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The Effect of Chronic Alcohol Abuse on the Benzodiazepine Receptor System in Various Areas of the Human Brain

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Abstract

Objective: Alcohol abuse induces neuroadaptive changes in the functioning of neurotransmitter systems in the brain. Decrease of GABAergic neurotransmission found in alcoholics and persons with a high risk of alcohol dependence. Benzodiazepine receptor (BzDR) is allosterical modulatory site on GABA type A receptor complex ($GABA_AR$), that modulate GABAergic function and may be important in mechanisms regulating the excitability of the brain processes involved in the alcohol addiction. The purpose of this study was to investigate the effects of chronic alcohol abuse on the BzDR in various areas of the human brain.

Materials and Methods: Investigation of BzDR properties were studied in synaptosomal and mitochondrial membrane fractions from different brain areas of alcohol abused patients and non-alcoholic persons by radioreceptor assay with using selective ligands: [^{3}H] flunitrazepam and [^{3}H] PK-11195. Brain samples obtained at autopsy urgent. In total 126 samples of human brain areas were obtained to study radioreceptor binding, including a study group and control group.

Results: Comparative study of kinetic parameters (Kd, Bmax) of [^{3}H] flunitrazepam and [^{3}H] PK-11195 binding with membrane fractions in studding brain samples was showed that affinity of BzDR was decreased and capacity increased in different areas of human brain under influence of alcohol abuse. More alters of synaptic BzDR "central" type (CBR) appeared in prefrontal cortex, mitochondrial BzDR "peripheral" type (PBR) – in n.caudatus and cerebella cortex.

These results showed that alcohol addiction induce more expressive alterations in PBR than CBR in the brain structures that agree with maintaining function of glial cells in CNS under influence of different toxic factors.

Conclusion: Chronic exposure to ethanol results in harmful effects on the human brain: causing nonuniform adaptive changes of BzDR in various areas of the brain, which can modulate $GABA_AR$ and reduce neuromediation GABA in various areas of the brain which can cause alcohol addiction.

Graphical Abstract: Alcohol abuse induces neuroadaptive alters of benzodiazepine receptor system in various areas of the brain in patients with alcoholism that can modulate $GABA_AR$ and mediation of GABA in the brain, which can cause alcohol addiction.

Keywords: Alcohol; Alcoholism; Brain; Benzodiazepine receptors; GABA; Synapse; Neuroadaptation

List of Abbreviations: AWS: Alcohol Withdrawal Syndrome; BzD: Benzodiazepine; BzDR: Benzodiazepine Receptor (binding site for benzodiazepine); CNS: Central Nervous System; CL^-: Chloride Ion; GABA: Gamma-AminoButyric Acid; $GABA_AR$: Receptor for Gamma-Aminobutyric Acid type A; $GABA_A$/BzDR: Gamma-Aminobutyric Acid type A receptor, Coupled with Allosteric Binding site for Benzodiazepine (receptor complex); GABRA1: Gene Gamma-Aminobutyric Acid type A Receptor alpha1 subunit; GABRA2: Gene Gamma-Aminobutyric Acid Type A Receptor alpha2 Subunit; GABRA4: Gene Gamma-Aminobutyric Acid type A Receptor alpha4 subunit; GABRB1: Gene Gamma-Aminobutyric Acid type A receptor beta1 subunit; GABARG1: Gene Gamma-Aminobutyric Acid type A Receptor Gama1 Subunit; HPA: Hypothalamic-Pituitary-Adrenal axis; RRA: Radio Receptor Analysis; Kd: Dissociation constant of the Ligand-Receptor Complex; Bmax: Density of Binding sites for Selective Ligand

Introduction

Alcohol abuse - a chronic relapsing disease characterized by compulsive addiction to alcohol, the formation of addictive behavior and alcohol dependence [1-4]. The problem of the treatment of alcohol addiction is very difficult due to the occurrence of relapses and the complexity of understanding the mechanisms of their formation.

Functional study of the brain of humans and animals allowed establishing the relationship of certain areas of the brain in response to the effects of alcohol [4-10]. The existence of a central pathophysiological mechanism based on the formation of alcohol dependence in humans and animals caused by genetic and neurochemical changes providing deep mediator processes in the brain structures, which determines the biological basis of susceptibility to alcohol [5,9,11-13].

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One of the theories of alcoholism involves a shift in the general excitability of the brain as a result of reduced inhibition processes. Patients with alcohol dependence noted increased excitability, impulsiveness, extravagance and other disorders associated with these processes [3]. Imbalance of excitation and inhibition in the brain may be the basis of formation of alcohol addiction [1,4,14]. Strengthening of β-activity in the deeper parts of the frontal cortex of the brain, recorded on the EEG, is a predictor of relapse in patients with alcoholism [15]. GABA is an inhibitory neurotransmitter and receptor GABA type A ($GABA_AR$) provides rapid processes (phase) and long (tonic) inhibition in the CNS.

$GABA_AR$ are targets for alcohol because they are the major inhibitory neurotransmitter in the central nervous system, and occupy a central role in mediating the effects of ethanol [16-21]. Alcohol facilitates activation of $GABA_A$ receptors, possesses anxiolytic properties [17,18], and in connection with its use of this ability is a form of self-medication when an alarm occurs and the development of anxiety [22]. Decrease of GABAergic neurotransmission found in alcoholics and persons with a high risk of alcohol dependence [7,21]. Some studies have shown a link between alcoholism and microsatellite marker locus GABARG1 chromosome 4p. This cluster of genes encoding subunits of $GABA_A$ receptor: GABRG1, GABRA2, GABRA4 and GABRB1 [12,13]. $GABA_A$ receptor polymorphism is based on differences in the response to alcohol in humans and animals of different lines.

$GABA_AR$ allosteric binding sites are targets for various drugs that modulate GABAergic function; BzD, barbiturates, alcohol and endogenous neurosteroids, allosterically modulate these receptors [9,19,23-25]. Site for specific recognition of GABA on $GABA_AR$ closely associated with the allosterical modulatory site for benzodiazepines – benzodiazepine receptor (BzDR). BzDR is allosterical modulatory site on GABA type A receptor complex, that modulate GABAergic function and may be important in mechanisms regulating the excitability of the brain processes involved in the alcohol addiction. $GABA_A$/BzD receptors are characterized by a high level of structural and functional heterogeneity [22,23,26]. They have a rich pharmacology, and this is dependent upon the particular subunits that are present within the receptor pentamer complex [27]. An important point in the functioning of the $GABA_A$ receptor complex is that this oligomeric protein complex contains various allosteric binding sites modulating the activity of the receptor [24]. These allosteric binding sites are the targets for a variety of agents, including benzodiazepines and ethanol. Benzodiazepines, binding with specific sites BzDR on $GABA_A$ receptors – alter its conformation and affinity [27,28] and play an important role on neuropharmacology of inhibitory processes in CNS – modulatory fast and tonic inhibition [28].

Potentiating of the inhibitory effect of GABA underlies the sedative and anxiolytic effects of alcohol and BzD [17,19]. Currently have not been established endogenous ligands for BzDR, as for opiate receptors and others, but their functional role is very significant in neuropharmacology of inhibitory processes in the CNS. There are cross-reactions (tolerance and dependence) between alcohol and BzD, which confirms the interaction of ethanol with BzDR [20].

Besides BzDR "central" type (CBR) associated with $GABA_AR$ and having synaptic localization, known BzDR "peripheral" type (PBR), non-$GABA_AR$ and localized in the mitochondrial membrane, mainly in the glial cells of the brain. These receptors allow the transfer of cholesterol into the mitochondria, affecting the regulation of the synthesis of neurosteroids that is endogenous modulators of the $GABA_A$/BzDR in the CNS [29]. BzD, anxiolytics, anesthetics and alcohol are implementing some of its effects through the PBR, regulating production of neurosteroids and their metabolites, which are critical components of normal brain function [29].

Signaling mechanisms that are required for our understanding how these processes are impaired by ethanol resulting in harmful consequences to the brain are very important. Understanding of the basic mechanisms regulating the excitability of the brain processes involved in the formation of alcohol addiction, definition of target effects of alcohol can contribute to the creation of new drugs acting on these targets, develop a potentially effective therapy to deal with the consequences of alcohol abuse and alcohol withdrawal.

In this connection it is relevant to further study the mechanisms of receptor systems in the brain, particularly benzodiazepine receptor system in conditions of chronic ethanol exposure, their role in alcohol addiction, which may contribute to the further elucidation of the etiopathogenesis of the disease and the search for promising new drugs needed for pharmacotherapeutic correction.

The purpose of this study was to investigate the effects of chronic alcohol abuse on the benzodiazepine receptor system in various areas of the human brain.

Materials and Methods

The study was conducted on autopsy human brain. Samples of human brain were obtained during the early anatomical dissections (not later than 5 hours after death) in 21 cases of death male patients suffering from alcoholism at age 33-54 years (study group). In addition to medical history to form a core group used objective biological criteria for chronic alcohol abuse human - the presence of physical signs of alcohol abuse - fatty liver, cirrhosis, and others. The control group consisted of 21 people standardized to the main group in age who did not have during the life of neurological and psychiatric disorders.

The autopsy diagnosis takes into account the clinical history, clinical manifestations diseases, including somatic (liver condition, etc.). Patients suffering alcoholism were under the supervision by psychiatrists of Mental Health Research Institute and had a diagnosis according to ICD - 10: "Mental and behavioral disorders due to use of alcohol, dependence syndrome» (F10.232) and "Mental and behavioral disorders due to use of alcohol, withdrawal state" (F10.302). Alcoholism flow type in the surveyed patients wore secondary progression of the character. Patients with other psychiatric disorders, or who use psychoactive drugs that could affect the results of the study in this study were not included. The lethal outcome in patients suffering from alcoholism and without such disorders occurred as a result of acute heart failure. The study included only patients not undergoing resuscitation, as a result of acute heart failure.

During the term of autopsies of the brain samples isolated from different structures: the cerebral cortex (prefrontal area – prefrontal cortex), cerebellar cortex, caudate nucleus and the head of the caudate nucleus of the brain, frozen and stored before the test in liquid nitrogen thermos. In total 126 samples of different human brain regions were obtained to study radioreceptor binding, including a study group and control group. Separation of human brain synaptosomal and mitochondrial membrane fractions was carried out by preparative ultracentrifugation. The obtained membrane fractions were frozen and stored at t = -80°C.

Investigation of BzDR properties was performed by radio receptor assay (RRA) by binding to synaptosomal and mitochondrial membrane

fractions with selective ligands: [^{3}H] flunitrazepam and [^{3}H] PK-11195.

[^{3}H] flunitrazepam binding procedure with specific binding sites on the synaptosomal membrane derived from autopsy samples of human brain

Properties of BzDRs "central" type (CBR) from autopsy samples of different areas of human brain examined by RRA binding of [^{3}H] flunitrazepam (85 Ci/mmol, Amersham, UK) with synaptosomal membrane fraction of the brain tissue during 60 min. at t=0°C. Concentrations of [^{3}H] flunitrazepam were 0.2 - 15 nM in incubation volume. The concentration of the membranes was 0.2 mg protein/ml in 0.25 ml samples of the incubation. Nonspecific binding was performed with flunitrazepam cold in concentration 10 μM in incubation volume.

[^{3}H] PK-11195 binding procedure with specific binding sites on the mitochondrial membrane derived from autopsy samples of human brain

Properties of BzDRs "peripheral" type (PBR) from autopsy samples of different areas of human brain was investigated by RRA binding of [^{3}H] PK-11195 (85.5 Ci/mmol, NEN, USA) (0.2 - 25.0 nM in incubation volume) with mitochondrial membrane fraction of the brain tissue for 90 min. at t= 0°C. The concentration of the membranes was 0.2 mg protein/ml in 0.25 ml samples of the incubation. Nonspecific binding was performed with PK-11195 cold in concentration 10 μM in incubation volume.

Bound ligands was separated in all cases filtration through GF/B filters ("Whatman", UK) following vacuum filtration using a system "Harvester - Skatron" (USA) in 15 ml Tris - HCl (50 mM, pH=7.4 at t =0°C), the filters were placed in glass vials containing 10 ml of scintillator. Radioactive analysis of the amount of bound ligands was carried out in β-scintillation counter - "Rack-beta" (LKB, Sweden). Nonspecific binding (<10%) was similar in control and test samples. The dissociation constant (Kd) and the maximum number of specific binding sites (Bmax.) was determined by analysis of saturation curves in Scatchard coordinates. Kd expressed in nM, Bmax – in fmol/mg protein. Linear Scatchard blots were analyzed in all cases which confirm the presence of only a specific population of binding sites. Distribution of signs did not differ significantly from normal, so the statistical data used parametric method of variation statistics (t-test) using Statistika 10.0, the differences were considered significant ($p<0.05$).

Experimental work was carried out in the Laboratory of Neuroimmunology and Neurobiology Mental Health Research Institute (Tomsk) and Laboratory of Clinical Biochemistry Research Center for Mental Health Sciences (Moscow). All the studies were approved by the Ethics Committee of the Mental Health Research Institute.

Results

I. Study of the binding characteristics of [^{3}H] flunitrazepam with synaptosomal membrane fractions prepared from autopsy samples of different brain regions showed that properties of central-type BzDR (CBR) are heterogeneous and differ in various brain structures studied alcoholic patients (study group) and in the control group.

Greatest affinity of CBR has been identified in the caudate nucleus, lower affinity receptor of the cerebral cortex (area of the prefrontal cortex) and cerebellar cortex in patients in the control group. Receptor density in these structures of the brain was also different - maximum receptor density (Bmax) was found in the caudate nucleus, smaller in the prefrontal cortex and in the cerebellar cortex. Thus, our results indicate heterogeneity of CBR in different areas of the human brain in the control group (Table 1, Figures 1 and 2).

Comparative analysis of a receptor binding to [^{3}H] flunitrazepam showed a significant increase in the Kd values of the brain structures studied alcoholic patients (study group) compared to control patients. However, these changes were also heterogeneous in different areas of the brain in patients with alcoholism. The greatest changes of Kd values of CBR were found in prefrontal cortex, caudate nucleus, and to a lesser extent - in the cerebellar cortex in study group compared with the control (Table 1 and Figure 1). The data indicate that chronic use of alcohol reduces the affinity (1/Kd) of CBR in various brain structures of patients with alcoholism (study group) which may be the basis of a reduced neuromediation of GABA in the brain of patients suffering from alcoholism, as CBR can allosterically modulate $GABA_A$ receptor function in the brain.

Receptor density (Bmax) was significantly increased in varying degrees in the studied areas of the brain of patients with alcoholism (study group). CBR density was significantly higher in patients who abuse alcohol and to a greater degree in the prefrontal cortex, caudate nucleus to a lesser extent - in the cerebellar cortex (Table 1 and Figure 2) in comparison to the control group of patients.

Thus, we have identified changes indicate a reduction in affinity for CBR in brains of patients under the influence of alcohol addiction and increase their density as compared with the control. Moreover, it should be noted varying degrees of these changes: the greatest changes to the CBR observed in the prefrontal cortex and caudate nucleus, to a lesser extent - in the cerebellar cortex. Increase in receptor density can be considered as a compensatory effect associated with decreased affinity of CBR and decrease neuromediation GABA in the brains of patients suffering from alcoholism.

According to our research, the most sensitive to the effects of chronic alcohol on the human brain was the central-type BzDR related $GABA_AR$ in the prefrontal cortex and caudate nucleus, neuroadaptive or neuroplastic changes of which were most pronounced.

II. Study of the binding characteristics of a selective ligand [^{3}H] PK-11195 with mitochondrial membrane fractions obtained from different regions of human brain showed that the properties of peripheral-type benzodiazepine receptors (PBR) different in various areas of the human brain in both investigated groups: alcoholic patients (study group) and in the control group. Thus, these data support the PBR heterogeneity in different areas of the human brain in the investigated groups (Table 1, Figures 3 and 4).

As a result of our research the highest affinity of PBR was detected in the caudate nucleus, lower affinity of PBR has been identified in the prefrontal cortex and cerebellar cortex of control patients (Table 1 and Figure 3). The maximum density of PBR was detected in the prefrontal cortex, and they are represented by a smaller amount in the cerebellar cortex, caudate nucleus in control group (Table 1 and Figure 4).

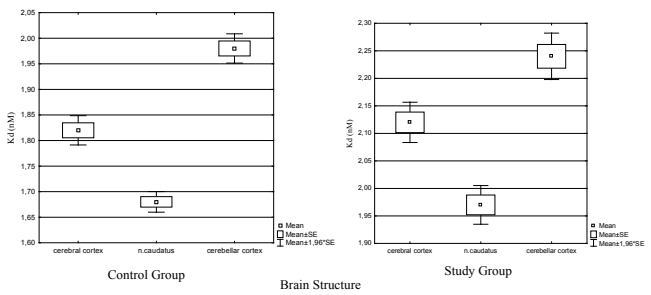
Comparative analysis of the binding characteristics of [^{3}H] PK11195 with mitochondrial membranes prepared from various structures of the brain of patients showed a significant increase in the Kd in the monitored areas of the brain in patients chronically consumed alcohol, as compared with patients in the control group, indicating that reducing the affinity of the PBR.

Receptor density (Bmax) was significantly increased in the structures of the brain in patients with alcoholism (Table 1 and Figure 4).

Area of the brain	[^{3}H] flunitrazepam binding to synaptosomal membranes				[^{3}H] PK-11195 binding to mitochondrial membranes			
	Control group (n=21)		Study group (n=21)		Control group (n=21)		Study group (n=21)	
	Kd^1 (nM)	Bmax^1 (fmol/mg protein)	Kd^1 (nM)	Bmax^1 (fmol/mg protein)	Kd^2 (nM)	Bmax^2 (fmol/mg protein)	Kd^2 (nM)	Bmax^2 (fmol/mg protein)
Prefrontal cortex {M ± SE}	1.82 ± 0.07	1772 ± 79	2.12 ± 0.09*	3165 ± 565*	2.45 ± 0.17	1824 ± 11	3.12 ± 0.13**	2245 ± 168**
N.caudatus {M ± SE}	1.68 ± 0.05	948 ± 112	1.97 ± 0.09*	2817 ± 386*	1.12 ± 0.09	724 ± 36	2.31 ± 0.16**	1895 ± 77**
Cerebellar cortex {M ± SE}	1.98 ± 0.1	1048 ± 67	2.2 ± 0.21*	1845 ± 217*	2.61 ± 0.21	1209 ± 98	3.32 ± 0.19**	2479 ± 123**

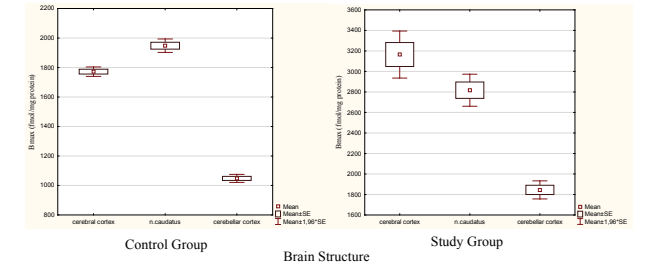
Notes: Bmax^1 - density of binding sites [^{3}Hflunitrazepam with synaptosomal membranes; Kd^1 - constant of dissociation ligand-receptor complex [^{3}H]flunitrazepam with CBR; Bmax^2 - density of binding sites [^{3}H]PK-11195 with mitochondrial membranes; Kd^2 - constant of dissociation ligand-receptor complex [^{3}H]PK-11195 with PBR; n - the number of cases studied; * - statistically significant difference indicators binding [^{3}H]flunitrazepam and ** - [^{3}H]PK-11195 between study and control groups, p<0.05

Table 1: Properties of [^{3}H] flunitrazepam and [^{3}H] PK-11195 binding to the synaptosomal and mitochondrial membranes from different areas of the human brain in alcoholic patients and control.



Affinity [^{3}H] Flunitrazepam binding to synaptosomal membranes in different areas of the human brain in the control and alcoholism.

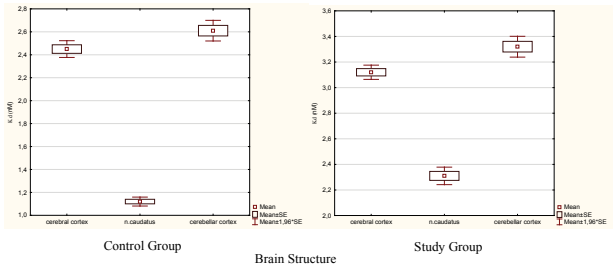
Figure 1: Statistical analysis of [^{3}H] flunitrazepam binding parameters {Kd (nM) – constant of dissociation ligand-receptor complex] with synaptosomal membranes in different areas of the human brain in control group and study group (alcoholic patients).



Density [^{3}H] Flunitrazepam binding to synaptosomal membranes in different areas of the human brain in the control and alcoholism.

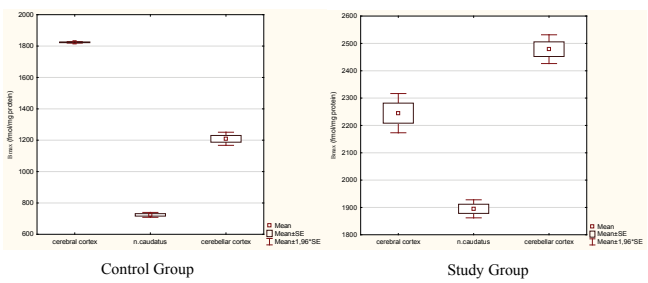
Figure 2: Statistical analysis of [^{3}H] flunitrazepam binding parameters [Bmax (fmol/mg of protein) – density of binding sites] with synaptosomal membranes in different areas of the human brain in control group and study group (alcoholic patients).

Comparative analysis of the binding characteristics of the selective ligands: [^{3}H] flunitrazepam and [^{3}H] PK-11195 with synaptosomal and mitochondrial membranes prepared from different regions of the human brain showed that the severity of changes in properties of BzDR varies in these structures of the brain of patients who had a history for alcoholism. The results indicate a non-uniform change in



Affinity [^{3}H] PK-11195 binding to mitochondrial membranes in different areas of the human brain in the control and alcoholism.

Figure 3: Statistical analysis of [^{3}H] PK-11195 binding parameters {Kd (nM) – constant of dissociation ligand-receptor complex] with mitochondrial membranes in different areas of the human brain in control group and study group (alcoholic patients).



Density [^{3}H] PK-11195 binding to mitochondrial membranes in different areas of the human brain in the control and alcoholism.

Figure 4: Statistical analysis of [^{3}H] PK-11195 binding parameters [Bmax (fmol/mg of protein) – density of binding sites] with mitochondrial membranes in different areas of the human brain in control group and study group (alcoholic patients).

the properties of BzDR (affinity and density of binding sites) selective ligands in human brain under the influence of chronic alcoholism, which confirms the hypothesis of adaptive neuroplasticity receptor heterogeneity and physiological response in different areas of the brain

to the effect of chronic alcohol consumption.

Thus, we have found that chronic alcoholism causes heterogeneous changes of benzodiazepine receptor system in different areas of the human brain, which is expressed:

a) reduction in the affinity of the "central" (CBR) and "peripheral" (PBR) receptor types: Kd values of CBR increase 1.2 times - in the prefrontal cortex, caudate nucleus, to a lesser extent - in the cerebellar cortex; Kd values of PBR increase 2.1 times in the caudate nucleus, 1.3 times - in the cerebellar cortex and prefrontal cortex of the brain, in the study group compared with the control;

b) increase the density of receptors (Bmax): the greatest change in density CBR found in the prefrontal cortex - 179%, cerebellar cortex - 176%, to a lesser extent in the caudate nucleus - 145%; PBR - in the caudate nucleus - 262%, in cerebellar cortex - 205%, in prefrontal cortex - 123% compared with the control group, which confirms the hypothesis of a differentiated response to physiological effects of alcohol on central nervous system as a result of its chronic use or as a possibility of developing alcohol dependence.

Discussion

Change in the $GABA_A$/BzDR systems in various areas of human brain is one of the most important mechanism in the development of addiction and the formation of alcohol relapse during abstinence [2,4,9,14].

Our studding of the properties of BzDR in various areas of the human brain showed that in the various brain structures: prefrontal cortex, the head of the caudate nucleus and the cerebellar cortex receptor properties of the "central" and "peripheral" type vary and in alcoholic patients affinity for the “central” and “peripheral” types receptor was significantly reduced in all investigated brain regions. The increased density of the “central” and “peripheral” types BzDR in various brain regions of patients with alcoholism identified in our study, can reflect a compensation for reduced receptor function (decreased affinity), that contributes to the development of tolerance to alcohol and the formation of the alcohol withdrawal syndrome.

Similar changes in the binding database were identified in the brains of patients with alcoholism, were in a state of abstinence quite a long period of time before the survey, as well as in individuals who consumed alcohol, but did not have alcohol dependence. This confirms that neuroplastic adaptive changes of BzDR in various areas of the brain occur earlier than the result of the expression formed the toxic effects of alcohol on the brain [9,30].

Chronic alcohol use causes various neuroadaptive changes that may be important in the development of alcohol dependence. Chronic ethanol exposure elicits changes in the subunit composition of $GABA_A$Rs, which, in turn, likely contribute to changes in receptor function associated with the altered pharmacological and behavioral sensitivity characteristic of ethanol tolerance and dependence. The high heterogeneity of the distribution of receptor subunits on the areas of the brain provides a functional differentiation $GABA_A$R in different nuclei of the basal ganglia, limbic areas and prefrontal cortex of the brain that determines the sensitivity of different receptors, and provides a differentiated degree of influence of $GABA_A$R modulators, including ethanol in various brain structures.

Binding database has differential sensitivity to α subunits; increased binding may be due to changes in the subunits composition that are observed in response to the alcohol withdrawal after chronic alcohol use. In the frontal cortex of alcoholics (postmortem studies) α1 subunit mRNA was increased, whereas mRNA α2, α3. α4 receptor subunits did not change significantly [19,31]. Previously it was shown that the effect of chronic ethanol exposure may lead to a decrease in the structure of the subunits of $GABA_A$/BzD receptors sensitive to benzodiazepines, increase the sensitivity of receptors to inverse agonist that causes a decrease $GABA_A$ receptor function [20].

Down regulation of $GABA_A$R containing β2 and β3 subunit leads to disruption of GABAergic function and inhibition processes in these areas of the brain that may cause the hyperexcitability, including seizure activity in the development of AWS [31].

Changes in the expression of neuronal elements induced by alcohol, leading to changes in neurotransmitter function adaptation systems in the brain associated with neuroplasticity [26]. CBR are sites of specific binding ligands BzD, alcohol and neurosteroids on the $GABA_A$R allosterically modulate its function, adjusting the balance of excitation and inhibition in the brain structures, affecting the activity of various neurotransmitter systems, including dopaminergic activity in brain structures involved the process of natural reinforcements. Exposure to ethanol causes a change in PBR, non-associated with $GABA_A$R but localized in the mitochondrial membrane, mainly in the glial cells of the brain, and ensuring the transfer of cholesterol into the mitochondria [29], thus influencing, the regulation of the synthesis of neurosteroids are endogenous modulators of the $GABA_A$/BzDR in the CNS [10,24]. Benzodiazepines, anxiolytics, anesthetics and alcohol are implementing some of its effects through the PBR, regulating production of neurosteroids and their metabolites, which are critical components of normal brain function. Ethanol may also modulate function of $GABA_A$/BzD receptor complex by increasing the de novo synthesis of neurosteroids in the brain through the PBR in a manner independent of the HPA axis. This latter mechanism may play an important role in the central effects of ethanol [19]. Thus, PBR indirectly affect GABAergic function in the brain, mainly in response to various neurotoxic effects and injury of the brain.

Chronic alcohol use causes different neuroadaptive changes in the properties of BzDR in various areas of the brain of alcoholic patients that may be important in the development of alcohol dependence. Changes in the expression of neuronal elements induced by alcohol and leading to changes in the adaptation of functions of neurotransmitter systems in the brain associated with neuroplasticity [26]. Alcohol abuse causes induction of neuroplasticity in the CNS, which may underlie the differences in susceptibility to alcohol and result in the emergence of compulsive behavior (compulsive, irresistible desire to drink alcohol) in patients with alcohol addiction. Alcohol may enhance the function of the $GABA_A$/BzDR with extrasynaptic localization, which revealed the relative insensitivity to the database, low ion conductivity CL^-, causing a decrease in GABAergic mediation in the brain [20].

Our results are consistent with other studies that showed a reduction in the function of $GABA_A$/BzDR in the prefrontal cortex in patients with alcohol dependence [9,25,30], which confirms the involvement of $GABA_A$/BzD receptor complex in the formation of anxiety disorders. Several studies and our data suggest that the low affinity of BzDR may be a marker of neuronal formation and development of anxiety and conditions associated with chronic alcoholism and AWS.

Our data confirm the existence of regulatory mechanisms that mediate the relationship between the properties of the $GABA_A$/BzDR caused receptor neuroplasticity and alcohol addiction [26]. Plasticity of ion channels regulated by ligands plays critical role in the development

of the nervous system, the formation and improvement of neuronal networks and processes associated with learning and memory, as well as various physiological and pathological processes. Receptors linked to ion channels that are dependent plasticity homeostatic preventing destabilization of the neuronal function in various physiological and pathophysiological processes.

Conclusion

Chronic exposure to ethanol results in harmful effects on the human brain: causing nonuniform adaptive changes of BzDR in various areas of the brain, which can modulate $GABA_AR$ and reduce neuromediation GABA in various areas of the brain which can cause alcohol addiction.

Conflict of interest: None declared

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