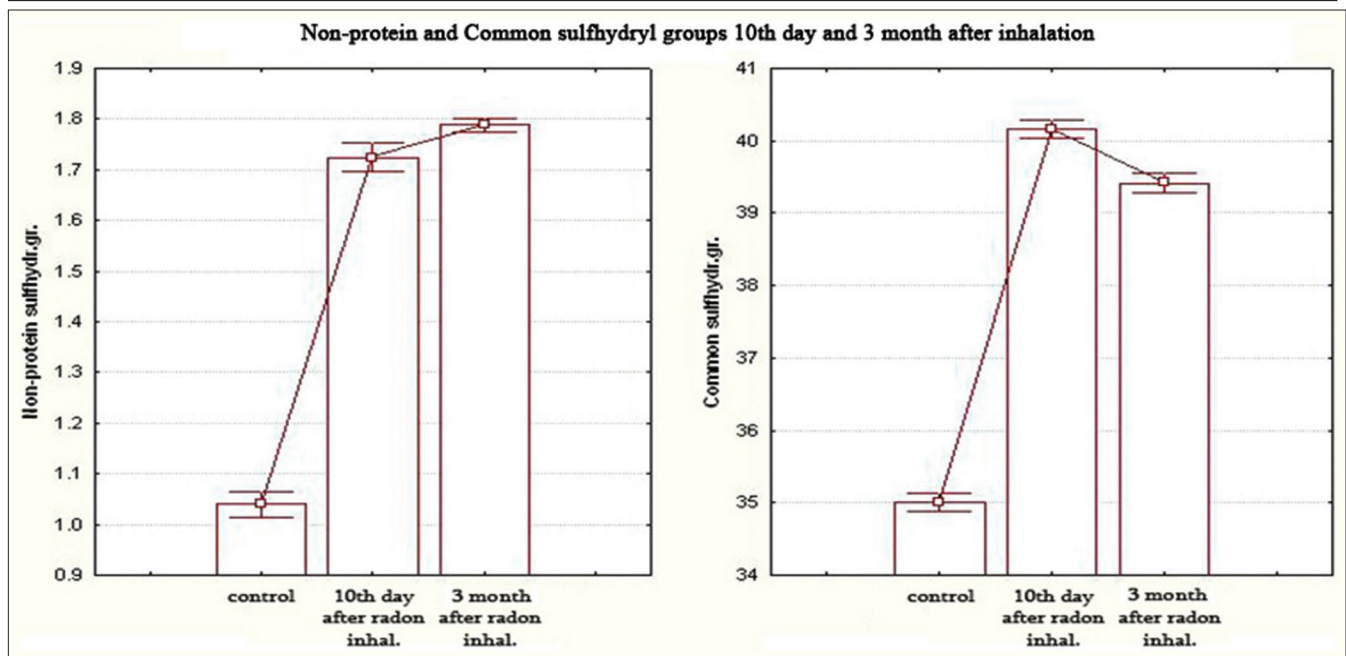


Figure 3: Sulfhydryl amount (mcg/g in the brain), ( $p < 0.05$ ) in the control and experimental rat group on the 10<sup>th</sup> day and 2–3 months after inhalation

Table 3: Non-protein and common sulfhydryl groups 10<sup>th</sup> day and 2–3 months after inhalation

Group	Control	Study group on the 10 <sup>th</sup> day after radon inhalation	3 months after radon inhalation
Non-protein sulfhydryl groups	1.04 ± 0.121	1.72 ± 0.107**	1.79 ± 0.109**
Common sulfhydryl groups	35.01 ± 1.23	40.16 ± 1.44**	39.46 ± 1.43**

\*\*p < 0.05. n=28 (14 in each group)

Table 4, on the 10<sup>th</sup> day after inhalation, an increase in Na<sup>+</sup>/K<sup>+</sup> + ATPase is observed [3], [20]. In the following experiments, Na<sup>+</sup>/K<sup>+</sup> ± ATPase activity was studied on the 10<sup>th</sup> day after radon inhalation and 2–3 months after radon inhalation.

Table 4: Na, K-ATPase activity, Na, K-ATP [Mg2<sup>+</sup>] = [ATP] = 0.31 mM, [MgATP] = 1.69 mM

Examination rat	Na, K-ATPase activity	%
Control	11.68 ± 0.34	100
After radon inhalation	14.67 ± 0.68	125.6 (increased by 25.6% compared to control)
After radon inhalation (Who had a seizure)	8.99 ± 0.92	77 (decreased by 23% compared to control)

As shown from Tables 4 and 5, Na<sup>+</sup>/K<sup>+</sup> + -ATPase activity on the 10<sup>th</sup> day after radon inhalation increased by 72%, and after 3 months – by 125.6% (increased by 25.6% compared to control), what else times shows that 2–3 after inhalation with radon – by 77% (decreased by 23% compared to control) (Figure 4).

Table 5: Months after inhalation of radon, the level of amino acids in the hippocampus (mikg/ml)

Hippocampus	Control A	Epilepsy B (A-B)	Epilepsy+radon C (B-C)
Amino acids in plasma			
Glutamic acid	0.085 ± 0.002	0.098 ± 0.002**	0.055 ± 0.001**
Glycine	0.098 ± 0.001	0.048 ± 0.003***	0.089 ± 0.001**
GABA	0.122 ± 0.02	0.128 ± 0.020	136 ± 0.01*
Tryptophan	0.084 ± 0.001	0.064 ± 0.001**	0.083 ± 0.004***

In Tskaltubo, OS decreases and remains within the normal range.

The activity of Na<sup>+</sup>/K<sup>+</sup> ± ATPase is manifested in a decrease in the first and second wild runs in animals

(Figure 5), including an increase in the pause and a decrease in clonic-tonic convulsions after 3 months, and on the 10<sup>th</sup> day, it sharply decreases and remains within (2 ± 1.1) s, and after 3 months - (1 ± 1.1).

Table 4 shows that there is a clear change in the level of amino acids – namely, compared with the control, there was a decrease in the blood plasma of proline, methionine, tyrosine, glutamic acid, aspartic acid, and glycine causing an “inhibitory” effect on neurons, an increase in the concentration in neurons “exciting” amino acids such as glutamic acid, and increased GABA concentrations. Glycine also binds to specific NMDA receptor sites and thus elicits sensations through the excitatory neurotransmitters glutamate and aspartate. The NMDA receptor is thought to play an important role in a wide range of nervous system functions, including neuronal migration, synapse formation, learning, and memory. In addition, it is involved in excitotoxic neuronal death, which occurs in various acute and chronic neurological disorders [1], [10], [26]. Experimental and clinical evidence that disorders of neurotransmission (primarily glutamate and GABAergic) may be the primary pathogenetic mechanisms for the development of epilepsy. At the same time, the formation of an epileptic focus and (or) generalized epileptic activity with transformation into epileptic seizures (EP) is associated primarily with the phenomenon of “disinhibition” of neurons. GABA contributes to the preservation of the structural and functional organization of biomembranes, the transport of neurotransmitters, and the improvement of synaptic transmission; it increases the content of dopamine in the brain; it causes an increase in the compensatory activity of aerobic glycolysis and a decrease in the degree of inhibition of oxidative processes in the Krebs

cycle under hypoxic conditions with an increase in the content of ATP, creatine phosphate and activation of energy-synthesizing functions of mitochondria, stabilization of cell membranes [5], [6], [27].

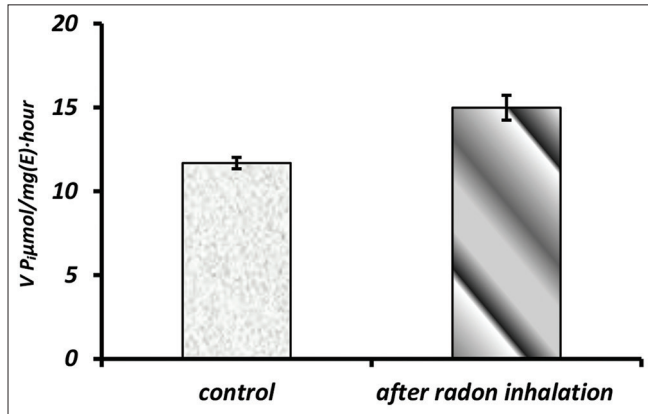


Figure 4: Rat brain plasma membrane Na, K-ATPase activity  
Reaction medium:  $[MgATP] = 1.69 \text{ mM}$ ;  $[ATP]_i = [Mg^{2+}] = 0.31 \text{ mM}$  ( $MgATP$  – substrate)

While excitatory neurotransmitters stimulate the potential for neuronal activity, inhibitory

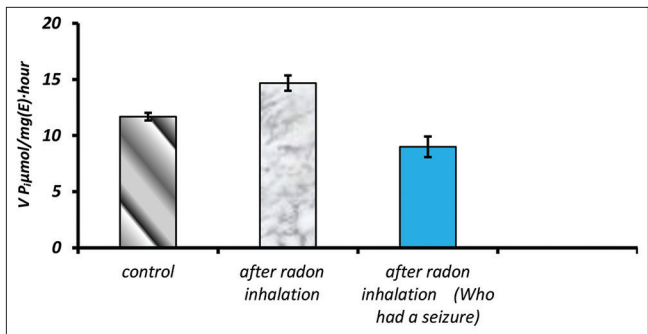


Figure 5: Rat brain plasma membrane Na, K-ATPase activity  
Reaction medium:  $[MgATP] = 1.69 \text{ mM}$ ;  $[ATP]_i = [Mg^{2+}] = 0.31 \text{ mM}$  ( $MgATP$  – substrate)

neurotransmitters such as GABA inhibit neurons, reduce their activation, and reduce the corresponding activity in the central nervous system. We see that inhalation of radon leads to an increase in the amount of GABA, and when the action potential reaches the end of the axon of the GABA-ergon neuron, GABA is released into the synaptic cleft, where it binds to postsynaptic receptors that open chloride ion channels. The action of GABA on receptors is the penetration of negatively charged chloride ions into the receptor neuron, which becomes more negative inside the cell membrane. In this case, the probability of activating the action potential is much less. It all goes on in milliseconds, then GABA slowly breaks down at the receptor so that inhibition continues. However, this inhibitory effect is manifested in the brain in combination with GABA. Drugs with anticonvulsant effects can cause increase the post-synaptic action of GABA.

It should be emphasized again that the glutamine system produces the most important mediators for the brain tissue, such as GABA (glutamic acid – a precursor of GABA) and glutamate (formed during the transamination of GABA). The latter plays the role of an excitatory mediator. This increases the permeability of the membrane for potassium ions. It is not difficult to imagine that disturbances in the glutamine system can lead to a variety of disorders – electrolyte, metabolic, and transmitter, contributing to the epileptization of neurons. Apparently, this is precisely what can explain the decrease in oxygenation of the brain tissue, the increase in residual and glucose oxidation of venous blood, and the increase in the content of lactate and pyruvate in the blood, that is, signs of disruption of redox processes and carbohydrate metabolism in the brain.

The effect of radon is also expressed in the activation of the amino acid deamination reaction, during

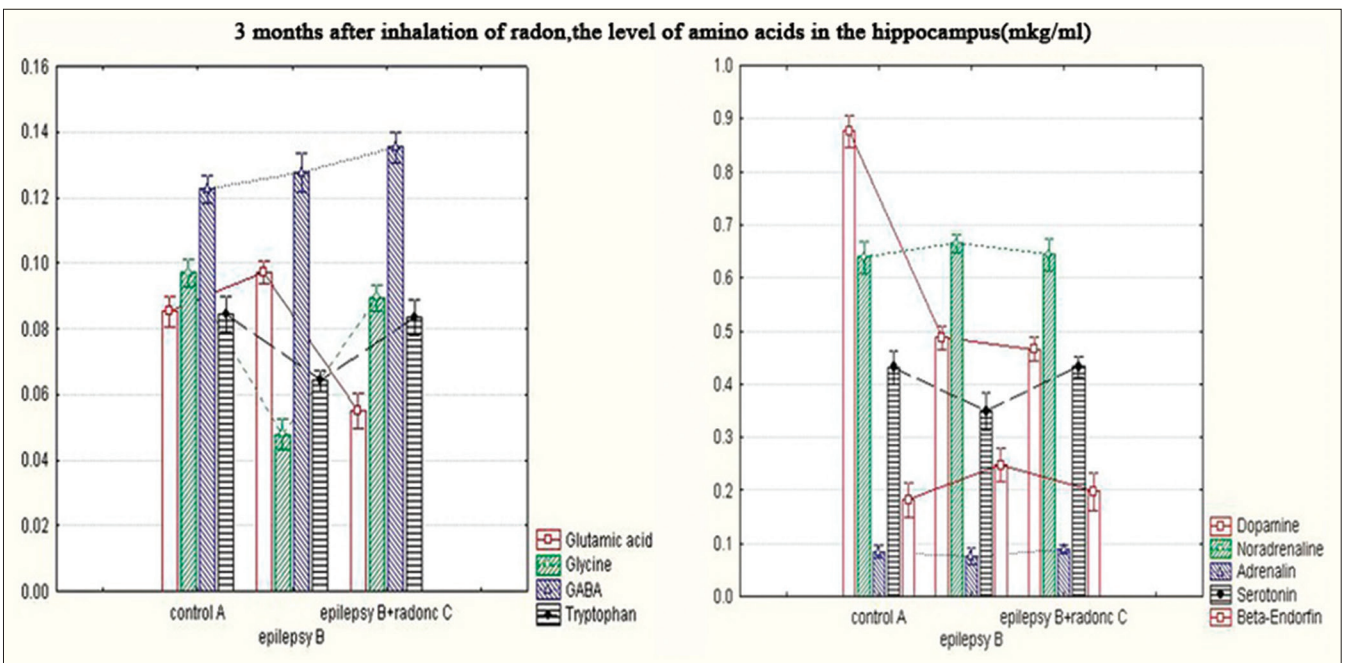


Figure 6: 2–3 months after inhalation of radon, the level of amino acids and biogenic amin in the hippocampus (mkg/ml)

which ammonia and keto acids are formed, which are included in the citric acid cycle. As a result, the carbon skeleton is consumed as an energy material, and ammonia is neutralized by the synthesis of glutamine or urea [5], [6], [10], [15], [19], [28]. Deamination of amino acids under the effect of radon reduces and normalizes the SA fund, which is necessary for the implementation of certain behavioral acts, which takes place 2–3 months after inhalation of radon. It regulates these processes leading to an increase in the amount of GABA and a decrease in glutamate (Figure 6).

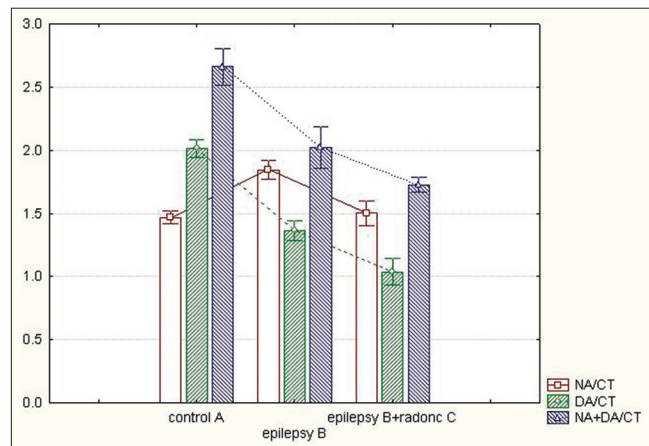


Figure 7: Ratio of catecholamines to serotonin

As for biogenic amines, dopamine markedly regulates neuronal excitability in the dentate gyrus [24] as well as other areas of the limbic system [2], [5], [21] through D1-like and D2-like signaling pathways. The liberated NE then binds to the appropriate receptors for synaptic transmission. These receptors are classified as G protein-coupled receptors with inhibitory or stimulatory effects. NE plays an anticonvulsant role in epilepsy. In addition, a decrease in NE has been associated with increased susceptibility to the absence of epileptic seizures and/or AGSs and neuronal damage to limbic regions [19], [26], [27], [28]. The fact that NE mediates synaptically mediated excitability through modulation of ion channel conductance or indirectly through GABAergic and, in addition, the glutamatergic transmission may help explain its role in epilepsy [11], [9], [26], [27].

Positive 5-HT levels are elevated after generalized seizures compared to pre-interictal levels. This relationship between levels of 5-HT in nervous system tissue suggested that 5-HT may play an important role in episodes [19], [20]. On the other hand, the inverse correlation between interictal serum 5-HT levels and the time of observation of postictal generalized EEG suppression suggests an important relationship between interictal serum 5-HT levels and 5-HT, Figure 7.

We find that the ratio of norepinephrine to serotonin, which is much higher than that of dopamine to serotonin, and that of catecholamines to serotonin is even greater than that of individual catecholamines to serotonin, is of great importance (Tables 6 and 7). It is their ratio, which allows us to think that what matters

here is not the increase or decrease in the number of any catecholamines, but the ratio between them, which gives us a cascade of epileptic seizures.

Seizure activity is partially regulated by inhibitory neuronal processes and catecholamines, DA and NE, which act as central seizure suppressors. These results indicate that NE may reduce CNS susceptibility to epileptiform events.

Quantitative shifts in beta-endorphins compared to biogenic amines in blood plasma during epilepsy have also been shown. Beta-endorphins are known to be pain relievers and play an important role in the human body's defense processes but their quantitative increase has been shown to cause seizures. An increase in beta-endorphins causes a decrease in the amount of dopamine, while dopamine is a prolactin inhibitor, and beta-endorphin causes androgen stimulation – hyperandrogenemia [4], [17]. All this, to a certain extent, is expressed in hyperprolactinemia, the cause of which, on the one hand, is a decrease in dopamine, and on the other hand, an increase in endorphin.

It is known from the literature that an increase in prolactin secretion leads to an increase in insulin, an increase in the adrenocortical tropic hormone, an increase in glucose-6-phosphatase-dehydrogenase, and a decrease in vasopressin. That causes an increase in blood pressure at the expense of vascular smooth muscle and all this leads to the development of seizures, and 2–3 months after exposure to radon, there is an increase in dopamine and a decrease in beta-endorphins, which leads to the disappearance of seizures.

## Discussion

As our study showed, the dysfunction of the so-called glutamatergic system plays a special role in the development of audiogenic epilepsy, and it also regulates many processes in the brain. Glutamic acid (Glu) is the main excitatory neurotransmitter. By enhancing the conduction of a nerve impulse from neuron to neuron in our brain, glutamate is involved in such mental functions as learning and memory, vision, hearing, and movement. A constant companion of glutamate is GABA, which plays the opposite role of an inhibitory neurotransmitter. Insufficient activity of GABAergic neurons, as well as an imbalance of Glu and GABA, can cause epilepsy. In rats with audiogenic epilepsy at the height of the seizure, the levels of important neurochemical indicators of brain activity clearly change, and in the KM rats, there are deviations in the background levels of a number of neurotransmitters [10], [20], [25].



Monoamines are the major neuromodulatory system in the central nervous system, and the overwhelming evidence accumulated over the past 30 years has also established their crucial role in epilepsy [1], [3], [8], [12] [24]. Beta-endorphins cause an increase in prolactin secretion and an increase in insulin levels, which, in turn, leads to the development of seizures in the excitatory and inhibitory processes of glutamate and GABA [1], [14], [15].

The involvement of the monoamine system in the pathogenesis of epilepsy suggests that depression, bipolar disorder, and other neuropsychiatric disorders classically associated with monoamine dysfunction may increase the risk of seizures [16].

Thus, as a result of epilepsy in rats, a certain change in the level of individual amino acids is observed in the nervous tissue, which itself or through derivatives affects the functional state of the central nervous system, the level of GABA, and glycine increases. It is accompanied by a decrease in the level of serotonin, which is manifested by a decrease in glutamate and aspartate in the nervous tissue with a parallel decrease in GABA and glycine.

A significant change in the qualitative ratio of the amino acid composition, followed by a change in the balance of functionally opposite mediators, the pathogenic significance of which is the regulation of the functional activity of the central nervous system, is of great importance. In the nervous tissue, there is a decrease in the level of serotonin. The ratio of the levels of dopamine and norepinephrine to serotonin revealed a significant increase in this indicator, which convincingly indicates a decrease in the relative amount of serotonin, that is, there is a pronounced activation of the kynurenine pathway of tryptophan metabolism, which leads to an imbalance of functional processes. Deviation from the optimal level and the ratio of biogenic amines and free amino acids, caused by biochemical and physiological processes, is directly or indirectly related to the implementation of behavioral acts. The change in the level of amino acids and their derivatives is one of the most important causes of multiple pathologies, which manifests itself not only in the dysfunction of the nervous system but also contributes to the development of epileptic seizures and a number of nervous and mental disorders [8], [24].

In this aspect, radon deserves special attention. In recent years, there have been trends in the study of natural substrates (traditional medicine) and their discussion in the modern scientific and practical context around the world. Because such methods are characterized by fewer side effects and, therefore, if chronic use is necessary, they cause less harm to a person. The method of treatment with Tskhaltubo water is a type of drug that has passed the basic phase of research on experimental models of epilepsy, which corresponds to the experimental research phase defined for studying the effectiveness of drug methodologies [7], [16], [20], [21], [23].

**Table 6: 2–3 months after inhalation of radon, the level of Biogenic Amin and Ratio of catecholamines to serotonin in the hippocampus (mikg/ml)**

Hippocampus			
Biogenic amines in plasma	Control A	Epilepsy B A-B	Epilepsy+radon C (B-C)
Dopamine	0.867 ± 0.01	0.488 ± 0.011*	0.465 ± 0.009**
Noradrenaline	0.639 ± 0.01	0.658 ± 0.024**	0.645 ± 0.012***
Adrenaline	0.08 ± 0.002	0.071 ± 0.005*	0.087 ± 0.003**
Serotonin	0.429 ± 0.01	0.352 ± 0.01*	0.430 ± 0.021**
NA/CT	1.489 ± 0.024 p < 0.001	1.869 ± 0.056 p < 0.001	1.5 ± 0.068**
DA/CT	2.0209 ± 0.013 p < 0.001	1.386 ± 0.032 p < 0.001	1.081 ± 0.042**
NA+DA/CT	2.66 ± 0.038 p < 0.001	2.044 ± 0.067 p < 0.001	1.726 ± 0.071**

Exposure to radon contributed to a certain regulation of the changes caused by epileptic seizures. In the group of rats treated with radon inhalations, there is a decrease in glutamate and aspartate, which is accompanied by an increase in GABA and glycine, which helps to optimize the ratio of activating/inhibiting amino acids. Obviously, a change in the balance of functionally opposite mediators contributes to the process of regulating metabolic shifts. The above problems in epilepsy, the effect of radon inhalation on the level of individual biochemical constants of the body, and, the holistic behavior of animals requires further study [11].

**Table 7: Determination of beta-endorphins and biogenic amines in blood plasma (pcg/ml M ± m)**

Beta-Endorphins	Rats	After 2 months	After 3 days	Control
	26.5 ± 0.7***	22.3 ± 0.5**	19.8 ± 0.9	18.0 ± 1.2
Amines				
Dopamine	20.5 ± 1.56***	34.5 ± 1.05*	38.2 ± 1.08	42.3 ± 2.73
Noradrenalin	502.2 ± 5.06***	392.4 ± 3.21**	385.0 ± 3.27	378.9 ± 2.48
Serotonin	89.5 ± 2.93***	68.6 ± 2.7**	57.4 ± 2.32	56.2 ± 2.20

\*\*\*p < 0.001, \*\*p < 0.01, \*p < 0.05 Comparison with control

Meanwhile, according to modern data [22], epileptic seizures is largely associated with the primary violation of the functional processes of the CNS, including primary cerebrovascular disorders [17]. In this regard, the study of epileptic seizures against the background of radon inhalation may be of practical importance.

The data obtained indicate that a decrease in OS after a certain time after radon inhalation is due not only to residual effects of organic changes in brain structures associated with the organization of behavior, in particular, specific glutaminergic neurons of the “attack center” of the hypothalamus [5] but also with activation the entire adaptive-compensatory system.

The clarification of these issues will deepen the modern understanding of epileptic seizures and the effect of radon inhalation on neurochemical correlates and dysfunction of the nervous system of animals.

## Conclusion

Further research is needed to elucidate the mechanism of radon effect on the antioxidant processes but based on the results of the experiment, it can be

concluded the following: The studies on experimental animals have shown that inhaling Tskhaltubo water develops hormesis, which regulates oxidative processes in the brain due to the activation of antioxidants. This is expressed in a decrease in existing epileptic seizures and is expressed in the activation of Na/K-ATPase and specific glutaminergic neurons of the “attack center” of the hypothalamus, but also with the activation of the entire adaptive-compensatory system.

Inhalation of Tskhaltubo water can be considered as a method of treatment with an anticonvulsant effect confirmed by experimental studies.

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