ISSN No:-2456-2165

Functioning of Mechanisms Regulating the Microcirculation in Some Tissues of Oral Cavity (Experimental Study)

[1]Dekanosidze M., [2]Saganelidze Kh., [3]Mitagvaria N.
[1]Doctoral Student, New Vision University, Tbilisi Georgia
[2]Professor in Medical Rehabilitation, New Vision University, Tbilisi Georgia
[3]Head, Department of Cerebral Circulation and Metabolism,
I.Beritashvili Center for Experimental Biomedicine, Tbilisi, Georgia

Abstract:- The identification of the role of Nitric Oxide (NO) in the regulation of the vascular tone caused a partial integration of the Neurogenic and Metabolic theories of this regulation.

A similar significant role was played by the establishment of a Calcitonin Gene-Related Peptide (CGRP) role in the contractile activity of the smooth muscle cells in a number of organs.

Based on the own in-vitro experimental studies, the possible interactions of these two factors in regulation of microcirculation in the tissues of the oral cavity (in the bed of rabbit lingual artery) are described in this paper.

Keywords:- *NO, CGRP, DMSO, microcirculation, lingual artery, vascular contractility, free oxygen radicals, rabbit.*

I. INTRODUCTION

Neurogenic vasodilation in the cardiovascular system of vertebrates is mainly due to Nitric Oxide (NO) [5]. Study of the nature of neurogenic dilatation of the lingual artery [Arteria Lingualis] – one of the main arteries of the oral cavity (feeding blood to tongue and gums with numerous rami) has demonstrated that it has a two-component character: the first component is atropin sensitive, i.e. of cholinergic nature, and another one is non-cholinergic. Such a mixed dilatation mechanism is not only characteristic of the lingual artery, but it also has been described for arteries of various tissues [18].

Calcitonin Gene-Related Peptide (CGRP) consists of 37 amino acids. It is widely represented in both central and peripheral nervous systems, including along the vascular system in nervous fibers. Along with Nitric Oxide (NO), it is considered as the main component of the neurogenic vasodilation mechanism [3]. The action of both of the above vasodilators in the body, as it turns out, has a sharply expressed regional character. In this respect, the lesser known is the relaxation mechanism of the lingual artery. Particularly, whether the Calcitonin Gene-Related Peptide (CGRP), or NO are involved in functioning of this mechanism, and if so, how is this participation expressed.

It is known that disruption of normal functioning of the vascular relaxation mechanism, i.e. violation of organic blood circulation regulation in a number of pathological conditions is caused by a large number of free oxygen radicals, but their role in the violation of oral cavity blood supply regulation is practically not studied [12].

II. GOALS AND OBJECTIVES OF THE STUDY

Based on the above, the goals of the presented study were as follows:

- Find out the role of Calcitonin Gene-Related Peptide in the functioning of vasodilating mechanism of lingual artery;
- Find out the role of Nitric Oxide in the functioning of vasodilating mechanism of lingual artery;
- Find out – whether free oxygen radicals violate the functioning of vasodilating mechanism of lingual artery and if it is so – what is the mechanism of this violation.

To achieve the above goals, it was needed to accomplish the following specific objectives:

- Selection of such methodological approach for the study that would provide objective and unequivocal data.
- Study of the response of the endothelium removed arcuate segment preparations of rabbit lingual artery to the electrical stimulation of different frequency (amplitude – 10V, frequency – 4, 8 and 16Hz, series of 2 msec rectangular pulses of 45 seconds duration) under the conditions of the tone activated by norepinephrine and without it.
- Determination of the effect of atropin, propranolol and nitro L-Arginine Methyl Ester (L-NAME) on the relaxation caused by electric stimulation of the lingual artery arcuate segment of the deendothelized and intact endothelium preparations under the norepinephrine activated smooth muscles tone conditions.
- Effect of Calcutonin Gene Related Peptide (CGRP) and its receptors antagonist (CGRP – 8-37) on the relaxation caused by electric stimulation of lingual artery arcuate segment.
- Effect of the Calcitonin Gene-Related Peptide receptors antagonist (CGRP – 8-37) on the relaxation of the lingual artery arcuate segment caused by Calcitonin Gene-Related Peptide (10^{-8}M).
- Effect of the Calcitonin Gene-Related Peptide receptors antagonist of the (CGRP – 8-37) on the vasodilation of the lingual artery arcuate segment caused by isoproterenol.

ISSN No:-2456-2165

- Effect of preliminary, 30-minute impact of Capsaicin on the relaxation caused by electric stimulation of the lingval artery arcuate segment.
- Effect of free oxygen radicals and their scavenger Dimethylsulfoxide on the relaxation response of the lingval artery preparation caused by Calcitonin Gene-Related Peptide.
- Influence of free oxygen radicals on the Nitroglycerin induced relaxation reaction of the lingual artery preparation.

III. OBJECT OF THE STUDY

One of the objective methods for the analysis of blood vessel smooth muscles appeared to be the measurement of constriction parameters of blood vessels isolated preparations by means of mechanotronic converters [1]. This method makes it possible to measure a degree of increase or decrease in the tone of blood vessels in conditions of different impacts. As a result of such a methodical approach it is possible to analyze some mechanisms of smooth muscle regulation without the interference of centrogenic neurohumoral signals in it. It also gives the experimenter the inexhaustible means for studying the effect of sequential or combined action of various biologically active substances on smooth muscle reactivity.

The tests were conducted on isolated preparations of the 3,5-4 kg chinchilla rabbit lingual artery.

After the anesthesia of animals with sodium ethanol (40 mg/kg) their euthanasia was performed using rapid bloodletting.

Lingual artery, which is the largest collateral ramus of the external carotid artery, was separated under the binocular magnifier. The artery was divided into several arcuate segments (length of about 1.5 mm). All the segments were immediately placed into the cooled Ringer-Krebs solution. If necessary, the material can be stored in the refrigerator for 24 hours at the temperature of $+5C^0$.

Then the preparation (segment) was placed on two small hooks of tensometric mechanotron (Fig. 1). One hook was rigidly fixed to the rod of 6 MXIC type mechanotron**.** The preparation was stretched and a constant stretching value was chosen according to the results of testing of arterial smooth muscle compression. The testing was performed using standard solutions, containing potassium in the concentration of 80mol and was an average of 5.1mN. Before the measurement for reaching equilibrium condition the preparation stayed in Ringer Solution for 1.5 hours at the temperature of 37ºC.

The electric signal received from mechanotron was transmitted to amplifiers. The calibration of mechanotron was performed in milli Newtons (mN). For this the horizontal rod was loaded by standard small weights and the deviation from an initial level was recorded. The value of preparation stretch is usually normalized according to the maximum contractile response (100%) of the preparation to the action of hyper potassium (800 mMol/L) solution.

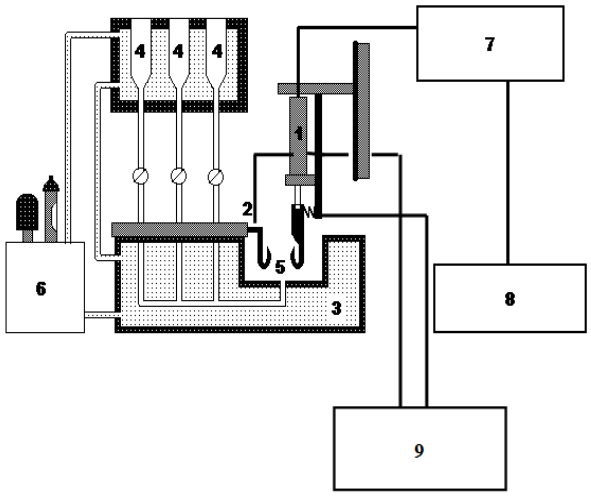

Fig 1:- The block-scheme of the device: 1 – mechanotron; 2 – the mechanism of stretching and calibration; 3 – thermostated chamber; 4 – the flasks with Krebs solution; 5 – the working chamber; 6 – ultrathermostat; 7 – the block of amplifiers; 8 – recorder; 9 – electric stimulator**.**

IV. THE PREPARATION OF SOLUTIONS, CONTROL OF PH AND TEMPERATURE

The Ringer-Heilit flow solution was used as a nutrient solution, the content of which was the following (mmol/L): NaCl – 118.0; KCl – 4.7; $NaHCO_3$ – 14.9; KH_2PO_4 – 1.18; $MgSO_4.7H_20$ – 1.17; $CaCl_2;2H_2O$ – 2.5; Glucose – 11.0.

The experiments were carried out under pH control, the measurement of which was directly performed before each impact by means of pH-meter (or ionometer). Solution pH change was allowed within 7.35-7.45.

During the experiment the permanence of solution temperature was performed by means of ultrathermostat at 37±0.5ºC level, which pumped the warmed water into special flasks with water and temperature-controlled chamber, which were united in a common, continuous, flowing system (Fig. 1).

A. Impacts used

For the analysis of the arteries contractility, the study solutions of pharmaceutical substances and metabolites should be prepared directly before each test and added into the Ringer-Krebs nutrient solution. Duration of impact and concentration of the used substance is selected by a researcher as needed. Substances are introduced to the bath working chamber with 15-30 minutes interval. This allows us to study the metabolic regulation factors action on the isolated vascular preparations and determine their value in comparison with other regulatory factors.

ISSN No:-2456-2165

Specifically, the following substances were used in our experiments:

In order to suppress neurogenic vasoconstriction, we added adrenergic receptors blocker - guanetidin ($5x10^{-6}$M) into the bath, and to increase the initial tone of the blood vessel preparation we used norepinephrine (10^{-5}M), after introduction of which we tested deendonthelization by lack of relaxation response to acetylcholine (10^{-5}M).

The maximal relaxation level of preparation is determined by introduction of papaverine ($2x10^{-4}$ M).

Preliminary measurements have shown that relaxation caused by electric stimulation of the preparations under the action of guaneteidin and activated tone with norepinephrine, is performed in sustainable way and lasts for approximately 5 hours.

B. Hydroxyl radicals generating system

It is known that free oxygen radicals after H_2O_2 dismutation form hydroxyl radicals (HO-), which are strong oxidants, as the experimental data show, cause long-term vasodilation and vascular oxidative damage [3].

For the purpose of generating of hydroxyl radicals, the so-called Fenton reaction (H_2O_2 and iron salts mixture gives HO^-, which was first described by Fenton – see [7] was used:

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + HO^- + OH^-$$

Specifically, $3x10^{-4}$M H_2O_2 and $2x10^{-4}$M $FeSO_4$ were used. It is known that immediately after adding $H_2O_2/FeSO_4$ into the solution, generation of hydroxyl radicals starts with the maximum intensity, which lasts for about 40 minutes [21].

C. Protocol of experiments

In the first series of the experiments, the reaction of the endothelium-removed preparations of lingual artery on electric stimulation was studied.

Response of the endothelium removed arcuate segment preparations of rabbit lingual artery to the electrical stimulation (amplitude – 10V, frequency – 4, 8 and 16Hz, 2 msec rectangular pulses of 45 seconds duration) was studied under the conditions of the tone activated by norepinephrine (10^{-6} M) and without it.

From the perspective of the quality control of deendothelization, we used exposure to acetylcholine (10^{-5} M) before electric stimulation.

In this series of experiments, there were 12 experiments, out of these – 6 on the intact endothelium preparations and 6 – on endothelium-removed preparations.

The second series of the experiments was performed in order to determine the effect on the relaxation induced by electric stimulation of the lingual artery arcuate segment of arthropin, propranolol and nitro L-Arginin Methyl Ester (L-NAME). for the preparations that demonstrated sustainable increase of the tone after exposure to norepinephrine, 10^{-5} M concentration of atropin (6 intact and 6 deendothelized preparations) or 10^{-5} M concentration of propranolol (6 intact and 6 deendothelized preparations), or 10^{-4} M L-NAME (6 intact and 6 deendothelized preparations) were added to the bath with the Ringer-Krebs solution.

In the next, third series of the experiments, we tried to find out the effect of Calcitonin Gene-Related Peptide(CGRP) and its receptors antagonist (CGRP-8-37) on the relaxation of the preparations caused by electric stimulation of the lingual artery arcuate segment (6 experiments) and by Calcitonin Gene-Related Peptide(10^{-8} M) and isoproterenol (10^{-6} M) (6 and 6 experiments respectively). CGRP – (8-37) in the concentration of $2x10^{-8}$ M was added to the Ringer-Krebs solution 30 minutes prior to the introduction of norepinephrine.

In the fourth series of the experiments the effect of preliminary 30-minutes processing of the preparations with Capsaicin (10^{-6} M) on the relaxation resulted of the electric stimulation of the lingual artery ararcuate segment was studied. In this case as well 6 experiments were performed.

The fifth series of experiments were devoted to studying the suppressive role of hydroxyl radicals on the preparation relaxation. We have used such a powerful scavenger of HO^- as Dimethylsulfoxide (DSMO). Effect of this agent and the Fenton reaction (and its components) on the relaxation response of the endothelium removed arcuate segment of rabbit lingual artery induced by electrical stimulation of different frequency were measured (6 experiments for each case, total 60 experiments, out of these – 30 controls).

The last sixth series of the experiments were carried out again on the 6 preparations, on which the effect of Fenton reaction, i.e. hydroxyl radicals, on the relaxation of the rabbit lingual artery arcuate preparations caused by nitroglycerin (10^{-5} M) was measured.

D. Statistical processing of data and scope of the study

Obtained data on the level of smooth muscle tension was shown by mean values and standard errors. In the action of relaxation factors we received as the initial (100%) value maximum relaxation level caused by papaverine ($2x10^{-4}$ M).

Quantitative data received at a total of 138 experiments were evaluated using the Student's t-test for paired and not paired data. The difference was considered as statistically reliable incase of $P < 0.05$.

V. RESULTS

Reaction of the endothelium-removed preparations of lingual artery to electrical stimulation

Response of the endothelium removed arcuate segment preparations of rabbit lingual artery to the electrical stimulation (amplitude – 10V, frequency – 4, 8 and 16Hz,

ISSN No:-2456-2165

duration – 45 seconds) was studied under the conditions of the tone activated by norepinephrine (10^{-6} M) and without it.

Exsposure to guanetidin ($5x10^{-5}$M) was used for the preparations in which response by contraction to the electrical stimulation was confirmed, and after that (with a 20-minute delay) the electrical stimulation was repeated. The next procedure provided for the removal of guanetidin from the solution and the 30-minute stay of the preparation in the normal (without any additives) Ringer-Krebs solution for the restoration of initial conditions.

Electric stimulation was still used in these preparations under the conditions with norepinephrine induced active tone and without it. From the perspective of the quality control of deendothelization, we used exposure to acetylcholine (10^{-5} M) before electric stimulation.

As is evident from the obtained results, after exposure to guanetidin, the preparations, despite of washing and staying in the normal solution for 30 minutes, they do not respond to electrical stimulation if their tone is not pre-activated with norepinephrine, while in the latter case, the preparation reacts with the relaxation and the amplitude of the relaxation depends on the frequency of electrical stimulation.

Effect of atropin, propranolol and Nitro L-Arginine Methyl Ester (L-NAME) on the relaxation of the lingual artery arcuate segment caused by electrical stimulation

The preparations that had sustainable increase in the tone after exposure to norepinephrine were exposed to atropin (10^{-5}M) or propranolol (10^{-5}M) for 10 minutes before electric stimulation. Stimulation with 4-16 Hz frequency demonstrated atropin and propranolol-resistant relaxation both in endothelium-inactic and endothelium-removed preparations. The resulting relaxation effect expressed in the percentage of papaverin ($2x10^{-4}$ M) induced maximum relaxation is given in the following Table 1. The test exposure to papaverine was performed at the end of the experiment. As this data shows, the relaxation reaction of the lingual artery preparations caused by the electrical stimulation is endothelium independent. Furthermore, there was no effect of the use of L-NAME (10^{-4} M) on the response of the deendonthelized preparations to electrical stimulation, but in the case of intact endothelium, L-NAME in the same dose causes the reaction blocking in the preparations which react to acetylcholine by substantial relaxation.

Electric stimulation frequency (Hz)	Endothelium-intact preparation		Endothelium-removed preparation		
	Impact (%)		Impact (%)		
	Control	Atropin	Control	Capsaicin	Propranolol
4	45.4±6.2	45.5±8.5	46.6±7.2	9.5±3.3	44.2±6.2
8	53.5±8.2	57.6±4.2	57.7±6.6	17.5±6.6	56.6±5.6
16	70.1±4.3	70.6±6.6	71.3±7.2	17.3±9.5	71.2±4.4

Table 1. Relaxation reaction of endothelium intact and endothelium removed arcuate segments preparations of the rabbit lingual artery (in the percentage of maximum relaxation caused by papaverine) during electrical stimulation at different frequencies against background of atropin, capsaicin and propranolol

Calcitonin Gene-Related Peptide (CGRP) and its receptor antagonist (CGRP – 8-37) effect on the relaxation of the lingual artery arcuate segment caused by electrical stimulation

In the next series of experiments, after confirming the effect of guanetidine on the deendothelized preparations of the arcuate segment of the lingual artery, we studied the effect of the receptor antagonist (CGRP-8-37) of the Calcitonin Gene-Related Peptide (CGRP) on the relaxation of the preparations caused by electrical stimulation and by the Calcitonin Gene-Related Peptideitself. CGRP-8-37 with the concentration of $2x10^{-8}$M was added into the Ringer-Krebs solution 30 minutes before introduction of norepinephrine.

The obtained results clearly indicate that relaxation of the lingual artery preparation caused by the electrical stimulation should be conditioned by the effect of the CGRP. Additional evidence to this assumption was obtained in the tests, which clearly demonstrated that, the first: more than 90% relaxation is achieved by CGRP (10^{-8} M) and the second: it is strongly suppressed if this peptide receptors antagonist (with the indicated concentration) is added to the Ringer-Krebs solution before introduction of norepinephrine. Thus CGRP-8-37 suppresses not only the neurogenic relaxation induced by electric stimulation, but also relaxation caused by exogenous supplementation of CGRP in the endothelium removed arcuate segments of the rabbit lingual artery. At the

ISSN No:-2456-2165

same time, the mentioned preparation does not cause any change in the relaxative responses caused by isoproterenol (10^{-6} M).

In order to further specify the nature of the relaxation received during electrical stimulation of the arcuate segment of the lingual artery, we used preliminary treatment of these preparations with capsaicin (10^{-6}M) for 30 minutes. As the data provided in the Table 1 shows, as a result of this relaxation caused by electrical stimulation has significantly decreased. It is clear that suppression of the reaction to the electrical stimulation was caused by exhaustion of CGRP by capsaicin.

Impact of free radicals on the relaxation of the arcuate segment of lingual artery caused by electrical stimulation

It is known that the main effect of free oxygen radicals is vasodilation, but they can also cause vasoconstriction. It was previously shown that the endothelium damage caused by hydroxyl radicals suppresses the endothelium-dependent relaxation factor production as well as its effectiveness. It is clear that the excess production of free radicals breaks the vasodilatative function, which makes a favorable condition for the increase of vasoconstrictive stimulus effectiveness. Free radicals generated in the wall of the blood vessel can cause both immediate impact on smooth muscle cells and affect the biologically active endogenous vasoactive mediators forming in endothelial cells.

Based on the above, we studied the possible effect of free radicals on the relaxation reaction of lingual artery caused by CGRP, for which the well-known Fenton reaction was used.

It was found out that the relaxation of the arcuate segment of the lingual artery caused by the electrical stimulation in the conditions of sustainable tone caused by norepinephrine is completely suppressed if we act on the preparation by $H_2O_2/FeSO_4$ in advance. In order to determine the role of hydroxyl radicals in the formation of this suppressive effect we have used such a powerful scavenger of HO^- as Dimethylsulfoxide (DSMO). Effect of this agent and the Fenton reaction (or its components) on the relaxation response of the endothelium removed arcuate segment of rabbit lingual artery under electrical stimulation of different frequency is given in the Table 2. As the data recorded in this Table shows, DMSO effectively protects the preparation relaxation reaction from the suppressive effect of $H_2O_2/FeSO_4$, which indicates that hydroxyl radicals generated as a result of the Fenton reaction are involved in the inhibition of the blood vessel preparation reaction.

We have already mentioned suppression of the relaxation reaction of the lingual artery arcuate segments caused by capsaicin under the conditions of electrical stimulation. In this series of experiments, we have repeated exposure to capsaicin, which replaced the components of the Fenton reaction this time. If we compare the results obtained, there is huge similarity between the effects of capsaicin and hydroxyl radicals. The latter allows us to think that hydroxyl radicals can damage capsaicin-sensitive nerves and by this way act on the relaxation response of the lingual artery preparation caused by the Calcitonin Gene-Related Peptide.

Impact of free radicals on the relaxation of the arcuate segment of lingual artery caused by the Calcitonin Gene-Related Peptide

In the following series of experiments, we tried to find out what is the effect of hydroxyl radicals on the exogenous CGRP-induced relaxation reaction of the rabbit lingual artery arcuate segment. It was found that preparation with the tone activated by norepinephrine undergoes significant relaxation after the introduction of Calcitonin Gene-Related Peptide (CGRP), and after $H_2O_2/FeSO_4$ impact the preparation's response on the CGRP introduced with the same dose has been significantly suppressed. Inhibition of relaxation response of similar type and more pronounced was received in the case of addition of CGRP-receptors antagonist CGRP (8-37) into the Ringer-Krebs solution 30 minutes before the introduction of norepinephrine.

Hydroxyl radicals' inhibitory effect on the relaxation reaction caused by the Calcitonin Gene-Related Peptide is significantly blocked in the case of use of free oxygen radicals' scavenger Dimethylsulfoxide (DMSO). Results of such a series of experiments are presented in the Table 3.

Ringer-Krebs solution additive	Dose	Relaxation (in the percentage of maximum relaxation caused by papaverine ($2x10^{-4}$ M))					
		4Hz		8Hz		16Hz	
		Control	After impact	Control	After impact	Control	After impact
-	-	37.5±6.5	30.6±5.2	52.6±8.6	45.9±6.6	81.2±8.3	73.5±7.8
H_2O_2	$3x10^{-4}$ M	25.9±9.2	26.8±7.3	41.3±7.9	41.2±8.2	74.4±6.5	70.2±8.6
$FeSO_4$	$2x10^{-4}$ M	28.5±7.8	29.0±3.9	50.7±5.9	46.6±4.4	84.4±8.9	71.6±5.1
H_2O_2+$FeSO_4$		38.2±7.5	8.7±3.1	56.0±9.9	14.5±5.2	82.8±9.1	20.1±3.5
DMSO	100mM	27.8±8.2	28.4±7.6	46.7±9.5	47.3±9.5	75.4±8.5	72.1±8.8

Table 2. Influence of Fenton reaction and oxygen free radicals scavenger impact on the relaxation reaction of the rabbit lingual artery endothelium-removed arcuate segments, caused by electric stimulation of different frequencies

In the next series of experiments (6 experiments) the effect of hydroxyl radicals on the relaxation of the endothelium-removed arcuate segments of the rabbit lingual artery with the norepinephrine activated tone caused by nitroglycerin was tested. It is known that neurovasodilators act on sensory fibers and release CGRP, which then reaches the vascular smooth muscle through the diffusion, activates the soluble guanylate cyclase and leads to the vascular dilation [19]. The existence of such type of mechanism was examined by us on the rabbit lingual artery preparation. Relaxation caused by nitroglycerin (10^{-5} M) remained unchanged, although the CGRP resource was reduced by preliminary exposure to hydroxyl radicals that should indicate that free radicals should not violate the functioning of the dilation mechanism of the lingual artery dependent on the guanylate cyclase.

Ringer-Krebs solution additive	Dose	CGRP (10^{-8} M) induced relaxation (in the percentage of maximum relaxation caused by papaverine ($2x10^{-4}$ M))	
		Control (%)	Experimental impact (%)
-	-	90.7 ± 8.2	92.3 ± 4.4
H_2O_2	$3x10^{-4}$ M	93.1 ± 6.1	89.7 ± 5.4
$FeSO_4$	$2x10^{-4}$ M	82.3 ± 5.2	93.2 ± 6.1
H_2O_2+$FeSO_4$		80.1 ± 7.8	19.4 ± 3.9
DMSO	100mM	84.2 ± 9.9	81.8 ± 9.5

Table 3. Influence of Fenton reaction and oxygen free radicals scavenger impact on the relaxation reaction of the rabbit lingual artery endothelium-removed arcuate segments, caused by Calcitonin Gene-Related Peptide

ISSN No:-2456-2165

VI. DISCUSSION

Analysis of the obtained results shows that CGRP leads to a significant relaxation of the rabbit lingual artery and that it should be conditioned by activation of CGRP receptors. However, it should be noted that although it is recognized that Nitric Oxide and related components play an important role in transmitting information from the nervous system to vascular smooth muscles, as primary messengers [18], in the case of the rabbit lingual artery our results do not confirm this judgement and, as it was already mentioned, CGRP-receptors activation in its smooth muscles should be considered as the maim mechanism of relaxation in the case of this vessel. In addition, it is quite possible that the functioning of CGRP induced mechanism of relaxation of the rabbit lingual artery at least partially is due to the activation of ATP-sensitive K^+ channels. The following enables to make such a conclusion:

1. Electric stimulation under the conditions of norepinephrine-induced tone (against the background of guanethidine, which suppresses smooth muscle contraction) causes the relaxation of the deendonthelized preparation.
2. This relaxation is endothelium independent and does not change by action of neither propranolol (β-adreno-receptors antagonist) nor atropine (muscarinic cholinergic receptors antagonist) in the concentrations that suppressed the acetylcholine relaxative action but did not prevent the relaxation reaction of the endothelium-intact preparation on the electric stimulation.
3. L-NAME, non-selective inhibitor of the Nitric Oxide synthases in the concentrations that suppress the relaxation reaction of the deendothelized preparation on acetylcholine, can not have any effect on the relaxation of the same preparations caused by the electrical stimulation.
4. CGRP-receptors antagonist CGRP (8-37) in the concentration that almost entirely suppresses the action of the exogenous CGRP, inhibites the relaxation caused by the electrical stimulation of the lingual artery deendothelized preparations, and finally
5. Exhaustion of endogenous CGRP from perivascular nerves by capsaicine selectively suppressed neurogenic relaxation.

CGRP (8-37) was originally used as a possible antagonist of CGRP-receptors [13]. It suppressed the CGRP-induced contractual reactions of atrium in guinea pigs and relaxation of mesenterial artery. The latter work also shows that there may be two types CGRP-receptors: $CGRP_1$ and $CGRP_2$. Out of these the $CGRP_1$-receptors are sensitive to the CGRP (8-37), and the $CGRP_2$-receptors – are not.

In the experiments conducted by us, CGRP (8-37) almost completely suppressed relaxation of the lingual artery in the case of its electrical stimulation as well as in the case of addition of exogenous CGRP. Therefore, the responses of the rabbit lingual artery under the condition of electrical stimulation of its nervous elements should be conditioned by $CGRP_1$-receptors. As for the residual reaction that we have received as a result of the CGRP (8-37) inhibition, it should probably be caused by a relatively low concentration of inhibitor ($2x10^{-8}$ M), which does not give 100% inhibition guarantee or may reflect activity of small number of $CGRP_2$-receptors (which are not inhibited by the inhibitor used by us).

There is a substantial number of evidence that ATP-sensitive K^+ channels should play an important role in vasorelaxation [14]. Many researchers argue that the relaxation effect of K^+ channel opening (eg, cromacaline and pinacidil) is balanced by the increase in potassium extracellular concentrations [2]. It is shown that CGRP activates the adenylat cyclase and increases cyclical AMP in the aortic smooth muscles of the rat. Possible involvement of a cyclic AMP-dependent mechanism in the process of activation of ATP-sensitive K^+ channels in smooth muscles is also confirmed by the fact that activation of ATP-sensitive K^+ channels with cyclic AMP has been shown in the myocytes [15]. Thus, we believe that it is quite possible that activation of the ATP-sensitive K^+ channels is involved in the relaxation of the rabbit lingual artery induced by CGRP.

Wei et al. [19] show that cyclic guanosine monophosphate dependent mechanism is involved in the CGRP-induced dilatation of cerebral arterioles of felines. According to our data, in the case of the rabbit lingual artery it turned out that relaxation caused by the electric stimulation is of endothelium-independent nature, and L-NAME did not have any effect on the neurogenic relaxation of the lingual artery. Thus, neurogenic vasodilation in our case does not depend on the generation of Nitric Oxide, which in turn relates to cyclic guanosine monophosphate generation.

Since the relaxation of the lingual artery caused by electrical stimulation is significantly suppressed by capsaicin sensory neurotoxin, which exhausts CGRP in the nerves [17], we should assume that CGRP is in the sensory nerve fibers of the lingual artery and that as a result electrical stimulation, the release of CGRP causes vasodilatation. It is known that activation of the peripheral terminal of the sensory nerve causes antidromatic vasodilation via the local axon-reflex mechanism [8]. Arterial vascular reactivity to active substances, including peripheral neurotransmitters of sensory nerves, probably should be significant from the view of the regulation of tongue blood circulation.

Analysis of a series of experiments carried out by the Fenton's reaction allows us to conclude that oxygen free radicals act on the presynaptic as well as the postsynaptic level of the CGRP-ergic synapses. The following provides us the basis for it:

1. The CGRP-conditioned relaxation of the lingual artery preparations in response to the electrical stimulation was almost entirely inhibited as a result of $H_2O_2/FeSO_4$ action on the preparations. It indicates that hydroxyl

ISSN No:-2456-2165

radicals are capable to eliminate the presynapticaly localized endogenous CGRP.
2. As a result of Fenton's reaction preliminary impact CGRP-induced relaxation, suppressable by CGRP-receptors antagonist is reduced, which indicates that hydroxyl radicals damage CGRP-induced relaxation, which in turn depends on the activation of ATP-sensitive K^+ channels on the postsynapse site.
3. As a result of the use of free radicals scavenger stoppage of the inhibition of relaxation caused by electrical stimulation (which was conditioned by the impact of Fenton reaction) is received. The same result was obtained in case of the free radicals suppression of the exogenous CGRP-induced relaxation – here as well the free radicals scavenger DMSO almost completely restored this mentioned relaxation reaction.

This effect of dimethylsulfoxide (DMSO) indicates that we deal with the hydroxyl radicals, it could be possibility of H_2O_2, which is the hydroxyl radicals precursor (it is not excluded that the result obtained by the Fenton reaction was caused by hydrogen peroxide. It is known that DMSO is only hydroxyl radical's selective scavenger and its protective effect when using Fenton reaction in order to inhibit relaxation of the lingual artery, clearly indicates that HO and not H_2O_2 is an active agent in our case. Based on the above mentioned about the CGRP-receptors, probably, it would be logical to reiterate that in the case of the rabbit lingual artery we deal mainly with the $CGRP_1$ receptors and hydroxyl radicals should cause the dysfunction of these receptors. It should be noted here that the mechanism by which hydroxyl radicals simultaneously carry out dysfunction of CGRP-receptors and inactivation of ATP-sensitive K^+ channels is still unclear. Kukreja et al. [10] showed that action of the xanthine-xanthine oxidase generated oxygen free radicals on the dog cardiac sarcolemma vesicles causes muscarinic receptors ligand significant depression and expressed the opinion that there should be more change in the number of receptors, or receptor protein structure change and less – change in the receptors existence. This opinion leads to the assumption that the hydroxyl radicals target may be structural and functional unity of proteins. As early as in 1981, Katz and Messineo [6] showed that change of lipid microenvironment of the cell membrane has a greater effect on the cell functioning. Moreover, free oxygen radicals easily oxidize part of the membrane lipid polyunsaturated fatty acids. Thus, the hydroxyl radicals may act on the following:

1. On the membrane lipids, the peroxidation of which may secondarily cause modification of the membrane protein [11];
2. Only on those lipids that change the protein microenvironment or both on the proteins and membrane lipids separately and simultaneously [6].

The nature of the secondary messenger system of vasorelaxation conditioned by CGRP is not known. Wei et al. [19] considered that cyclic guanosine monophosphate dependent mechanism is involved in the CGRP-induced dilatation of the cerebral arterioles. Of course, it is of great interest to determine the mediator importance of the guanylate cyclase on the vascular smooth muscle cells in the action of CGRP. The main essence of the action of nitrodilators is that they act directly on a vascular smooth muscle and generate Nitric Oxide either spontaneously or by interaction with tissue components [4]. This substance further activates soluble guanylate cyclase either by direct action, or by the formation of intermediate nitrosothiol. This results in increase of cyclic guanosine monophosphate and protein kinase dependent on it, with further resulting relaxation of smooth muscles. If we share the opinion that violation of the Calcitonin Gene-Related Peptide-induced lingual artery relaxation mechanism by impact of the hydroxyl radicals means decrease of cyclic guanosine monophosphate formation by inhibiting of guanylate cyclase, then it should be also possible to suppress nitroglycerine induced relaxation of the same preparation as a result of the impact of hydroxyl radicals. But as our results show, such action of hydroxyl radicals does not actually have a place. This emphasizes the fact that its effect is not determined by the area of generation of guanosine monophosphate in the receptors and smooth muscle cells. The point is that sensitivity of the process of production of guanosine monophosphate in the smooth muscles conditioned by nitroglycerin to hydroxyl radicals (i.e. to lipid peroxidation) can be significantly different from the relaxation of the rabbit lingual artery that is induced by activation of the $CGRP_1$-receptors and opening of ATP sensitive K^+ channels. It is quite possible that hydroxyl radicals damage the postsynapse membrane in the CGRP-ergic synapses and not the secondary messenger system in connection with the formation of cyclic guanosine monophosphate in the smooth stomach cells. Residual vasodilation, which has been observed in our results, should be due to other mechanisms, may be associated with cyclical adenosine monophosphate related mechanisms. This is strengthened by the fact that cyclical adenosine monophosphate (AMP) in myocytes activates AMP-sensitive K+ channels [15]. Kubota and others [9] showed that in the rat aorta smooth muscle culture CGRP stimulates formation of cyclic AMP and not GMP (guanosine monophosphate). Although these results highlight the cyclical AMP's substantial role in vascular smooth muscles response to the Calcitonin Gene-Related Peptideaction, they do not claim that cyclic AMP is the one that conditions vasodilating action.

A number of mechanisms are known to help free oxygen radicals in dysfunction in blood vessels, skeletal muscles and myocardin (Okabe et al., 1991). Free radicals can influence the normal control of the vascular tone through the chemical destruction of endogenous vasoactive agents (catecholamines, nitric oxide) [20]; the powerful reactive hydroxyl radical is a significant mediator from the perspective of tissue damage. Tissue damage leads to activation of nonmyelinated C fibers release of vasoactive agents (maybe CGRP).

Under the long-term effect of hydroxyl radicals, endogenous CGRP, localized in the presynaptic site of CGRP-ergic nervous fibers of the lingual artery is dissolved

or permanently released with the result of final devastation. Despite the concrete mechanism, the results obtained by us show that hydroxyl radicals are the same strong toxic agent to the CGRP nervous fibers, as capsaicin. In our study was not studied direct inactivation of CGRP by hydroxyl radicals, as both the addition of exogenous CGRP and electrical stimulation, which leads to the release of CGRP, was performed only after stopping of Fenton reaction and washing of the preparation environment. Our results show just that the hydroxyl radicals can exhaust presynaptically located endogenous CGRP and damage CGRP-induced relaxation of the rabbit lingual artery preparation that is carried out by the activation of ATP-sensitive K^+ channels on the postsynaptic site and that the secondary messenger system of relaxation, conditioned even by cyclic guanosine monophosphate, is less sensitive to the destructive action of free oxygen radicals.

VII. CONCLUSION

1. Electric stimulation of the rabbit lingual artery arcuate preparation under the conditions of norepinephrine induced tone, against the background of adrenergic blocker action, causes the relaxation of the deendonthelized preparation.

2. This relaxation is endothelium independent and does not change by action of neither β-adreno-receptors antagonist nor muscarinic cholinergic antagonist in the concentrations that suppress the acetylcholine relaxation action.

3. L-NAME, non-selective inhibitor of the Nitric Oxide synthases in the concentrations that suppress the relaxation reaction of the deendothelized preparation on acetylcholine, cannot have any effect on the relaxation of the same preparation caused by the electrical stimulation.

4. CGRP-receptors antagonist CGRP (8-37) in the concentration that almost entirely suppresses the action of the exogenous CGRP, inhibites the relaxation caused by the electrical stimulation of the lingual artery deendothelized preparations.

5. Exhaustion of endogenous CGRP from perivascular nerves by capsaicine selectively suppresses neurogenic vasorelaxation.

6. Relaxational responses of the the rabbit lingual artery under the electrical stimulation of its nervous elements shall be conditioned by the $CGRP_1$-receptors.

7. Hydroxyl radicals have capability to exhaust endogenous, presynaptically localized CGRP that damages the relaxation mechanism of rabbit lingual artery preparation.

REFERENCES

[1]. Berlin, G. S., Petrov A. G., Kharkevich D. A., and Shorr V. A., “On the possibility of using mechanotron transducers in experimental biological studies”, *Bull. Experiment. Biol. and Medicine*, 88, 11, 1979, pp. 626-629 (in Russian).

[2]. Chiba T., Yamaguchi A., Yamatani T., Nakamura A., Morishita T., Inui T., Fukuse M., Noda T., Fujita T. Calcitonin gen-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance veselsof the rat. Nature, 1988, 335, 164-167.

[3]. Dieterle Anne, Michael JM Fischer, Andrea S Link, Winfried L Neuhuber and Karl Messlinger. Increase in CGRP- and nNOS-immunoreactive neurons in the rat trigeminal ganglion after infusion of an NO donor. International Headache Society 2011. DOI: 10.1177/0333102410375725].

[4]. Ignarro LJ. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol. 1990; 30: 535-60.

[5]. Jennings BL, Donald JA. Mechanisms of nitric oxide-mediated, neurogenic vasodilation in mesenteric resistance arteries of toad Bufo marinus. Am J Physiol Regul Integr Comp Physiol. 2010; 298 (3): R767-75.

[6]. Katz AM, FC Messineo. Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium.- Circulation Research, 1981. *48*: *1-16.*

[7]. Kremer M. The Fenton Reaction. Dependence of the Rate on pH. *J. Phys. Chem. A*, 2003, 107 (11), pp 1734–1741.

[8]. Krootila K. CGRP in relation to neurogenic inflammation and cAMP in the rabbit eye. Experimental Eye Research. 47, 2, 1988, 307-316.

[9]. Kubota M, J.M.Moseley L. Butera G.J. Dusting P.S.MacDonald T.J.Martin. Calcitonin gene-related peptide stimulates cyclic AMP formation in rat aortic smooth muscle cells. Biochemical and Biophysical Research Communications, Volume 132, Issue 1, 15, 1985, Pages 88-94.

[10]. Kukreja R.C., Eiichiro Okabe, Gayle M.Schrier, Michael L.Hess. Oxygen radical-mediated lipid peroxidation and inhibition of Ca^{2+}-ATPase activity of cardiac sarcoplasmic reticulumArchives of Biochemistry and Biophysics Volume 261, Issue 2, March 1988, Pages 447-457.

[11]. Lee C., Okabe E. Hydroxyl radical-mediated reduction of Ca2+-ATPase activity of masseter muscle sarcoplasmic reticulum. Jpn. J. Pharmacol, 1995, 67, 21-28.

[12]. Lobo V, A. Patil, A. Phatak, and N. Chandra Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010 Jul-Dec; 4(8): 118–126.

[13]. Maggi CA, Chiba T, Giuliani S. Human alpha-calcitonin gene-related peptide-(8-37) as an antagonist of exogenous and endogenous calcitonin gene-related peptide. Eur J Pharmacol. 1991 Jan 3;192 (1): 85-8.

[14]. Nelson M.T., Hung Y., Beyden J., Hescheler J., Standen N.B. Arterial dilations in response to calcitonin gene-related peptide involve activation of K+ channels. Nature, 1990, 344, 770-773.

[15]. Notsu T., Tanaka I., Mizota M., Yanagibashi K., Fukutake K. A cAMP-dependent protein kinase inhibitor modulates the blocking action of ATP and 5-hydroxydecanoate on the ATP-sensitive K+ channel. Life Sci., 1992, 51, 1851-1856.

[16]. Okabe E., Todoki K., Odajima C., Ito H. Free radicals-induced changes in mesenteric microvascular dimensions in the anesthetized cat. Jpn. J. Pharmacol, 1983, 33, 1233-1239.
[17]. Saito, H., Yukie, M., Tanaka, K., Hikosaka, K., Fukada, Y., and Iwai, E. 1986. Integration of direction signals of image motion in the superior temporal sulcus of the macaque monkey. J. Neurosci. 6, 145-157.
[18]. Toda N, Ayajiki K, Uchiyama M, Okamura T Nitric oxide-mediated neurogenic vasodilatation in isolated monkey lingual arteries. Am J Physiol. 1997, 272 (4 Pt 2): H1582-8.
[19]. Wei E.P., Moskowitz M.A., Boccalini P., Kontos H.A. Calcitonin gene-related peptide mediates nitroglycerin and sodium nitroprusside-induced vasodilatation in feline cerebral arterioles. Circ. Res., 1992, 70, 1313-1319.
[20]. Wolin M. S. Activated oxygen metabolites as regulators of vascular tone. Klinische Wochenschrift, 1991, 69, 21-23, pp 1046-1049.
[21]. Zweier JL Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. *J Biol Chem* 1988, 263:1353–1357.