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Comparison of Hemostatic and Neuro Protector Properties of Alkaloids N-Metiltsitizin and a Desoxypeganin in the Conditions of *in Vitro*

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Abstract

Modeling of sharp alcoholic intoxication induced animal 6 mg/kg of 50-70% ethanol by introduction. On this background studied effects of N-metilsitizin alkaloids and a desoxypeganin on ADP-induced aggregation of platelets and level of intracellular Ca^{2+} in the synaptosomes of a brain of rats. The obtained data render that the inhibiting effect of N-metilsitizin alkaloids and a desoxypeganin on ADP-induced aggregation of platelets is connected with oppression of a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets. Thus, N-metiltsitizin alkaloids and desoxypeganin block a gain of level of intracellular Ca^{2+} at the expense of increase in Ca^{2+} EPR pool, provoked by ethanol.

N-metiltsitizin doesn't compete with a glutamate for a binding site. Perhaps, action of N-metiltsitizin is caused by interaction with ionic channels of NMDA receptors. The neuronal of the receptors involved in the mechanisms which are the cornerstone of AAS (including convulsive attacks) and effectively to stop to possibility of application of N-metilsitizin in regulation of dihydropyridine-sensitive calcic channels of the main subtypes them. It is shown that the possible

competition between desoxypeganin and a glutamate for a site of binding of regulation of opening of ionic channels. Desoxypeganin directly doesn't affect calcic canals of a NMDA receptor. Perhaps, desoxypeganin like a glutamate causes overexcitation of NMDA receptors.

Keywords: desoxypeganin, N-metilsitizin, platelet, ADP, aggregation, argiolobate, synaptosome, ethanol.

Introduction

Ions of calcium are universal regulators and play the leading role in ensuring various cellular processes, including excitability of neurons, contractive activity of muscles, secretion of mediators and hormones, an expression of genes and proliferation of cages.

For realization of the majority of cellular processes in various cages certain Ca^{2+} a homeostasis in which regulation numerous Ca^{2+} the transporting systems of a cage take part is supported.

$[Ca^{2+}]$ in also intracellular depots – SR and EPR which have the specific Ca^{2+} -channels connected by IP3-and ryanodine receptors make an essential contribution to increase [1]. In all cases a moving force for providing transport of Ca^{2+} is the electrochemical gradient of Ca^{2+} which exists both between cytosols and extracellular space, and between cytosols and intracellular Ca^{2+} pools.

An important role in maintenance of intracellular Ca^{2+} of a -homeostasis plays also transporting Ca^{2+} - ATPase, localized on a plasmatic membrane and on membranes of intracellular organelle's. Thus low (100-150 nanometers) level of cytosolic Ca^{2+} at calm condition supported by active transport of ions of Ca^{2+} from cytoplasm in extracellular space with the help of Ca^{2+} ATPase of plasmatic membranes, and also mobilization of Ca^{2+} from cytosolic in intracellular compartment. Filling of intracellular pools happens at the expense of systems uni - and the anti-malt liquors localized in mitochondria and the secretory granules, and Ca^{2+} by ATPase of EPR and SR [2, 3].

It is quite obvious that the harmonious work of Ca^{2+}-transporting, which is provided both receipt of Ca^{2+} in a cage, and its removal from a cage, and also its mobilization in intracellular Ca^{2+} pools is the important factor providing maintenance of Ca^{2+}- of a homeostasis. In this regard violations of functional activity of separate links of Ca^{2+}- of the transporting systems often is followed by serious changes of level of intracellular Ca^{2+}, often is the main reason for pathogenesis of various diseases of cardiovascular and nervous systems. In particular, in warm and the smooth muscle leads increase in intracellular concentration of Ca^{2+} to change of electric properties of their membranes, violation of excitability and contractive activity. These changes, in turn, lead to development of such pathologies as a hypertension, stenocardia, coronary heart disease, a myocardial infarction and a stroke. At the same time, violations in work of Ca^{2+} of the transporting systems and a calcic overload of neurons are also the main reason for development of various pathologies of nervous system. In these conditions of change of electric parameters of membranes of neurons secretions of mediators and interaction between neurons are followed by violation of processes of generation and distribution of nervous impulses. Epilepsy, ischemia, various mental disorders, neuroses and depressions can be a consequence of such violations in neurons of a brain.

In this regard it is quite obvious that clarification of mechanisms of modulation of a calcic homeostasis and the related transport systems has not only important theoretical value, but also defines further development of the applied directions of biology and medicine.

One of the most actual problems of modern pharmacology and medicine is creation of new generation of medicines of the possessing highly effective and high-selective therapeutic action and not defiant serious side reactions. On the basis of the last achievements of combinatory chemistry, molecular biology, genetic engineering and biotechnology large-scale researches on working off of optimum strategy of the directed creation of new medicines are conducted. It is considered one of the main and defining principles of these strategy creations of bank of leading connections, structural and functional prototypes of future medicines. In this plan biologically active connections of a vegetable and animal origin the majority from which possess unique pharmacological properties and in centuries were used in traditional medicine, are considered as the most perspective for these purposes.

Similar versatility of pharmacological effects characterizes the majority of medicines of which under the action of interaction with ionic channels and neuroreceptors. This results from the fact that the same ionic channels, but to belonging functionally various cages and therefore the effects

caused by them aren't unambiguous can be a target for these connections. At the same time, it is revealed that some connections can work on various types of ionic channels and neuroreceptors [4]. So, Na^+ blocker channels disopiramide - can interact with a nicotinic cholinoretseptor. The blocker of K^+ of channels – amiodaron - is capable of inhibiting a muscarinic cholinoreceptor. Some blockers of Ca^{2+} channels can block also K^+ channels. Besides, it is shown that some blockers of NMDA of a receptor can interact with serotonins and adrenoreceptors. It is quite obvious that, possessing many-sided and ambiguous action on various types of cages and fabrics, the majority of these preparations along with positive therapeutic effect causes various undesirable side reactions. So, some antiarrhythmic means - Na^+ blockers channels cause increase of arterial pressure, violation of the speech, a depression and hallucinations. Some blockers of K^+ of channels applied as antiarrhythmic means [5], can also increase arterial pressure cause a depression of a hallucination and toxic affect a liver and lungs. α-adrenoceptors blockers can also cause violation of warm activity [6], bradycardia and a bronchospasm. Hypotensive preparations - blockers of Ca^{2+} of channels cause violation of warm activity, hearing and sight. Some blockers of NMDA of a receptor have negative effect on warm activity. Moreover, blockers of NMDA of a receptor can affect and receptors of adrenaline and serotonin and to cause various mental disorders and violations of physical activity [7].

It is known that abuse of alcohol causes violation of synthesis of various factors of the curtailing system, leads to decrease in its coagulative potential, developing of spontaneous bleedings, disseminates intra vascular folding's and malfunctions of platelets and a platelets link of a hemostasis [8, 9].

In preliminary researches it is established that at model rats both at sharp, and at chronic intoxication ethanol noted increase of ADP and adrenaline - the induced aggregation of platelets that testifies to increase of actually aggregation activity of platelets at alcoholic intoxication [10]. Perhaps, it is connected with activation of inductors of aggregation with increase in a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets.

At the same time chronic alcoholic intoxication causes compensatory increase in sensitivity of separate subunit of NMDA receptors, and also increases in density of receptors in various areas of a brain. At chronic effect of ethanol hyperproduction of NMDA receptors in membrane synaptosoma and the increase in their sensitivity connected with it to a glutamate is noted. The increase in an expression of NMDA receptors can be explained with the blocking effect of alcohol on the modulator glycine site and the subsequent compensatory increase in an expression of a receptor [11, 12].

The extremely dangerous consequences of alcoholism are established in activity of various parts of the nervous system where various mental disorders, depressions, epilepsy, and encephalopathy leading to degenerate disorders of cerebration, disability and disability develop. One of the neuronal of the mechanisms mediating sharp effects of ethanol is decrease in glutamatergic neurotransmission. Alcohol works as the antagonist of N-methyl-D-aspartate (NMDA) of receptors, one of subtype's glutamates the ionotropic receptors [12-14] that, undoubtedly, plays a role in the mechanisms which are the cornerstone of development of alcoholic intoxication and the alcohol abstinence syndrome (AAS) [15]. Clinical manifestations of AAS include psychopathological symptomatology, such as alarm, the depression, a dysphoria, irritability, sleep disorders and vegetative symptoms connected with the termination of alcohol intake [16, 17]. At chronic alcoholic intoxication ethanol cancellation, is followed, in particular, by the spasms connected with strengthening of the glutamatergic transfer happening at the expense of increase in release of a glutamate [18]. Antagonists of NMDA receptors in experiments on animals showed ability to block convulsive attacks during AAC [19]. Therefore it is supposed that the preparations blocking NMDA receptors or reducing glutamatergic neurotransmission by decrease in release of a glutamate can effectively stop AAS.

The solution of these questions occupied numerous scientific centers of the largest pharmaceutical companies, clinics and universities where leading experts not only pharmacologists and chemists, but also microbiologists, biochemists, electrophysiologists and biophysics are involved. In these centers on the basis of the last achievements of combinatory chemistry, molecular biology, genetic engineering and biotechnology large-scale researches on working off of optimum strategy of the directed creation of new medicines are conducted. It is considered one of the main and defining directions of this strategy the principle of creation of bank of leading

connections, structural and functional prototypes of future medicines. In this plan biologically active connections of a vegetable and animal origin the majority from which possess unique pharmacological properties, and for centuries were used in traditional medicine, are considered as the most perspective for these purposes.

Results of these researches are extremely important for establishment of new approaches of pharmacological regulation of Ca^{2+} a homeostasis in norm and at various pathologies nervous and platelet system and vascular.

The researches of pharmacological properties of vegetable alkaloids of a desoxypeganin and N-metilsitizin which are carried out at Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan on models of sharp alcoholic intoxication and chronic alcoholic dependence showed that among them there are perspective connections possessing potential tire-tread and anti-toxic action. However at what level their action is realized and what their molecular mechanisms remain not studied.

Research objective: Research action biological active substance on platelet and vascular, plasma links of a hemostasis and level of intracellular Ca^{2+} in the synaptosomes of a brain of rats, at the pathological changes observed at different conditions of alcoholic intoxication.

Materials and methods

Animals and Ethics statement: This study was carried out in the Laboratory Electrophysiology of Institute of Bioorganic Chemistry of Academy Sciences of the Republic of Uzbekistan on physically fit, adult, albino rats sexes (male) obtained from the vivarium of the Laboratory of Pharmacology. Animals had been fed with standard food and water in the vivarium. In all experiments albino rats weighing 200–250 g were used (n = 18). During the experiments, while working with experimental animals, International principles of the Helsinki Declaration and the rules of human attitudes towards animals were completely followed. Model experiments were made on 20 not purebred white rats males the weight (200-250 g) containing on a standard diet of a vivarium. All experiments carried out according to requirements of "The world society of protection of animals" and "The European convention on protection of experifcmental animals" [20]. Counted on each group of 20 rats background average daily consumption of 15% of ethanol for 1 kg of weight. To control group of animals in similar experimental conditions entered the distilled water. From them selected animals - chronic alcoholics in the period of mental and physical dependence on alcohol in the conditions of a free choice between 15% ethanol and water which is expressed in increase in a pathological inclination to alcohol. At modulation of sharp alcoholic intoxication the group of rats of alcoholic dependence was given 50-70% of ethanol.

Solvents and chemicals: Platelets allocated with a centrifugation method at 1500 rpm, within 15 min., for sedimentation of erythrocytes. The plasma enriched with platelets was centrifuged repeatedly within 10 min. at 3 thousand rpm. A deposit of platelets of a suspended in 5 ml of the environment containing *150 mm of NaCl, 2,7 mm of KCl, 0,37 mm of NaH_2PO_4, 1 mm of $MgCl_2$, 1 mm of $CaCl_2$, 5 mm glucose, 10 mm of HEPES-NaOH, pH 6,55, 50 of piece/ml of heparin, 0,35% of serumal albumine and 0,15 mg/ml of an apyrase*. Aggregation of platelets was registered on Born's method [21]. As inductors of aggregation of platelets used ADP (2 microns), adrenaline (5 microns) and thrombin (0,5 pieces/ml) (Sigma).

Synaptosomes allocated from a brain of rats with method of two-stage centrifugation [22]. All procedure of allocation was carried out at 4 °C.

For measurement of quantity of cytosolic Ca^{2+} ([Ca2 +] i) counted on Grinkevich's equation [23] synaptosomes, the rats allocated from a brain with chronic alcoholic intoxication placed on the middle, similar, that which was used for allocation of cages, were added by 20 microns of chlortetracycline (CTC). 60 min. for achievement of the maximum interaction of CTC with membrane-bound Ca^{2+}, as on plasmatic and intracellular membranes incubated. Wavelength of excitement of CTC – 405 nanometers, registration – 530 nanometers. Results expressed as a percentage, taking for 100% a difference between the maximum value of intensity of fluorescence (fluorescence of dye, saturated Ca^{2+}) and its minimum value (fluorescence of the indicator in lack of Ca^{2+}) received after addition EGTA ethylene glycol-encore-aminoetil-tetra acetate.

Aggregation on platelets was carried out on the photoelectric colorimeter (kfk-2), and also measurements of quantity of cytosolic Ca^{2+} in the synaptosomes were taken on the USB 2000 fluorimeter (Ocean Optics inc., First in PhotonicsTM. USB 2000. 2010. USA).

Data analysis the statistical importance of distinctions between controlled and skilled values was defined for a number of data, using the pair t-test where controlled and skilled values are taken together, and the unpaired t-test if they are taken separately. Value P<0, 05 indicated statistically significant distinctions. The received results are statistically processed on Origin 6.1 (Origin Lab Corporation, USA).

Results and discussion

Effect of alkaloid N - the metiltsitizin allocated from plants (*Thermopsis alterniflora*) and the desoxypeganin (2,3-trimetilen-3,4-digidrokhinazolina hydrochloride) allocated from plants of *Peganum harmala L* is investigated. On vascular platelets, plasma links of a hemostasis and level of intracellular Ca^{2+}in the synaptosomes of a brain of rats, at the pathological changes observed at different conditions of alcoholic intoxication.

At research of feature vascular platelets and plasma links of a hemostasis at model rats alcoholism, in a condition of sharp alcoholic intoxication of in vivo the blood plasma condensation is revealed. Thus thrombin (TT) and prothrombin time (PTT) in plasma with poor platelets was slightly extended, and also time of folding and formation of a fibrin clot in comparison with control (fig. 1). The mechanism of violation of folding of blood is a consequence of changes of a way as formations of fibrin, and its disintegration — a fibrinolysis. The end result of these violations at chronic alcoholism, despite all their complexity, there is a hypo coagulation which, possibly, is connected with a dysfibrinogenemia (qualitative defect of fibrinogen) or education and accumulation in a blood-groove of products of degradation.

However, in the plasma rich with platelets at model rats with chronic alcoholism of TT and PTT, on the contrary, folding time in comparison with control (fig. 1) is accelerated. Shortening of TT and PTT, as a rule, testifies to a hyperfibrinogenemia. In this case shortening of TT and PTT as earlier it was assumed, happens due to activation of factors of folding in a membrane of platelets that conducts to formation of the additional thrombin activating other coagulation factors (V, VIII, XIII, etc.).

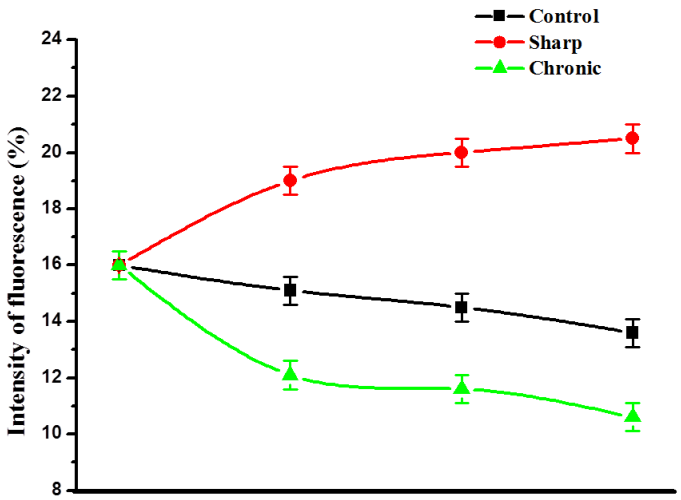

Figure 1. Influence of ethanol on factors of a fibrillation of model rats with chronic and sharp alcoholic intoxication. Reliability indicator: P<0, 05

At research of model rats with chronic alcoholism and in a condition of sharp alcoholic intoxication high rates of spontaneous aggregation of platelets are found. At research of plasma with rich platelets of blood of model rats with chronic alcoholism on adrenaline and ADP-induced aggregation also their dysfunction in response to the induced aggregation is revealed.

Dysfunction in response to the induced aggregation in plasma of blood of rats with chronic alcoholism, possibly, is connected with malfunction of receptors of membranes and secretion from platelets of activators of aggregation.

It is known that ADP and adrenaline are physiological inductors of aggregation and differ in mechanisms of activation of platelets. ADP, contacting glycoprotein receptors on a plasmatic membrane of a platelet, inhibits activity of an adenylate cyclase and by that reduces the cAMP level in cytoplasm, stimulates release of arachidonic acid and formation of a thromboxane of A_2 [24]. The effect of adrenaline on platelets is expressed much more weakly in comparison with ADP and activation of adrenoreceptors is connected with modulation of membranes and change of their permeability for ions of Ca^{2+}. Proceeding from above stated, it is possible to assume that effect of ethanol leads to decrease in level of cytosolic Ca^{2+} as a result of blocking of its entrance to a cage and its influence on activity of an adenylate cyclase and α-adrenoreceptor a plasmatic membrane.

When studying influence of ethanol on change of level of intracellular Ca^{2+} it was revealed that it causes dose dependent increase in intensity of fluorescence of CTC by 15-30% (fig. 2). It testifies that addition of ethanol leads to increase in quantity of Ca^{2+}, the rat associated with membranes of platelets.

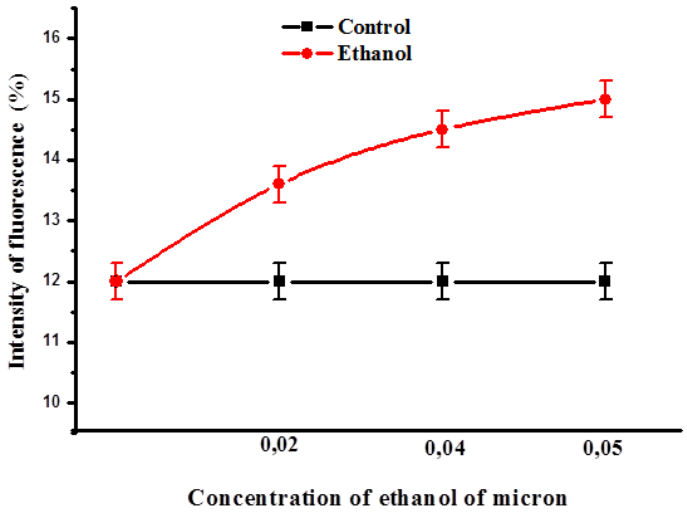

Figure 2. Influence of ethanol on change of level of membrane-bound Ca^{2+}.
As a probe CTC was used

It is known that the necessary level of ions of Ca^{2+} in cytoplasm is controlled by several systems of active transport of these ions through a plasmalemma and in intracellular Ca^{2+}depo (EPR and mitochondrion's). Thus, using the corresponding inhibitors, it is possible to estimate influence of ethanol on the size of intracellular Ca^{2+} pools. It is possible to judge the size of a reticular pool on effect of BHQ (a butylhydroxylchenon, inhibitor Ca^{2+}-ATPase EPR) which causes an exit of Ca^{2+}in cytosol that is registered as decrease in fluorescence of CTC.

In these conditions it was shown that effect of ethanol on the level Ca^{2+} in EPR had dose dependent character. Thus it is revealed that inhibitor Ca^{2+}- ATPase – BHQ caused bigger decrease in intensity of fluorescence of CTC in the cages processed by ethanol in comparison with control (fig. 3).

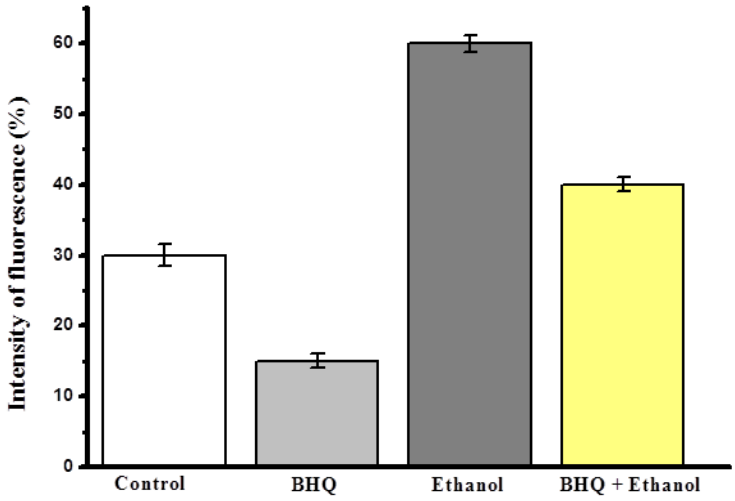

Figure 3. Influence of ethanol on intracellular Ca^{2+} a pool against BHQ. As a probe CTC was used. Reliability indicator: P<0, 05

So, in control decrease in intensity of fluorescence averaged 30%, and in the presence of 0,02-0,1 g/ml of ethanol – about 45-60%. It can be interpreted as increase in Ca^{2+} EPR pool, caused by inhibition of an exit of ions Ca^{2+} from a sarcoplasmic reticulum. Also it was shown that addition of inhibitors of breath (Rotenone) to the cages processed by ethanol doesn't cause considerable change of intensity of fluorescence of CTC. It allows assuming that ethanol has no essential impact on mitochondrial Ca^{2+} a pool.

Thus, at model rats with chronic alcoholism the activation of parameters of folding caused by the activation of a platelet hemostasis connected with violations of functional activity of platelets at the expense of increase in Ca^{2+} EPR pool that defines relevance of studying of mechanisms of a gain of cytoplasmatic concentration of Ca^{2+} comes to light.

At research of influence of N-metilsitizin alkaloids and a desoxypeganin against ethanol (0,05 g/ml) their dose dependent ant thrombin action is revealed. As ant thrombin action of a desoxypeganin and N-metilsitizin is shown more in the plasma rich with platelets, perhaps, their action is connected with secretion inhibition from platelets of activators of a fibrillation (a thromboxane of A2, ions of Ca^{2+}, the factor of activation of platelets (FAP), fibrinogen and many others) (fig. 4).

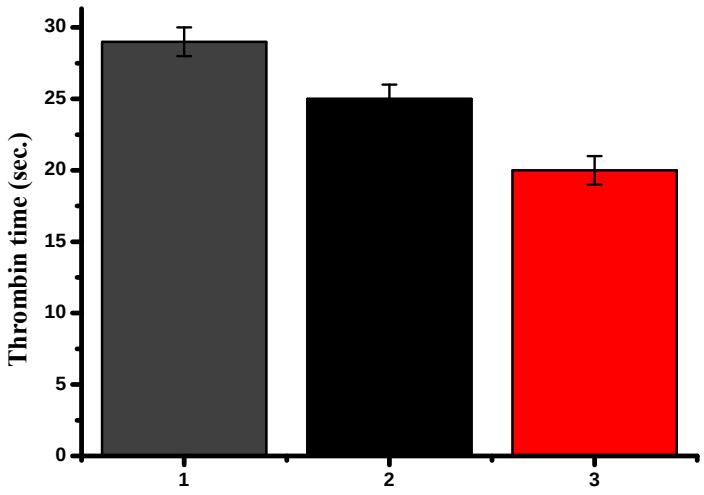

Figure 4. Dose dependent influence of alkaloids on a thrombin time
1 – Control; 2 - N-metiltsitizin; 3 – desoxypeganin. Reliability indicator: P<0, 05

At research of effect of alkaloids of a desoxypeganin and N-metilsitizin on functional activity of platelets it is revealed that N-metiltsitizin dose dependent inhibits adrenaline and ADP-induced aggregation of platelets. Thus the most inhibiting effect N-metiltsitizin alkaloid had, and its inhibiting properties are shown at ADP-induced aggregations. N-metiltsitizin at concentration of 50 microns causes 50% suppression ADP-induced of aggregation of platelets. Further increase of concentration of N-metilsitizin to 80 microns and 100 microns led to almost full inhibition ADP-induced of aggregation of platelets (fig. 5).

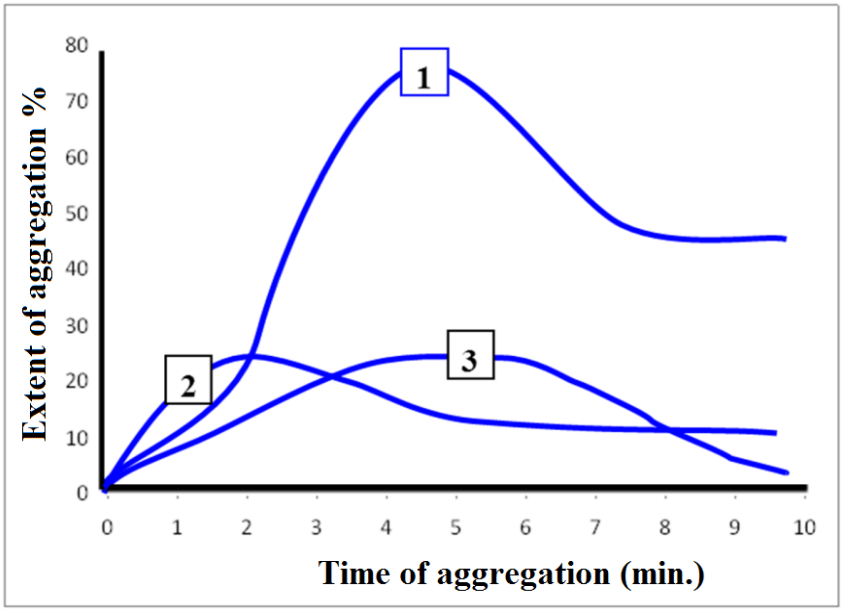

Figure 5. Influence of alkaloids on ADP-induced aggregation of platelets
1 – ADP-induced aggregation (control)
2 – ADP-induced aggregation against N-metilsitizin
3 – ADP-induced aggregation against a desoxypeganin

It is known that ADP leads to sharp increase in intracellular concentration $[Ca^{2+}]_i$, and this increase is carried out as at the expense of its entrance outside, and release from intracellular storages.

It is shown that against verapamil (a blocker of calcic channels) and a forskolin (the adenylate cyclase activator) in the concentration, for 50% reducing ADP-induced aggregation of platelets, inhibiting effects of N-metilsitizin and desoxypeganin amplified. The received results show that N-metiltsitizin alkaloids and desoxypeganin suppress activity of an adenylate cyclase and reduce level intracellular $[Ca^{2+}]_i$, perhaps their effects are connected with inhibition of a gain of cytoplasmatic Ca^{2+} as at the expense of its entrance outside, and release from intracellular storages.

With the purpose of specification of some mechanisms of antiaggregantis effect of N-metilsitizin alkaloids and a desoxypeganin their influence on the level of intracellular and membrane-bound Ca^{2+} with use of fluorescent probes Fura-2/AM and chlortetracyclin (CTC) was investigated. It is known that ADP leads to sharp increase in intracellular concentration $[Ca^{2+}]$. To define, whether effect of N-metilsitizin alkaloids and a desoxypeganin is based on a gain of cytoplasmatic concentration of Ca^{2+}, the induced ADP, experiment was made in the presence of physiological concentration of Ca^{2+} and without addition of Ca2 +. In control in both cases the fluorescence gain Fura-2/AM and CTC induced by ADP is revealed.

At research of effect of N-metilsitizin alkaloids and a desoxypeganin on a fluorescence gain Fura-2/AM induced by ADP in lack of extracellular Ca^{2+} it is revealed that N-metiltsitizin alkaloids and desoxypeganin dose dependent oppress release of Ca^{2+} from intracellular depots. Thus full suppression of a gain of cytoplasmatic concentration of Ca^{2+} wasn't observed. At the same time against N-metilsitizin alkaloids and a desoxypeganin, in the presence of extracellular Ca^{2+}, the fluorescence Fura-2/AM induced by ADP was much more, than in lack of extracellular Ca^{2+} that says that N-metiltsitizin alkaloids and desoxypeganin oppress only release of Ca^{2+} from intracellular depots. These assumptions are confirmed in researches of action of N-metilsitizin and

desoxypeganin against a blocker of Ca^{2+}- verapamil. Against N-metiltsitizin verapamil and desoxypeganin slightly oppressed a gain of level of intracellular Ca^{2+}, the induced ADP.

At linking of ADP with the corresponding receptors on a membrane of platelets, intermediate connections which stimulate release of calcium from depot are formed. At research of effect of N-metilsitizin alkaloids and a desoxypeganin against a forskolin (the adenylate cyclase activator) it is revealed that N-metiltsitizin alkaloids and desoxypeganin dose dependent strengthened the inhibiting action of a forskolin on ADP-induced increase of intracellular calcium.

In a case with use of fluorescent probes of CTC against N-metilsitizin alkaloids and a desoxypeganin considerable oppression of fluorescence of membrane-bound Ca^{2+} in lack of physiological concentration of Ca^{2+} was also observed. Perhaps, oppression of fluorescence of membrane-bound Ca^{2+} is connected with inhibition N-metiltsitizin alkaloids and desoxypeganin release of calcium from depot.

It is known that neurotoxic effects, and also some other manifestations of AAS are result of violation of balance between the inhibiting and exciting neurotransmitter systems and are partly mediated by glutamatergic neuromedia even system, change in particular of NMDA receptors and the level of intracellular Ca^{2+}.

The following work with synaptosomes of a brain of rats. Preincubation N-metiltsitizin alkaloids reduced fluorescence and respectively a level of cytosolic calcium at action of a glutamate on CTC-synaptosom's (fig. 6) complex.

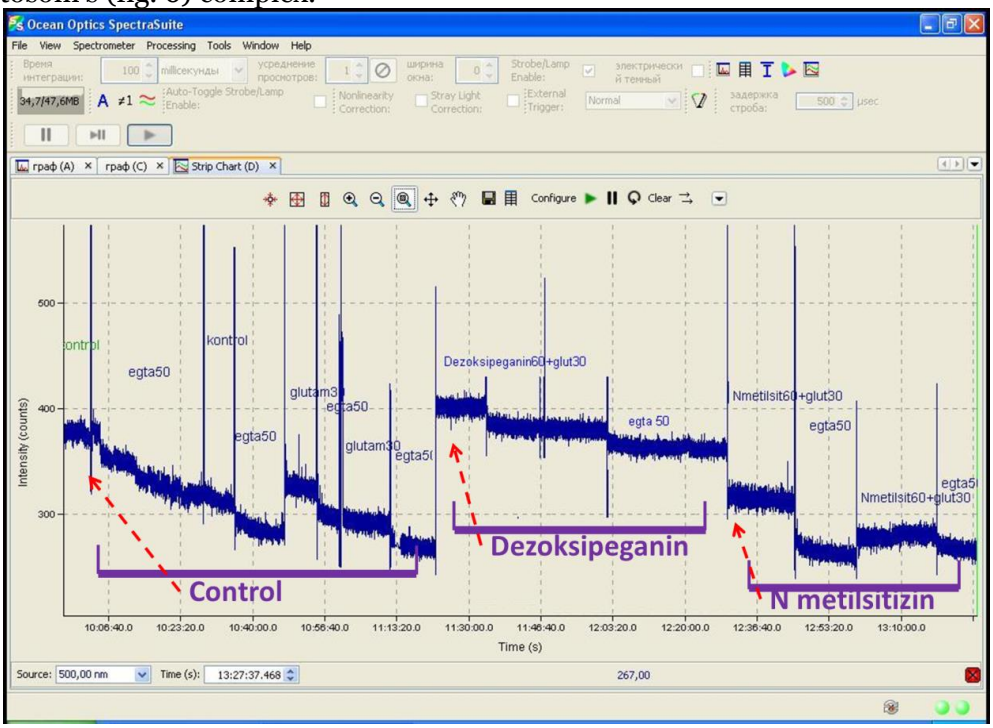

Figure 6. Influence of alkaloids of Desoxypeganin and N-metilsitizin on glutamatergic neuromedia even system at chronic alcoholic intoxication

At research of action of N-metilsitizin on synaptosoma of a brain of model rats with chronic alcoholic intoxication it is revealed that N-metiltsitizin considerably reduce fluorescence, respectively a level of cytosolic calcium in comparison with control. At the same time the increase in concentration of N-metilsitizin from 10 to 100 microns against a glutamate didn't lead to further decrease in effect of a glutamate.

The received results show that N-metiltsitizin alkaloids don't compete with a glutamate for a binding site. Perhaps, their actions are caused by interaction with ionic channels of NMDA receptors.

For identification, possible interaction of N-metilsitizin with sites of overexcitation of the NMDA receptors responsible for opening of calcic channels, their action against noncompetitive antagonists, such as magnesium ions, argiolobate and a blocker of the calcic channel – nifedipine is investigated.

In these researches it is shown that in the presence of N-metilsitizin the inhibiting action of ions of magnesium (10 microns) isn't observed. Possibly, it is caused by the competition between Mg^{2+} and N-metiltsitizin for sites stimulating opening of ionic channels.

Desoxypeganin at concentration of 10-50 microns differently influenced CTC-synaptosoma's complex. At a preincubation of a desoxypeganin with CTC-synaptosoma's complex of a brain of rats of control groups, its influence on fluorescence level isn't revealed. At the same time desoxypeganin induced decrease in fluorescence and respectively increase in level of cytosolic calcium in the presence of a glutamate in CTC-synaptosom's complex.

At research of action of a desoxypeganin on synaptosoma of a brain of model rats with chronic alcoholic intoxication it is revealed that desoxypeganin slightly increases fluorescence, respectively a level of cytosolic calcium in comparison with control (fig. 6). At the same time the preliminary preincubation of desoxypeganin (10 microns) synoptic membranes, and then led addition of the CTC-glutamate to decrease in fluorescence and respectively a level of cytosolic calcium. The Dose dependent increase in concentration of a desoxypeganin from 10 to 100 microns, led to dose dependent decrease in effect of a glutamate (fig. 7).

By results of the conducted researches it is possible to assume, the possible competition between of desoxypeganin and a glutamate for a site of binding of regulation of opening of ionic channels.

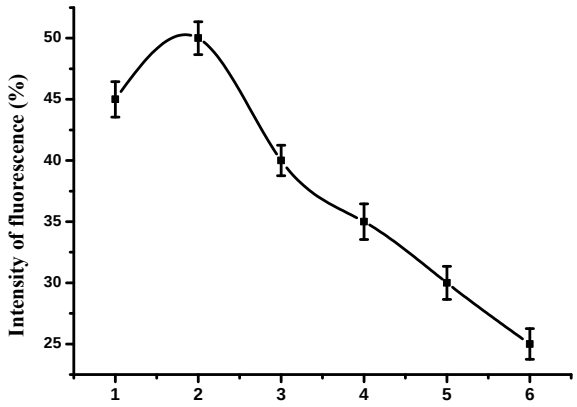

Figure 7. Influence of Alkaloid of a Desoxypeganin on Fluorescence and level of cytosolic calcium in the synaptosomes of a brain of rats

1 – Control (fluorescence of a complex of CTC-synaptosom) from a brain of rats at chronic alcoholic intoxication;

2 – Addition to CTC-synaptosom's complex of 10 microns of a desoxypeganin;

3–6 - action of a glutamate in concentration of 50 microns, against a preliminary incubation desoxypeganin in concentration 25, 50, 75, 100 microns

At research of action of N-metilsitizin on calcium - dependent processes of a NMDA receptor were studied against nifedipine (a blocker of Ca^{2+}-channels of L-type) in the synaptosomes from a brain of rats at a condition of AAS.

Preincubation of nifedipine with CTC-synaptosom's complex, led to decrease of fluorescence. Preincubation N-metilsitizin with CTC-synaptosom's complex, also led to decrease of fluorescence. Preincubation N-metilsitizin against nifedipine with CTC-synaptosom's complex, led to insignificant decrease of fluorescence (fig. 8) that points to the competition between alkaloids and nifedipine for a site of regulation of dihydropyridine-sensitive calcic channels.

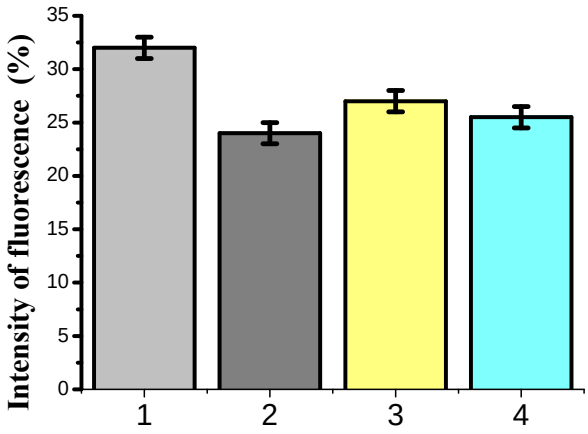

Figure 8. Influence of N-metilsitizin on calcium - dependent processes of a NMDA receptor against nifedipine

1 - Control (CTC-synaptosom's complex in conditions of AAS);
2 - nifedipin preincubation with CTC-synaptosom's complex;
3 - N-metilsitizin preincubation with CTC-synaptosom's complex;
4 - N-metilsitizin preincubation against nifedipine with CTC-synaptosom's complex;

It is known that glycine stimulates effects of a glutamate, and competitive antagonists of a receptor, such as AP_5, AV-2-1 toxin, can prevent activation of a glutamate. Other preparations and ions of Mg^{2+} can block the open channel by means of noncompetitive antagonism. To these preparations belong experimental neurotyre-tread preparation MK-801 and argiolobate [25].

To check whether it affects desoxypeganin to a site of linking of a glutamate with NMDA receptors or opening of the Ca^{2+}-channel oppresses, the following experiments are made.

Preincubation of alkaloid of a desoxypeganin with glycine shows stimulation of fluorescence and respectively strengthening of answers of a NMDA receptor that testifies that desoxypeganin like a glutamate causes overexcitation of NMDA receptors that leads to opening of calcic channels. Perhaps, desoxypeganin shows properties as agonist, and the antagonist.

For identification of possible interaction of a desoxypeganin with sites of overexcitation of the NMDA receptors responsible for opening of calcic channels, its action against noncompetitive antagonists – ions of magnesium and an argiolobatin is investigated.

It is shown that magnesium ions in the millimolar concentration considerably inhibit fluorescence of a complex glutamat-CTC-synaptosoma. The inhibiting action of ions of magnesium of fluorescence of a complex of CTC-synaptosom in the presence of a desoxypeganin didn't change. It is also shown that action of an argiolobatin on calcic canals of a NMDA receptor in the presence of a desoxypeganin doesn't change (fig. 9).

The received results testify that desoxypeganin directly doesn't affect calcic canals of a NMDA receptor. Perhaps, desoxypeganin like a glutamate, causes overexcitation of NMDA receptors.

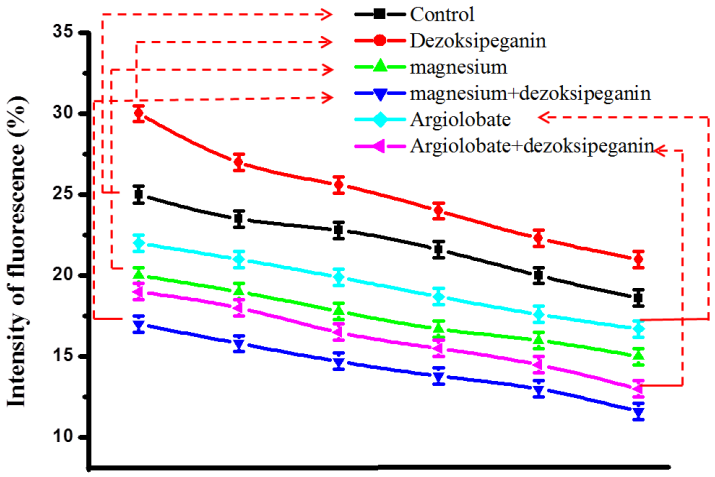

Figure 9. Influence of ions of magnesium, an argiolobatin and desoxypeganin on fluorescence and level of cytosolic calcium in the synaptosomas of a brain of rats

In the following experiments action of a desoxypeganin on synaptosoma of a brain of model rats with chronic alcoholic intoxication, after alcohol cancellation is investigated. In these researches it is shown that desoxypeganin considerably increases fluorescence, respectively a level of cytosolic Ca^{2+}, in synoptic membranes in comparison with control (fig. 10).

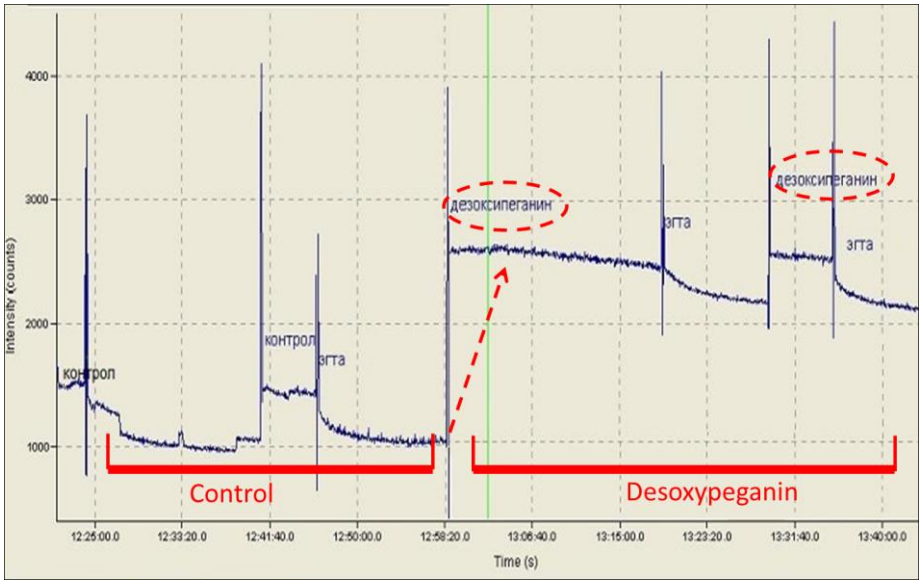

Figure 10. Influence of alkaloid of a desoxypeganin on fluorescence and level of cytosolic calcium in the synaptosomas of a brain of rats with chronic alcoholic intoxication, after alcohol cancellation

The received results testify that desoxypeganin directly doesn't affect calcic canals of a NMDA receptor. Perhaps, desoxypeganin like a glutamate causes overexcitation of NMDA receptors.

In case of ASS desoxypeganin considerably increases fluorescence, respectively a level of cytosolic Ca2 + and it can effectively be used as an exciting neurotransmitter preparation at chronic alcoholic intoxication.

Conclusion

The received results show that the inhibiting effect of N-metilsitizin alkaloids and a desoxypeganin on ADP-induced aggregation of platelets is connected with oppression of a gain of

cytoplasmatic concentration of Ca^{2+} from depot of platelets. Preliminary researches showed that ethanol at concentration of 0,01-0,03 mg/ml accelerates thrombin time of a fibrillation and causes spontaneous aggregation of platelets. Against N-metilsitizin alkaloid ethanol didn't influence process of a thrombogeneses and functional activity of platelets.

Thus, the received results confirm that alkaloids N-metiltsitizin and desoxypeganin block a gain of level of intracellular Ca^{2+}at the expense of increase in Ca^{2+} a pool of EPR provoked by ethanol.

The obtained results show that N-metiltsitizin alkaloids don't compete with a glutamate for a binding site. Perhaps, their actions are caused by interaction with ionic channels of NMDA receptors. The neuronal of the receptors involved in the mechanisms which are the cornerstone of AAS (including convulsive attacks) and effectively to stop to possibility of application of N-metilsitizin in regulation of dihydropyridine-sensitive calcic channels of the main subtypes them.

By the results of the conducted researches it is possible to assume, the possible competition between desoxypeganin and a glutamate for a site of binding of regulation of opening of ionic channels. The received results testify that desoxypeganin directly doesn't affect calcic canals of a NMDA receptor. Perhaps, desoxypeganin like a glutamate causes overexcitation of NMDA receptors.

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Сравнение гемостатических и нейропротекторных свойств алкалоидов N-метилцитизина и дезоксипеганина в условиях *in vitro*

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Аннотация. Моделирование острой алкогольной интоксикации индуцировали путем введения животным 6 мг/кг 50-70 % этанола. На данном фоне изучали действия алкалоидов N-метилцитизина и дезоксипеганина на АДФ-индуцированную агрегацию тромбоцитов и уровня внутриклеточного Ca^{2+} в синаптосомах мозга крыс. Полученные данные оказывают, что ингибирующий эффект алкалоидов N-метилцитизина и дезоксипеганина на АДФ-индуцированную агрегацию тромбоцитов связан с угнетением прироста

цитоплазматической концентрации Ca^{2+} из депо тромбоцитов. Таким образом, алкалоиды N-метилцитизин и дезоксипеганин блокируют прирост уровня внутриклеточного Ca^{2+} за счет увеличения Ca^{2+} пула ЭПР, спровоцированный этанолом.

N-метилцитизин не конкурируют с глутаматом за участок связывания. Возможно, действие N-метилцитизина обусловлены взаимодействием с ионными каналами NMDA-рецепторов. Возможности применения N-метилцитизина в регуляции дигидропиридин-чувствительных кальциевых каналов основных подтипов нейрональных рецепторов, вовлеченных в механизмы, лежащие в основе ААС (включая судорожные припадки) и эффективно купировать их. Показано что возможную конкуренцию между дезоксипеганином и глутаматом за участок связывания регуляции открывания ионных каналов. Дезоксипеганин непосредственно не действует на кальциевые каналы NMDA-рецептора. Возможно, дезоксипеганин подобно глутамату, вызывает перевозбуждение NMDA-рецепторов.

Ключевые слова: дезоксипеганин, N-метилцитизин, тромбоциты, АДФ, агрегация, аргиолобатин, синаптосома, этанол.