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Research of Action of Preparat Rutan on Various Sites of GABA-Receptor at Chronic Alcoholic Intoxication

Key words: *synaptosomes, glutamate, rutan, picrotoxin, phenobarbital, ethanol.*

Annotation: *Action of a rutan on various sites of the GABA-receptor and level of cytosolic calcium in a complex CTC-synaptosomes taken from the brain of model rats with chronic alcoholic intoxication was studied, showed insignificant decrease in level of cytosolic calcium in comparison with control. If to consider that chronic alcoholic intoxication leads to decrease in the GABA level respectively, and activation of NMDA exciting neurotransmitter system in CNS. Insignificant decrease in level of cytosolic calcium is explained by inhibition of one of NMDA receptor sites.*

INTRODUCTION

The special role in formation of a neurologic picture of chronic poisoning with ethanol play multidirectional violations in a gamma-amino butyric acid (GABA) – and in the glutamatergic systems of a brain (1, 2). The system of GABA-ergic neurotransfer participates in formation of tolerance to ethanol.

Chronic influence of alcohol reduces the level of the inhibition of nervous cages induced by GABA. Consequence of it may appear in hard cases of development of spasms against an abstinence syndrome. In their genesis plays a role increase of functional activity of glutamatergic system and modification of receptors of N - methyl - D - aspartate (NMDA) – a subtype. Long alcohol intake leads to increase in population of the central NMDA - receptors and, as a result, to decrease of the activity of GABA-ergic system, that forms the violation of balance between exciting (glutamatergic) and inhibiting (GABA-ergic) neuromedia systems lying as the base of manifestations of a state of dependence (2).

Besides, it is shown that alcoholic dependence takes part in these mechanisms, and also in the GABA-ergic systems (3, 4).

Even low doses of alcohol start activity of inhibitory GABA — systems of a brain (5). This process also leads to the sedative effect which is followed by relaxation of muscles, a somnolention and euphoria (feeling of intoxication). Genetic variations of receptors of GABA can influence tendency to alcoholism (6).

The oppressing effect of ethanol on CNS is caused first of all by stimulation of receptors GABA and antiglutamatergic activity (7).

Use of the pharmacological preparations which is selectively influencing the most important links of glutamatergic and GABA-ergic mediation will allow to reveal new ways of the prevention of neurotoxic defeats and restoration of the broken functions of the central nervous system (CNS) as a result of alcoholic intoxication.

Research objective. Studying of action of a preparation of a rutan on various sites of the GAMK-receptor at chronic alcoholic intoxication.

MATERIALS AND METHODS

Model experiments were made on white impurebred rats (200-250 g). Counted on each group background average daily consumption 15% of ethanol on 1 kg of weight. Controlled group of animals in similar experimental conditions injected the distilled water. Synaptosoma allocated from a brain of rats with method of two-stage centrifugation [8]. All procedure of allocation was carried out at 4 °C.

For measurement of quantity of cytosolic Ca^{2+} in the synaptosomes, the rats allocated from a brain with chronic alcoholic intoxication placed in the middle, similar, which was used for allocation of cages, were added by 20 microns of chlortetracyclin (CTC).Incubated 60 min. for achievement of the maximum interaction of CTC with membrane-bound Ca^{2+} , as on plasmatic, and intracellular membranes. Length of wave 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 ethylene glycol-encore-aminoetil-tetraacetate(EGTA). Measurements were taken by means of the fluorimeter (Hitachi, Japan) and (Ocean Optics inc., First in PhotonicsTM. USB 2000. 2010. November 19.USA). 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 were taken together, and the unpaired t-test if they are taken separately. Values $p<0.05$ were pointed at statistical value difference. Data was analyzed by Origin Pro 6.1 (MicroCal Software, Northampton, MA).

RESULTS AND DISCUSSION.

Research of the inhibiting effect of ethanol on GABA-ergic system in the synaptosomes of a brain of rats at chronic alcoholic intoxication showed that the level of fluorescence of a complex of CTC-sinaptosom is lower in comparison with control (Figure 1).

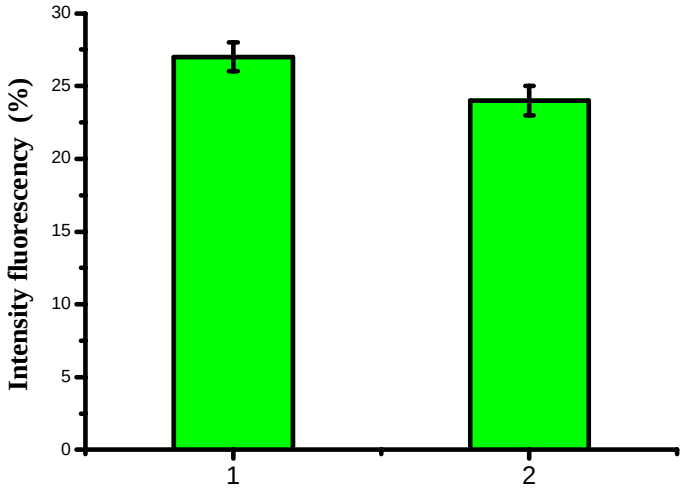


Figure. **1. Effects of ethanol on GABA-ergic system in the synaptosomes of a brain of rats at chronic alcoholic intoxication.** 1 – control; 2 – chronic alcoholic intoxication.

For an assessment of influence of ethanol on the complex GABA-receptor components as pharmacological "probes" used a preparation of the rutan (3,6-bis-O-galloil-1,2,4-tri-O-galloil-β-D-glucose) allocated from plants (Rhus coriária) for cytosolic Ca^{2+} in the synaptosomes of a brain of rats (Figure 2).

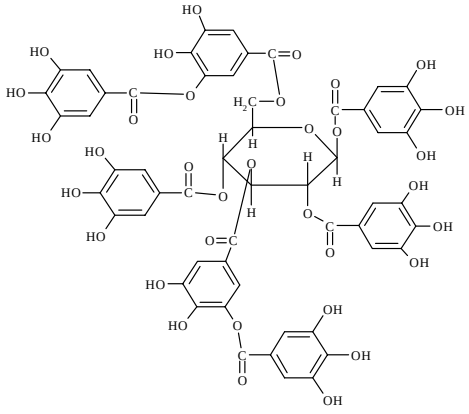
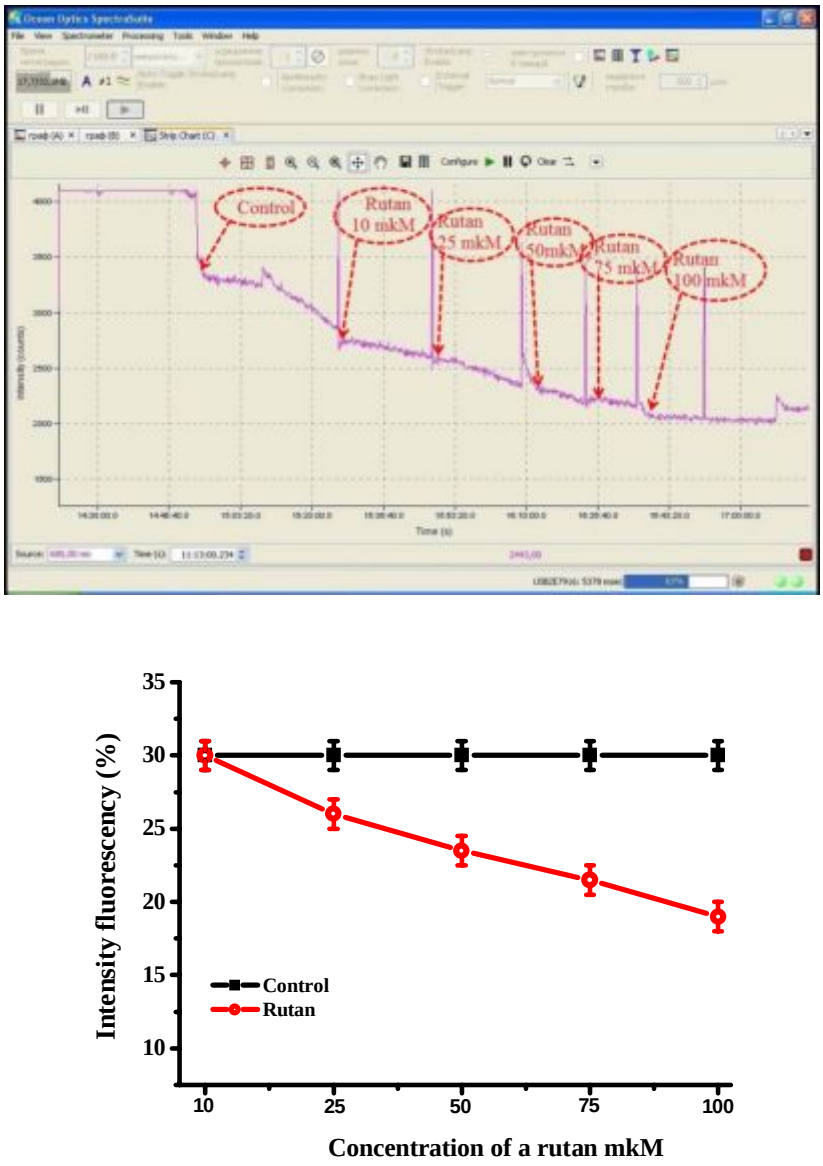


Figure. 2. Chemical formula of a rutan $C_{55}H_{40}O_{34}$**,** molecular weight 1244.

The preliminary preincubation sinaptosomy with different concentration of GABA, then addition of a glutamate led to dose-dependent reduction of level of cytosolic calcium and respectively, to decrease in NMDA exciting neurotransmitter system.

Preincubation of a rutan (10-100 microns) with CTC-sinaptosom's complex, didn't increase fluorescence level. At the same time rutan (50 microns) reduced fluorescence and respectively, led to increase in level of cytosolic calcium against GABA (50 microns), CTC-sinaptosom's complex allocated from a brain of intact rats, induced by a glutamate (Figure 3).



Figire. 3. Dose-dependent influence of a preparation of a rutan on a glutamate – the induced fluorescence against GABA.

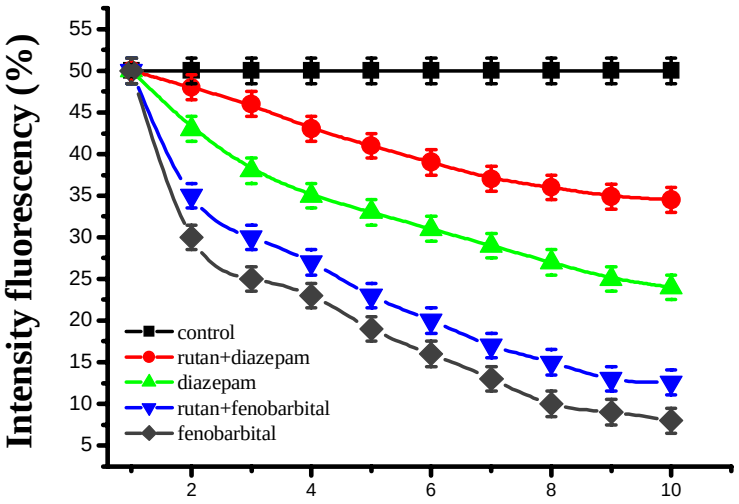
Research presented ,the studying of the inhibiting effect of ethanol on GABA-ergic system against blockers of the GABA- benzodiazepine receptor complex.

Preincubation of the antagonist of the GABA-receptor of a picrotoxine with a complex CTC-sinaptosoma against a rutan (10-100mkm), stabilized the locked condition of an ionofor and by that reduced fluorescence level. Thus the maximum decrease in level of cytosolic calcium against a glutamate (50 microns) it was observed in concentration (50mkm) of a rutan. The received result shows that against the antagonist of the GABA-receptor of a picrotoxine rutan reduces the level of cytosolic calcium where rutan doesn't affect inhibition the GABA-receptor.

In case of a preincubation of benzodiazepin-agonist of the GABA-receptor of a diazepam and phenobarbital in concentration of 50-100 microns against a preparation of a

rutan with CTC-sinaptosoma's complex allocated from a brain of intact rats slightly strengthened effect of agonist of the GABA-receptor (Figure 4).

Figire. 4. Action of benzodiazepin-agonist of the GABA-receptor in concentration of 50-100 microns against a preparation of a rutan with CTC-sinaptosoma's complex of a brain of intact rats.



At research of action of a rutan on the level of cytosolic calcium in CTC-sinaptosom's complex of a brain of model rats with chronic alcoholic intoxication, insignificant decrease in level of cytosolic calcium in comparison with control is revealed. If to consider that chronic alcoholic intoxication leads to decrease in the GABA level respectively, and activation of NMDA exciting neurotransmitter system in CNS. Insignificant decrease in level of cytosolic calcium is explained by inhibition of one of NMDA receptor sites.

The received results indicate possibility of application of a rutan, as the antagonist of the exciting neurotransmitter at chronic alcoholic intoxication and at alcohol cancellation.

Thus, research of pharmacological properties of some biologically active connections which are carried out on model rats with chronic alcoholic intoxication showed that among them there are perspective connections possessing properties of analeptic that gives the chance to their application for treatment of an alcoholic abstinence syndrome of AAS.

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