Ivane Javakhishvili Tbilisi State University

Faculty of Medicine

NINO CHIKOBAVA

THE ROLE OF NITROGEN OXIDE IN THE DISTURBANCE OF ARTERIAL VASOMOTOR REACTIONS DUE TO HYPERHOMOCYSTEINEMIA

(Experimental study on white rats)

Abstract of the dissertation for the academic degree of a doctor of medicine

Tbilisi
2019

The dissertation is performed at Ivane Javakhishvili Tbilisi State University and Ivane Beritashvili Experimental Center for Biomedicine

Scientific supervisors

Nana Doreulee
Doctor of Biological Sciences, Professor

Nodar Mitagvaria
Doctor of Biological Sciences, Professor

INTRODUCTION

During the years, the diagnostics of cardiac ischemic diseases was based on the determination of lipid exchange and hemostasis indices. But, in the available literature there appeared some references that the development of atherosclerotic and thrombotic processes in the myocardium is due to a "new factor" – an increase of homocysteine concentration in the blood – hyperhomocysteinemia, which appears to be the process dependent on many factors.

A normal level of homocysteine in blood serum is considered to be 5-12 mkmol/l. In conditions of a light hyperhomocysteinemia this index increases up to 15-30 mkmol/l, while hyperhomocysteinemia of an average degree is 31-100 mkmol/l, and a severe case is considered to be more than 100 mkmol/l.

The identification of homocysteine, as one of the risk-factors of vascular disease has begun since 1964, when Mudd (1964) has shown that the accumulation of homocysteine in the blood and then in urine, due to the deficit of cystathionine beta synthase enzyme, induces homocysteinuria. After this discovery, McCully and Wilson (1975) reported that patients with the above-said enzyme deficiency develop arterial blood vessel injuries. Based on this, they have concluded that homocysteine itself or one of its derivatives condition an arterial damage. This has led to the hypothesis that a moderate increase in homocysteine in the blood may become a risk-factor for the development of atherosclerosis.

At present it is undoubtedly that a significant congenital disorder of homocysteine metabolism leads to the damage to blood vessels, but the mechanism of this damage development is not well established up to now.

A significant relation between hyperhomocysteinemia and clinical cardiovascular cases is described in many fundamental theses. However, there are also such papers where such a connection is rejected (Ganguly, Alam, 2015). Despite this, at present hyperhomocysteinemia is considered as an independent risk-factor of atherosclerotic vascular disease (Boers, 2000).

The mechanism, by which hyperhomocysteinemia promotes the development of vascular damage, is still unknown. What is established can be shortly formulated in this way:

In vitro studies have shown that an increased concentration of total homocysteine affects the endothelial cells. Their damage, the activation of platelets, a damaging effect on thrombomudulin expression, the activation of tissue factor, an enhanced oxidativeness in low density lipid proteins – it is the list, in which any of above-said may be considered as possible mechanisms, by means of which homocysteine leads to atherosclerosis and thrombosis (Fay, 2008).

As a rule, the dysfunction of endothelial cells accompanies many types of cardiovascular diseases (Tousoulis et al., 2012). It has been established that a disorder of function of endothelial nitrogen oxide synthase or/and the reduction of nitrogen oxide delivery can become a reason for many clinical manifestations in patients with endothelial dysfunction (Schulman et al., 2006; Tousoulis et al., 2012). This consideration indicates that the synthase of endothelial nitrogen oxide may be influenced by hyperhomocysteinemia.

Although indirectly, the inhibitory effect of homocysteine on nitric oxide release from endothelial cells was confirmed. Taking into account a long-lasting discussion in the literature on a possible role of homocysteine in the development of atherosclerosis (Tehlivets, 2011), the above-said facts indicate that the decrease in nitrogen oxide bioresource and reduction of eNOS activity should induce the dysfunction of endothelial cells, which makes the development of cardiovascular complications significantly understandable.

Nitrogen oxide (NO) created by nitrogen oxide synthase (NOS) with three main isoforms (endothelial – eNOS, neuronal – nNOS and inducible – iNOS) has a highly important role in cardiovascular functioning. At the same time, it reveals the useful effects in the organism having an antibacterial, antiparasitic, antiviral, thomorrhoidic regulatory action on a number of functions in the organism. But, on the other hand, its high level if it is uncontrollable may be disastrous (Yen et al., 2002).

Based on the above-said, we considered it appropriate to formulate the goal and the following objectives of our study: to establish a role of nitric oxide in the disorder of arterial vasomotor reactions due to hyperhomocysteinemia in vitro and in vivo studies on white rats. Particularly:

- How a contractility of normal and deendothelized segments of the arterioles changes to norepinephrine, acetylcholine and histamine in control and hyperhomocysteinemia animals, and
- How a contractility of normal and deendothelized segments of the arterioles changes to norepinephrine, acetylcholine and histamine in control and hyperhomocysteinemia animals changes in case of nonselective inhibition of nitric oxide synthases.

Literature review

The identification of homocysteine, as one of the risk-factors of vascular disease has begun since 1964, when Mudd (1964) has shown that the accumulation of homocysteine in the blood and then in urine, due to the deficit of cystathionine beta synthase enzyme, induces homocysteinuria. After this discovery, McCully (1971) reported that patients with the above-said enzyme deficiency develop arterial blood vessel injuries. Based on this, he has concluded that homocysteine itself or one of its derivatives conditions an arterial damage. This has led to the hypothesis that a moderate increase in homocysteine in the blood may become a risk-factor for the development of atherosclerosis (McCully, Wilson, 1975).

A significant connection between hyperhomocysteinemia and clinical cardiovascular cases is described in many fundamental theses. However, there are also such papers where such a connection is rejected (Elkelboom et al., 1999). Despite this, hyperhomocysteinemia is considered as an independent risk-factor of atherosclerotic vascular disease (Boers, 2000).

What is homocysteine and what is the basis of cardiovascular disorders associated with it?

Homotsisteine is a sulfur containing amino acid, which is not used for protein synthesis. Homocysteine in food presents as microelements. It is formed during the metabolisation of an indispensable amino acid - methionine to cysteine. Its intracellular concentration is regulated precisely, while its excess amount is transferred to plasma, where it is subjected to the oxidation to disulfide approximately by 99%.

Hyperhomocysteinemia is determined when homocysteine level in the plasma exceeds 15 mkmol/l. It has a diverse etiology: genetic, kidney failure, taking certain drugs or deficiency of vitamins B6 and B12 in foods (Selhub, 1999). Even a moderate increase of homocysteine in plasma within the frames of 10-15 mkmol/l (or between the norm and a high level) increases a risk of origin of cardiovascular disturbances (Elkelboom et al., 1999).

The first and accurate evidence that an increased concentration of total homocysteine appears to be a causal risk-factor for atherothrombotic disease, was obtained from the patients, who had a pronounced congenital disorder of homocysteine metabolism.

A large-scale study carried out in 2002 (Ford et al., 2002) has shown that an increase of a risk of coronary blood vessels disease associated with the increase of total homocysteine concentration for each excess 5 mkmol makes up 20%.

Three different studies carried out on a different contingent (healthy people /Ridker et al., 1997/; systemic lupus erythematosus /Petri et al., 1996/, and patients with venous thrombosis /Elchinger et al., 1998/) showed a positive correlation between the development of the concentration of total homocysteine and venous thrombosis. It is considered that a primary thrombogenic effect of total homocysteine concentration explains its high risk for the patients suffering from the diseases of coronary blood vessels. As this contingent already has atherosclerosis of definite degree, thrombosis induced by the concentration of total homocysteine may become catastrophic from the point of view of blood vessels occlusion (de Bree et al., 2020).

Mc Cally (1969) formulated a hypothesis that homocysteinemia itself appears to be a main reason for arterial disorder. Despite many in vitro and in vivo studies dedicated to this hypothesis, the mechanism by means of which hyperhomocysteinemia leads to the development of vascular disorder is unknown up today.

As a rule, the dysfunction of endothelial cells accompanies many types of cardiovascular diseases. It has been established that disorder of function of endothelial nitrogen oxide synthase or/and the reduction of nitrogen oxide delivery can become a reason for many clinical manifestations in the patients with endothelial dysfunction (De Caterina et al., 1995; Dubey et al., 1995). This consideration indicates that the synthase of endothelial nitrogen oxide may be influenced by hyperhomocysteinemia.

Direct measurement of nitrogen oxide released from the culture of micro vessel endothelial cells by means of NO-selective electrode system has shown that 50 mkml homocysteine can significantly inhibit nitrogen oxide release despite by means of which way this process (release) was stimulated (Zhang et al., 1993). At the same time, it turned out that an oxidation of sulfhydryl group in eNOS induces a decrease in NO-generative activity of this enzyme. By means of indirect measurements it has also been established that this decrease is induced not by means of production inhibition, but by the reduction of relative bioresource. That is, although not in a direct way, but the inhibitory effect of homocysteine on the release of L-arginine-stimulated nitrogen oxide from endothelial cells was confirmed. Taking into account a long-lasting discussion in the literature on a possible role of homocysteine on the development of atherosclerosis (McCully, Wilson, 1975), the above-said facts indicate that the decrease in nitrogen oxide bioresource and the reduction of eNOS activity should induce the dysfunction of endothelial cells, which makes the development of cardiovascular complications significantly understandable. Of course hyperhomocysteinemia-induced dysfunction of endothelial cells deserves a detailed research and analysis.

The material and methods

The induction of moderate hyperhomocysteinemia was performed in male rats, weighing 120-160 mg (n=12) by adding L-methionine to drinking water (1 g/kg per day) during four weeks. The volume of daily consumed water by each animal was determined by the volume of average consumption of water in the norm. The control animals (n=12) took water *ad libitum.* At the beginning and completion of 4-week period, the mass of animal was determined. Blood sample was taken from hip artery, which was centrifuged during 20 minutes at 3000 g at 4^0C temperature. Before conducting analysis the serum isolated was kept at -20^0C. The total content of homocysteine was determined by using chromatographic method and fluorimeter determination.

One of the objective methods for the analysis of the function of blood vessels smooth muscles is considered to be the measurement of parameters of contractility of isolated vascular preparations by means of mechanotron conventors (Berlin et al., 1970). This method allows us to measure a degree of increase or decrease of vascular tone in conditions of various influences on it.

The experiments were carried out in the first row arterioles (diameter 130-10 mkm), isolated from gracilis muscle of a rat. At the end of the 4^{th} week from the beginning L-methionine taking, a systemic arterial pressure of the animal was measured under sodium pentobarbital (50 mg/kg) anesthesia and the blood sample was taken to measure the homocysteine content. Then the isolation of gracilis muscle from the surrounding tissues was performed. The mentioned muscle was cut and placed in Ringer-Heilith solution at 0-4^0C temperature. Under the binocular microscope the 1.5-2 mm length segment of the first row intramuscular arteriole was extracted, which by means of a special device (Climin, 1989) was placed in a small pool of fluid flow chamber with Ringer-Heilith solution, where the preparation was mounted on two steel small hooks (Fig. 2.1). One of the hooks was strongly attached to the rod of mechanotron. The preparation was stretched, and the size of constant stretching was selected according to the

results of the arteries smooth muscle contractility testing. The testing was performed by means of standard solutions, containing potassium in the concentration of 80 mol and make up 5.1 min in average. For the achieving an equilibrium state, before the measurements the preparation was present in Ringer's solution at 37^0C during 1.5 h.

Recording of mechanical activity of smooth muscles of vascular preparations

Recording of contractile activity of isolated blood vessels is possible on strain gauge device using 6 MXIC type mechanotrons in isometric mode.

Electric signals received from mechanotrons were transferred to the amplifiers, using the block diagram, given on Fig. 2.3. The calibration of mechanotrons was performed in millinewtons (mN). For this reason a horizontal rod was loaded with standard, small weights and a deviation from the initial level was registered with pen recorder.

Preparation of solutions, control of pH and temperature

As a nutrient solution, the Ringer-Heilith flowing solution containing the following substances (mMoll/l) has been used:

NaCl – 11.0; KCl – 4.7; $NaHCO_3$ – 14.9; KH_2PO_4 – 1.18; $MgSO_4.7H_20$) – 1.17; $CaCl_2.2H_20$ - 2.5; glucose – 11.0.

The experiments were conducted under the control of solution pH. During the experiment the constancy of solution temperature was maintained at 37 ± 0.5^0C level by means of ultra thermostat.

The experiment protocol

The contractile reactions were studied on normal and deendothelized segments of arterioles. The reaction of contractile arterioles of control and hyperhomocysteinemia group animals was examined before and after the deendothelization of segments.

In the next series of experiments a peak reaction of arterioles was studied at cumulative doses of acetylcholine (10^{-10} – 10^{-5} mol/l) and sodium nitroprusside (10^{-10} – 10^{-5} mol/l) in the solution surrounding the segments. After this the segments were incubated during 30 minutes by the use of L-NAME solution (10^{-4} mol/l) (nonselective inhibitor of nitrogen oxide synthase) and again the reaction of segment on acetylcholine and sodium nitroprusside mentioned doses was measured.

The same series of experiments were carried out to study the reaction on histamine (10^{-4} mol/l) before and after injection of L-NAME. The endothelium intact and deendothelized segments were measured.

All the above mentioned substances were administered into solution flowing chamber, where the segments of arterioles were placed. After this reaction Ringer-Heilith pure solution was administered into the system.

Statistical analysis of the data obtained

The data obtained were expressed in their average values and standard error. The statistical analysis was conducted by the use of ANOVA. Statistical reliability was examined by Student's t-criterion. $P<0.05$ was considered to be statistically reliable.

The obtained data

Methionine diet evoked a significant increase (3-fold) of homocysteine concentration in animals. The difference of the systemic arterial pressure values and animals' weight between control and hyperhomocysteinemia group animals was not practically observed.

The reaction of arterioles to norepinephrine

In the conducted experiments it has been revealed that norepinephrine (10^{-9} – 10^{-5} mol/l) induces a dose-dependent reaction in the arterioles of both control and hyperhomocysteinemia group animals. Though in these last animals this reaction was significantly more pronounced.

The removal of endothelium significantly increased the reaction of isolated arterioles on norepinephrine in control animals, while the deendothelization of the arterioles in hyperhomocysteinemia group animals had no considerable influence on norepinephrine-induced reaction.

The reaction of arterioles to acetylcholine

Acetylcholine (10^{-9} – 10^{-6} mol/l) induced a dose-dependent dilation of arterioles, more pronounced in the vessels of control animals, as compared to homocysteine ones. After 30 min washing out of the vessels by Ringer-Heilith solution, at the background of the administration of nonselective inhibitor of nitrogen oxide synthase L-NAME the conducting of the same test induced a significant decrease in dilation degree of arterioles in control animals, but in the arterioles of hyperhomocysteinemia group animals the dilation remained almost the same.

The reaction of arterioles to sodium nitroprusside

Sodium nitroprusside also induced a similar dose-dependent dilation of arterioles both in control and hyperhomocysteinemia group animals. The inhibitor of nitrogen oxide synthase practically did not change either degree, or the character of dilation in arterioles both in control and hyperhomocysteinemia animals.

The reaction of arterioles to histamine

Histamine (10^{-6} – 10^{-4} mol/l) induced a dose-dependent dilation of arterioles in control and hyperhomocysteinemia group animals. Dilation degree of arterioles of control animals was more pronounced than in case of hyperhomocysteinemia group animals.

After washing out of blood vessel preparations, at the background of nonselective inhibitor of nitrogen oxide synthase the test with histamine revealed a significant decrease in dilation degree of control animals' arterioles, as compared with the arterioles of hyperhomocysteinemia group animals.

In case of deendothelized arterioles, their dilation reaction to histamine in control group animals was significantly reduced, while in case of hyperhomocysteinemia group animals the reaction remained almost unchanged.

The discussion of the data obtained

The main results of experimental study (without their analysis) can be summarized so that the increase of homocysteine in blood plasma (induced by the increased content of methionine in drinkable water) associates with blood vessels constriction induced by norepinephrine, as well as with decreased endothelium-dependent dilation in muscle arterioles. What should be the mechanism inducing this reaction and to what its formation should be connected?

The increase of homocysteine in plasma appears to be an independent risk-factor of atherothrombotic disease. There are some opinions how the increased concentration of homocysteine in blood plasma can induce atherothrombotic diseases in cardio-vascular system. In particular, it is considered that hyperhomocysteinemia damage effect can be expressed in morphological change in blood vessels' wall, an increased activity of platelets (Durand et al., 1966, 1997), in the stimulation of the proliferation of smooth muscle cells. Based on the existing investigations, one of the reasons, inducing the above-said changes is the fact that a high concentration of homocysteine induces the disorder of vascular endothelium function, though the impact of increased concentration of homocysteine commonly on the function of micro vessels is not still established.

In our experiments methionine (homocysteine precursor) was added to drinkable water of animals. As a result the concentration of homocysteine in plasma has been increased almost by three times and reached the level, which is connected with high risk of vessels' disease in humans.

Generally, an increase of methionine concentration (or some other amino acids) does not associate with a damaged effect of blood vessels. But there are enough evidences that an

increased concentration of homocysteine appears to be a significant reason for the damage of blood vessels endothelium.

As the blood circulation of skeletal muscle appears to be a basic constituent of hemodynamic impedance, its disturbance during hyperhomocysteinemia may be considered as a significant component in the development of peripheral vascular disease. For this very purpose we have studied the changes in endothelium vasoregulatory function of blood vessels in normal and methionine-diet induced hyperhomocysteinemia animals.

First of all, contractile reactions of control and hyperhomocysteinemia group animals to norepinephrine have been studied and it has been established that hyperhomocysteinemia induces norepinephrine-induced enhancement of vasoconstriction.

As previously shown, the injection of norepinephrine into the arterioles of skeletal muscle is accompanied by the release of nitrogen oxide from the endothelium, which modulates a degree of contraction induced by norepinephrine. We assume that the reason for an increased reactivity of vessels to norepinephrine in hyperhomocysteinemia group animals should be a disorder of nitrogen oxide synthase. And indeed, deendothelization in the arterioles of control animals induced an increase of a reaction to norepinephrine, while the reaction of the same arterioles to norepinephrine in hyperhomocysteinemia group animals was unchanged. This fact confirms our assumption. For further verification of the above-said, the tests of reaction to acetylcholine and histamine were used. As known, the mentioned agents induced the generation of nitrogen oxide. As seen, a vasodilation of blood vessels of skeletal muscle induced both by acetylcholine and histamine in control animals was decreased, as compared to the reaction of arterioles in hyperhomocysteinemia group animals. Moreover, the inhibition of nitrogen oxide production by means of L-NAME decreased a dilation induced by acetylcholine and histamine of isolated arteriole only in control animals, while the injection of L-NAME in hyperhomocysteinemia animals did not give us any significant effect. In addition, a

deendothelization eliminated a histamine-induced vasorelaxation difference between the arterioles of control and hyperhomocysteinemia animals.

As turned out, in conditions of hyperhomocysteinemia a sensitivity of blood vessels' smooth muscles to nitrogen oxide did not change, as the reaction of arteriole to nitrogen oxide at the administration of its donor – sodium nitroprusside did not change. This result testifies that at hyperhomocysteinemia only the disorder of a bioresource of endothelial nitrogen oxide takes place.

As shown previously, the process of release of relaxing factor (i.e. nitrogen oxide) of endothelium-dependent muscles is disturbed at hyperhomocysteinemia (Celemajer et al., 1993; Taniwaki et al., 1998). Our results agree with these data and spread them to the level of arterioles.

The disorder of nitrogen oxide synthase induces a change in the action of endothelium-dependent dilators to those small caliber crown arteries and arterioles, which do not suffer from atherosclerotic disorders (Kuo et al., 1972).

Summarizing the above-said allows us to assume that an endothelial dysfunction is one of the early component of the complex phenomena that take place in the process of development of blood vessels' diseases.

As nitrogen oxide, besides vasodilation function, has a significant role in the implementation of anticoagulation and anti-thrombotic reactions, endothelial dysfunction should be considered as an important link between hyperhomocysteinemia and atherothrombotic diseases.

It should be also taken into account that in hyperhomocysteinemia conditions a nitrogen oxide deficit may become reason for smooth muscles proliferation, and in its turn, endothelial dysfunction promotes an adhesion of leucocytes and platelets on blood vessels wall.

The mechanisms of endothelial damage induced by hyperhomocysteinemia undoubtedly need the further study, but the experimental material obtained by us gives an opportunity to conclude that the blood micro vessels suffer from a significant influence of hyperhomocysteinemia (that relatively affects tissue blood supply).

Therefore, an increase of homocysteine concentration due to diet, obtained in our experiments, associates with the enhancement of norepinephrine-induced contraction and the decrease in acetylcholine-induced dilation. These changes, most likely should be connected with the elimination of arterial vasomotor reactions due to nitrogen oxide and appears to be an important early stage in the development of vascular diseases related to hyperhomocysteinemia.

What kind of interpretation can be given to the literature data, which indicate that the angina pectoris and acute myocardial infarction in severe patients against the background of strengthening thrombotic processes, hyperhomocysteinemia, as compared to homocysteinemia within normal limits, cardiac dysfunction and coronarostenosis are more strongly pronounced, which appear to be the significant risk-factors of aggravation and complications of the disease.

The cardioprotective effect of ischemic precondition is an already well-known phenomenon and currently an intensive study of its possible mechanisms is undergoing. The main essence of this phenomenon is that an organism that has undergone pre-ischemic “preparation” (pre-conditioning), subsequently more easily stands an ischemic attack, than the organism, which has not undergone such pre-conditioning (Bernaudin et al., 2002).

Recently obtained data indicate that the preconditioning of the myocardium against a severe attack is formed by means of the generation of nitrogen oxide in pre-ischemic attacks. The cellular mechanism of this preconditioning is unknown (Samoilov, Mokrushin, 2003; Semenza, 2004) and its study is one of the topical task of modern cardiology.

It is believed that a principal conditioning factor of myocardial preconditioning is ischemia-induced NOS gene expression, followed by an increased formation of nitrogen. It is evident that

here we are talking about activation of isoform of induced nitric oxide synthase. Nitrogen oxide isolated in result can have both facilitating and aggravating effect. Therefore the given cellular mechanism of preconditioning phenomenon needs the further study and clarification. But we considered it necessary to discuss it, as yet no other mechanism has been proposed in the literature.

Mainly, we should take into account that preliminary ischemia by still unknown way facilitates the possible further ischemic attack. Taking into account that because of atherosclerosis developed in hyperhomocysteinemia conditions, to some extent the organism is in ischemic condition, it may be that at ischemic attack it will be more adaptive to the situation (the myocardium consumes less amount of oxygen), than a healthy organism ("not prepared") in the same conditions.

CONCLUSIONS

The analysis of our experimental results and the data existing in the literature, allows us to draw the following conclusions:

1. During cardiac ischemic disease, an increase of homocysteine at enhanced atherogenesis and post-infarction period indicates the repetitive angina attacks more, than an increase of indices of lipid metabolism and hemostasis.

2. In case of cardiac ischemic disease, at hyperhomocysteinemia the atherogenic processes prevail in blood vessels of patients, while in case of existing normal homocysteine - thrombotic processes.

3. An increase of homocysteine concentration evokes a disorder of arterial vasomotor reactions due to nitrogen oxide and appears to be a significant early stage in the development of vascular diseases related to hyperhomocysteinemia.

4. Endothelial dysfunction should be considered as one of the early component in complex phenomena of the development of blood vessel diseases, as well as an important link between hyperhomocysteinemia and atherothrombotic diseases.

5. During hyperhomocysteinemia, a disorder of endothelial nitrogen oxide bioresource takes place.

The list of scientific works published on the dissertation theme

1. Homocysteine and its role in the functioning of the organism. Proc. of Georgian National Academy of Sciences, Biomed. Series, 2016, 42, 5-6, 23-29 (co-authors: N. Doreulee, N. Mitagvaria) (in Georgian).

2. Direct measurement of contractility of isolated small arteries preparations. Georgian National Academy of Sciences, Bull., 2017, 11, 3, 146-150 (co-authors: M. Dekanosidze, N. Mitagvaria).

3. The role of nitrogen oxide in the regulation of blood local flow in oral tissues of a rat. Proc. of Georgian National Academy of Sciences, Biomed. Series, 2017, 43, 5-6, 215-224 (co-authors: M. Dekanosidze, Kh. Saganelidze, N. Mitagvaria) (in Georgian).

4. The role of nitric oxide in the changes of blood vessels acetylcholine induced dilation in the conditions of hyperhomocysteinemia. International Journal of Research (IJR), 2018, 5, ISSN: 2348-6848, #44396, 2035-2040 (co-authors: N. Doreulee, N. Mitagvaria.
5. Changes in arteriole reactivity to noradrenaline under conditions of hyperhomocysteinemia. Georgian Medical News, 2019, 7-8, 292-293, 92-95 (co-authors: N Doreulee, M. Mitagvaria).
6. A possible mechanism of the changes in arteriole contractility under conditions of hyperhomocysteinemia. Proc. of the Georgian National Academy of Sciences, Biomedical Series, International Congress of Georgian Ivane Beritashvili Society of Physiologists Proceedings, 2019, 45, 3-4, 453-455 (co-author N. Doreulee).