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REVIEW

The Influence of Ethanol on the Functional Status of $GABA_A$ Receptors

A. I. Golovko[1]*, S. I. Golovko[1], L. V. Leontieva[2], and S. Yu. Zefirov[1]

[1]Regional Therapeutic and Diagnostic Medical Center "Bekhterev", Korabelnaya ul. 6, St. Petersburg, 198096 Russia; fax: (812) 328-1324; E-mail: prgolovko@mail.ru
[2]West Virginia University, Box 9151, Morgantown, WV 26505-9151, USA; fax: (304) 293-0265; E-mail: Luba 105@hotmail.com

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Abstract—Gamma aminobutyric acid (GABA) is one of the main inhibitory neurotransmitters in the mammalian brain. Its effects are realized via $GABA_A$, $GABA_B$, and $GABA_C$ receptors. $GABA_A$ is the most abundant type of GABA receptors. It consists of six classes of subunits, α, β, γ, δ, ε, and χ. Acute and chronic exposures to ethanol are accompanied by changes in structure and function of $GABA_A$ receptors. These changes may be a basis for altered behavior seen in alcoholism.

Key words: GABA receptors, ethanol, subunits, expression

Narcotic effects of ethanol are associated with its non-electrolytic action. The latter includes inhibition on the central nervous system resulting from the interaction of ethanol with neuronal membranes [1, 2]. However, toxicological characterization of ethanol should take into consideration its modulating effects on structure–metabolic complexes of the brain [3]. Ethanol can influence energy metabolism, genome functioning, plasticity, biological membranes, and neurotransmitter systems [4-6]. The effects of ethanol on neurotransmitter systems are especially important.

First of all, ethanol-induced changes in the functional status of neurotransmitter systems are responsible for formation of various types of behavioral impairments including both pathological craving for ethanol or its inhibition [4, 7-10].

Secondly, structure–functional impairments of brain neurotransmitter systems are important elements of mechanisms underlying tolerance/sensitization to ethanol itself or to various pharmacological preparations (cross tolerance/sensitization) [11-15]. Consequently, possible changes in neurotransmitter systems should be taken into considerations during blockade of acute ethanol intoxication or medical treatment of chronic alcoholism.

Pre- and postsynaptic receptors are important elements of neurotransmitter systems. They can be considered as potential targets of toxic effects of ethanol [7-9, 15]. Modification of lipid environment and direct effects on supramolecular glycoprotein receptor complex are the simplest possible routes of ethanol effects on functioning of neuroreceptors.

During chronic ethanol intoxication changes in neurotransmitter system functioning, particularly their receptors, can stem from altered gene expression [10-12]. In the present review we have considered ethanol effects on receptors of γ-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter in the mammalian brain.

GABA RECEPTORS AND THEIR RESPONSES TO ACUTE EFFECTS OF ETHANOL

Central GABA receptors are subdivided into three classes: $GABA_A$, $GABA_B$, and $GABA_C$ receptors. The former is the most studied type of GABA receptors. Some evidence exists that $GABA_A$ receptor consists of 5 subunits. Several types of subunits, α, β, γ, δ, ε, and χ have been identified. They are subdivided into subtypes. Six subtypes of α-subunit (α_{1-6}), four subtypes of β-subunit (β_{1-4}), and four subtypes of γ-subunit (γ_{1-4}) have been isolated and the list of subunits has not yet complete. Subunits γ_2 exists as two subtypes which differ in the length of polypeptide chain. They are denominated γ_{2L} (long) and γ_{2S} (short). Various combinations of polypeptides provide diversity of $GABA_A$ receptors [5, 16, 17].

* To whom correspondence should be addressed.

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The subunits $\alpha_{1\text{-}6}$, $\beta_{1\text{-}4}$, $\gamma_{1\text{-}4}$, ε, and δ have been isolated from mammalian brain. Peripheral $GABA_A$ receptors also contain special a χ-subunit. The latter was discovered in the electric conducting system of human embryo. It shared 25-42% identity with previously studied α_4, β_2, γ_2, and γ_4 [18].

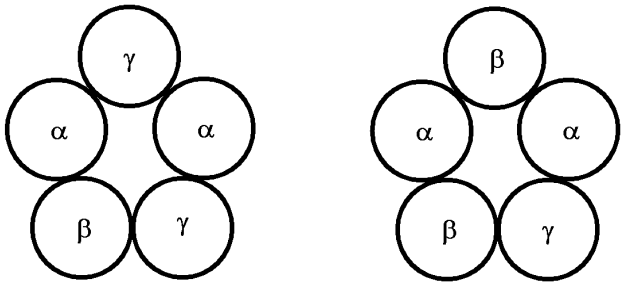
Molecular masses of several subunits have been estimated: α_1 = 50-51 kD, α_2 = 52-53 kD, α_3 = 58-61 kD, α_4 = 66-67 kD, α_5 = 53-55 kD, α_6 = 57-58 kD, β_1 = 57 kD, β_2 = 54-57 kD, β_3 = 57 kD, γ_1 = 45-51 kD, γ_{2S} = 45 kD, γ_{2L} = 47 kD, γ_3 = 43-46 kD, δ = 52-54 kD[1] [16, 17, 19].

The hydrophilic subunit surfaces form a chloride selective channel. An inhibitory effect of $GABA_A$ receptor mediated neurotransmission is related to increased membrane permeability to chloride. The latter is unequivocally distributed in the central nervous system of mammals: intracellular chloride concentration is 10-20 times lower than in the extracellular space. Opening of the chloride channel results in chloride influx, hyperpolarization of membranes and reduced excitation of neurons.

Central $GABA_A$ receptors usually consist of three types of subunits: α, β, and γ. The most frequent combinations of these subunits include the following oligomers: $2\alpha + 1\beta + 2\gamma$, $2\alpha + 2\beta + 1\gamma$, and $1\alpha + 2\beta + 2\gamma$ (figure) [20]. Usually receptor molecules contain two identical subunits (e.g., $\alpha_1 + \alpha_1$, $\beta_2 + \beta_2$, $\gamma_{2S} + \gamma_{2S}$, etc.) [16]. It is possible that some brain $GABA_A$ receptors include four types of subunits. It is also possible that some receptor molecules comprise just one or two subunits. Certain evidence exists that chloride channel sensitive to general anesthetics and GABA may be formed by one β_1- or β_3-subunit [21-23].

Each $GABA_A$ receptor subunit contains several domains. The large extracellular N-terminal domain represents nearly half of the molecular mass of the whole subunit. There are four transmembrane domains TM: TM1-TM4. A small extracellular site is localized between TM2 and TM3. A large intracellular domain located between TM3 and TM4 is a target for intracellular modulators including protein kinases [24]. A small C-terminal domain is facing the extracellular space [16, 25, 26].

The subunit surfaces are binding sites for numerous ligands. This includes specific binding sites for $GABA_A$ agonists and $GABA_A$ antagonists [27, 28], benzodiazepines (BD) [29, 30], chloride channel inhibitors, barbiturates, neurosteroids (such as synthetic alphaxalone and 3α-hydroxy-3β-methyl-5α-pregnane-20-one, progesterone metabolite 5α-pregnan-3α-ol-20-one, etc.), butyrolactones, melatonin, ethanol, antidepressants, polyunsaturated fatty acids, ions of zinc, terbium, lanthanum, etc. [16, 23, 31-35]. These ligands specifically interact with certain parts of a subunit (or subunits), but



The most frequent subunit combinations in central $GABA_A$ receptors (transverse section of the receptor) [16]

the efficacy of such interaction also depends of other subunits constituting the $GABA_A$ receptor molecule that are not involved in such binding. For example, benzodiazepine binding requires the presence of certain type of α-subunit. The affinity of the receptor also depends on type of γ-subunit. Only the β-subunit is inert in terms of binding of $GABA_A$ receptor ligands [16].

When a certain subunit is considered as a site of specific binding for some pharmacological agent, the final conclusion on discovery of a new receptor binding site is often corrected during subsequent studies. Now it is firmly recognized that many (more than ten) amino acid residues are involved in benzodiazepine ligand binding and the list is far from completion [29]. Specific binding sites for some compounds that influence GABA/benzodiazepine/chloride channel functioning still have not been precisely localized. This is the case of ethanol.

Acute treatments with ethanol lead to increase of $GABA_A$ receptor functioning and activation of GABA-ergic neurotransmitter systems. This is accompanied by inhibition of N-methyl-D-aspartate-glutamate (NMDA) receptors, mediating neurotransmitter effects of excitatory amino acids [14, 36-38]. Chronic exposure to ethanol and especially abstinence cause opposite changes [30, 39]. In other words, during acute effects of ethanol inhibition processes predominate in the central nervous system, whereas abstinence increases functional activity of excitatory neurotransmitter systems, particularly glutamate-ergic. These changes have been recognized not only in experiments employing cloned receptors and long-term alcohol but also in clinical practice. For example, we found quite reasonable pharmacological activity of flumazenil for the treatment of acute ethanol poisoning. This pharmacological agent inhibits GABA-ergic systems and benzodiazepine receptors [40, 41]. However, medical treatment of abstinent syndrome requires employment of pharmacological agents possessing opposite effects.

Augmentation of $GABA_A$ receptor functioning during acute treatments with ethanol is associated with direct and indirect effects of ethanol on the glycoprotein receptor complex. Indirect modulation of $GABA_A$ receptors is

[1] Some variations of molecular masses of subunits obviously reflect interspecies differences.

attributed to ethanol effect on membrane lipids; involvement of some other (chemically distinct) neurotransmitter systems is also possible. Membrane effects take place in neurotoxic mechanisms underlying acute ethanol poisoning. Perhaps this is a common feature of the non-electrolytic effects of other alcohols [2].

Thus, acute ethanol intake stimulates $GABA_A$ receptor functioning. This was demonstrated in *in vitro* experiments on evaluation of chloride channel functioning. As a rule these experiments were carried out using cloned receptors, neuronal receptors of brain sections, dissociated neuron populations, and synaptoneurosomes [42-44]. Augmentation of $GABA_A$ receptor mediated neurotransmission during acute ethanol treatment was also demonstrated in *ex vivo* experiments [45]. However, some authors failed to demonstrate stimulation of $GABA_A$ receptor functioning by ethanol [26, 46-48]. This suggests that a direct effect of ethanol on $GABA_A$ receptor complex may depend on many factors which vary in different laboratories. These include species, gender, and age of laboratory animals, brain region studied, characteristics of neuron populations within certain brain region, subunit composition of receptor complex and biological systems expression (cloned) receptors, spatial orientation of receptors in membrane (whether receptors are anchored in clusters or they are distributed over the postsynaptic membrane), the existence of phosphorylating sites receptor molecule for phosphorylation by protein kinase C, etc. (Table 1). The range of doses (concentrations) of ethanol used in such experiments is also important.

The use of gene engineering and cloning of receptor complexes explained some biochemical aspects of the heterogeneity of $GABA_A$ receptor reactions to ethanol. Subunit composition of receptor complexes, mutual receptor orientation in neuronal membrane, and the existence of special intracellular biochemical system mediating the ethanol effect on receptor are important factors underlying $GABA_A$ receptor responsiveness to ethanol. Certain evidence exists that protein kinase C is involved in the stimulating effect of ethanol on the chloride channel activity of $GABA_A$ receptor [42].

A stimulating effect of ethanol was found on those GABA receptor complexes which include the main subunits, α, β, and γ. The effectiveness of the ethanol effect depends on types of α- and β-subunits. However, in some studies correlation between receptor composition and its responsiveness to ethanol was not found [43, 46].

Good evidence exists that the γ-subunit plays an important role in the $GABA_A$ receptor responsiveness to ethanol. Receptors containing γ_{2L}-subunit exhibit the highest sensitivity to ethanol. For example, ethanol increased chloride current induced by $GABA_A$ receptor agonist, muscimol, only in receptor complexes containing the γ_{2L}-subunit [26, 43, 46].

Thus, subunit composition of receptors is crucial for responsiveness to acute effects of ethanol. However, subunit ratio in the receptor molecule may be changed during intoxication due to altered gene expression. For example, 30 min after a single administration of a large dose of ethanol (4 g/kg, intraperitoneally, i.p.) to C57BL/2J mice increased the level of mRNA for α_1- and β_3-subunits was found in cerebellum, whereas levels of mRNA for α_6-, β_2-, and γ_2-subunits remained virtually unchanged. In that study changes in subunit composition were evaluated by alterations of specific binding of various radioligands [44]. However, it should be emphasized that changes $GABA_A$ receptors attributed to gene expression are more typical for long term alcohol consumption.

$GABA_A$ RECEPTORS DURING CHRONIC ETHANOL TREATMENT AND ABSTINENCE

Long term treatment with ethanol *in vitro* and *ex vivo* was accompanied by changes in functional characteristics of $GABA_A$ receptors. First of all, chronic treatment with ethanol was accompanied by reduced channel permeability for chloride anions [13, 50, 52].

Prolonged exposure of neurons or transfected cell cultures to ethanol was also accompanied by altered sensitivity of GABA/benzodiazepine/chloride channel complex to allosteric modulators and to acute effect of ethanol (Table 1).

The data of Table 1 suggest that functional changes of $GABA_A$ receptors during chronic treatments with ethanol may be due to changes in gene expression and/or posttranscriptional and posttranslational events [50, 58]. This suggestion was confirmed in animal experiments and also during examinations of alcoholics. Changes of $GABA_A$ receptor functioning were recognized during chronic ethanol intoxication [19, 59, 60] and in abstinence [19, 45, 60, 61]. Some authors believe that formation of alcohol abstinent syndrome involves attenuation of GABA-ergic neurotransmission with simultaneous increase of functional activity of N-methyl-D-aspartate receptors [39, 40].

The effects of alcohol treatment, alcohol withdrawal and abstinence on mRNA levels of various $GABA_A$ receptor subunits have been investigated in numerous studies (Table 2). These treatments exerted different effects on mRNAs. Moreover, changes in mRNA levels do not necessarily correlated with content of corresponding protein subunit. For example, treatment of male rats with ethanol (daily dose 10-12 g per kg) for 14 days resulted in significant (40%) reduction of α_1-subunit right after alcohol withdrawal, whereas the content of α_4-subunit increased by 27%. These changes were maintained during the subsequent 6-8 h. Changes in content of α_1- and α_4-subunits correlated with changes in mRNA levels only at the period of alcohol withdrawal but not during abstinence. The levels of β_2-, β_3-, and γ_1-subunits were significantly high-

Table 1. Effects of ethanol on the functional status of $GABA_A$ receptors

No.	Research object	Mode (and doses) of ethanol effect	$GABA_A$ receptor functioning after treatment with ethanol	Reference
1	2	3	4	5
1	$GABA_A$ receptors of brain synaptoneurosomes and synaptic membranes of male Fischer-344 rats	acute treatment with 50 mM ethanol *in vitro*	ethanol[a] stimulates uptake of radioactive chloride induced by 10 mM GABA[b]	[14]
2	$GABA_A$ receptors (subunit composition $\alpha_1\beta_1$) cloned in *Xenopus laevis* oocytes	acute treatment with 30-100 mM ethanol *in vitro*	lack of ethanol effect on GABA-induced chloride current	[26]
3	$GABA_A$ receptors (subunit composition $\alpha_1\beta_1\gamma_{2S}$) cloned in *Xenopus laevis* oocytes	acute treatment with 30-100 mM ethanol *in vitro*	ethanol stimulates GABA-induced chloride current	[26]
4	$GABA_A$ receptors of hippocampal neurons of male Sprague–Dawley rats	acute treatment with 40-160 mM ethanol *in vitro*	ethanol increases GABA-induced post-synaptic inhibitory potentials; this effect increased after precooling of hippocampal slices[c]	[42]
5	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2L}$) cloned in *Xenopus laevis* oocytes	acute treatment with 20 mM ethanol *in vitro*	ethanol stimulated chloride current induced by 20 μM GABA	[43]
6	$GABA_A$ and benzodiazepine receptors of cerebellar neurons of female C57BL/6J mice	single dose administration of ethanol (4 g/kg, i. p.)	increase of specific binding of [^{3}H]muscimol and [^{3}H]flunitrazepam	[44]
7	Male Wistar rats	single dose administration of ethanol (1 g/kg per os)	increase of GABA-ergic muscimol- and diazepam-induced neurotransmission in nucleus accumbens, hippocampus, and cerebellum	[45]
8	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2L}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_1\beta_3\gamma_{2S}$, $\alpha_5\beta_3\gamma_3$, $\alpha_6\beta_3\gamma_{2S}$, $\alpha_1\beta_1\gamma_{2L}$, $\alpha_1\beta_1$) cloned in L(tk-) cell line	acute treatment with 5-50 mM ethanol *in vitro*	ethanol increased muscimol-induced uptake of radioactive chloride only in receptor complexes containing subunit γ_{2L}	[46]
9	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2L}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_1\beta_3\gamma_{2S}$, $\alpha_5\beta_3\gamma_3$, $\alpha_6\beta_3\gamma_{2S}$, $\alpha_1\beta_1\gamma_{2L}$, $\alpha_1\beta_1$) cloned in *Xenopus laevis* oocytes	acute treatment with 30-100 mM ethanol *in vitro*	weak effect of muscimol-induced uptake of radioactive chloride	[46]
10	$GABA_A$ receptors (subunit composition $\alpha_1\beta_1$) cloned in X25 cell line	acute treatment with 10-100 mM ethanol *in vitro*	lack of ethanol effect on muscimol-induced uptake of radioactive chloride	[47]
11	$GABA_A$ receptors (subunit composition $\alpha_1\beta_1\gamma_{2L}$) cloned in PA3 cell line	acute treatment with 3-30 mM ethanol *in vitro*	ethanol increases muscimol-induced uptake of radioactive chloride	[47]

Table 1. (Contd.)

1	2	3	4	5
12	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2S}$, $\alpha_6\beta_2\gamma_{2S}$, $\alpha_6\beta_2\gamma_{2L}$) cloned in HEK-239 human embryonic kidney cell line	acute treatment with 10-100 mM ethanol *in vitro*	lack of ethanol effect on GABA-induced chloride current; ethanol attenuated GABA-induced desensitization of $\alpha_6\beta_2\gamma_{2S}$ receptors; lack of ethanol effect on the rate of GABA-induced desensitization of $\alpha_1\beta_2\gamma_{2S}$ receptors	[48]
13	$GABA_A$ receptors (subunit composition $\alpha_1\beta_1\gamma_{2L}$, $\alpha_1\beta_2\gamma_{2L}$) cloned in L(tk-) cell line	acute treatment with 10 mM ethanol *in vitro*	ethanol increased muscimol-induced uptake of radioactive chloride; lack of ethanol effect on cells pretreated with colchicine, taxol, and vinblastin[d]	[49]
14	$GABA_A$ receptors (subunit composition $\alpha_5\beta_3\gamma_3$, $\alpha_6\beta_3\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2L}$, $\alpha_1\beta_3\gamma_{2S)}$ cloned in L(tk-) cell line	acute treatment with 10 mM ethanol *in vitro*	ethanol increases muscimol-induced chloride current	[50]
15	$GABA_A$ receptors (subunit composition $\alpha_{1,2,3,4,5}$, $\beta_{1,2,3}$, γ_{2S}, and γ_{2L}) cloned in P19-N cell line	acute treatment with 50 and 100 mM ethanol *in vitro*	ethanol increases chloride current induced by 10 μM muscimol	[51]
16	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2L}$, $\alpha_1\beta_2\gamma_{2S}$) cloned in L(tk-) cell line	chronic treatment with 100 mM ethanol *in vitro* for 4 days	attenuation of radioactive chloride uptake induced by 0.5 μM muscimol and 10 μM flunitrazepam	[13]
17	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2L}$) cloned in L(tk-) cell line	chronic treatment with 100 mM ethanol *in vitro* for 24 h	attenuation of the inhibitory effect of reversed agonist of benzodiazepine receptors, DMCM[e], in uptake of radioactive chloride induced by 2 μM muscimol; attenuation of activating effect of neurosteroid pregnanolone on uptake of radioactive chloride induced by 0.5 μM muscimol	[13]
18	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_6\beta_3\gamma_{2S}$) cloned in L(tk-) cell line	chronic treatment with 100 mM ethanol *in vitro* for 24 h	maintenance of the activating effect of pentobarbital on uptake of radioactive chloride induced by 0.5 μM muscimol; maintenance of direct activating effect of pentobarbital on uptake of radioactive chloride	[13]
19	$GABA_A$ receptors (subunit composition $\alpha_1\beta_2\gamma_{2L}$) cloned in L(tk-) cell line	chronic treatment with 100 mM ethanol *in vitro* for 24 h	attenuation of activating effect of ethanol on uptake of radioactive chloride induced by 0.5 μM muscimol	[13]

Table 1. (Contd.)

1	2	3	4	5
20	$GABA_A$ receptors of cerebral cortex membranes of ethanol resistant mice	chronic (10 days) (per oral) treatment with ethanol (21.6 g per kg daily)	lack of changes in muscimol-induced uptake of radioactive chloride; lack of changes in muscimol- and flunitrazepam-induced uptake of radioactive chloride; lack of changes of the inhibitory effect of reversed benzodiazepine receptor agonists on uptake of radioactive chloride	[30]
21	$GABA_A$ receptors of cerebral cortex membranes of ethanol sensitive mice	chronic (10 days) (per oral) treatment with ethanol (20.5 g per kg daily)	lack of changes in muscimol-induced uptake of radioactive chloride; lack of changes in muscimol- and flunitrazepam-induced uptake of radioactive chloride; ethanol increased the inhibitory effect of reversed benzodiazepine receptor agonists on uptake of radioactive chloride	[30]
22	$GABA_A$ and benzodiazepine receptors of cerebellar neurons of female C57BL/6J mice	chronic treatment with ethanol for 14 days; ethanol represented 5-7.5% of daily liquid consumption	lack of changes of specific binding of [^{3}H]muscimol and reduced binding of [^{3}H]flunitrazepam	[44]
23	Male Wistar rats	chronic (per oral) treatment with ethanol (5.0 g per kg daily) for 21 days	lack of effect of diazepam-induced GABA-ergic neurotransmission in nucleus accumbens, hippocampus and cerebellum; inhibition of muscimol-induced GABA-ergic neurotransmission in nucleus accumbens, hippocampus and cerebellum	[45]
24	$GABA_A$ receptors (subunit composition $\alpha_5\beta_3\gamma_3$, $\alpha_6\beta_3\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2L}$, $\alpha_1\beta_3\gamma_{2S}$) cloned in L(tk-) cell line	chronic treatment with 92-100 mM ethanol *in vitro* for 3.3-24 h	attenuation of chloride currents induced by muscimol and flunitrazepam; lack of ethanol effect on muscimol-induced chloride currents	[50]
25	$GABA_A$ receptors (subunit composition $\alpha_1\beta_1\gamma_{2L}$) cloned in PA3 cell line	chronic treatment with 50 and 100 mM ethanol *in vitro* for 4 days	attenuation of radioactive chloride uptake induced by GABA and flunitrazepam (1 μM); lack of changes in specific binding of [^{3}H]flunitrazepam (including GABA-stimulated binding)	[52]
26	Long–Evans male rats	chronic per oral treatment with ethanol (12-13 g per kg daily) for 28 weeks	lack of changes in specific binding of $GABA_A$ receptor agonist [^{3}H]bicuculline (10 and 100 nM) in CA1 and dentate gyrus; increase of binding of 500 nM [^{3}H]bicuculline	[53]

Table 1. (Contd.)

1	2	3	4	5
27	$GABA_A$ receptors of microvesicles from cerebral cortex, hippocampus and midbrain of Sprague–Dawley male rats	chronic per oral treatment with ethanol (6 g per kg daily with two day interval) for 90 days	attenuation of radioactive chloride uptake induced by 30-100 μM muscimol was observed only in hippocampal preparations	[54]
28	$GABA_A$ receptors of CA1 neurons of male Sprague–Dawley rats	chronic per oral treatment with ethanol (6 g per kg daily with two day interval) for 90 days	attenuation of GABA-ergic postsynaptic inhibition	[54]
29	$GABA_A$ receptors of brain neurons of male C3H mice	chronic inhalation treatment with ethanol for 16 h (4 cycles with intervals for 8 h)	inhibition of radioactive chloride uptake induced by GABA (10 μM) and diazepam	[55]
30	$GABA_A$ receptors of neurons of cerebral cortex, hippocampus, amygdala, and other brain structures of ethanol-preferring male rats in comparison with Wistar rats (control)	chronic treatment with ethanol for 28 days; ethanol represented 10% of daily liquid consumption	lack of changes in specific binding of $GABA_A$ receptor agonist [^{3}H]SR 95531 in all brain structures of both groups of animals; increase of specific binding of $GABA_A$ receptor agonist [^{3}H]muscimol in cerebral cortex, lateral septum, striatum of ethanol-preferring rats (compared with control)	[56]
31	$GABA_A$ receptors of synaptosomes from cerebral cortex of male and female Sprague–Dawley rats	chronic treatment with ethanol for 15 days; ethanol represented 6-7.5% of daily liquid consumption	attenuation of radioactive chloride uptake induced by 20 μM GABA (both in male and female rats); lack of changes in radioactive chloride uptake induced by neurosteroid 3α,21-dihydroxy-5α-pregnane-20-one	[57]

[a] During evaluation of functional state of $GABA_A$ receptors ethanol was always present in the incubation medium.
[b] Lack of information on concentration used means that the experiments were carried out using a wide range of concentrations.
[c] Similar effect of "cold" stress is interpreted in term of activation of protein kinase C in cytoplasm of neurons.
[d] Colchicine, taxol, and vinblastin cause depolymerization of microtubules, which join postsynaptic receptors into clusters.
[e] Methyl-6,7-4-dimethoxy-4-ethyl-β-carboline-3-carboxylate.

er in all periods studied, whereas the content of γ_2-subunit remained within the range of initial values [19, 60].

Abstinence after long-term alcohol treatment of rats for 60 days (daily per oral doses of ethanol were 5-6 g per kg) did not influence mRNA levels of α_5-subunit in hippocampus (CA1, CA3 and dentate gyrus), II and III layers of brain cortex, and thalamus. However, this treatment caused an increase of mRNA level for α_4-subunit in these structures [63].

Charlton et al. [62] studied the effect of alcohol treatment of male rats on the content of α_1- and α_5-subunits in the following various brain regions: frontal parietal cortex, ventral tegmental area, nucleus accumbens, substantia nigra, and hippocampus. Ethanol represented 5% of daily liquid consumption. Animals were treated for 1, 2, 4, and 12 weeks. Expression of α_1- and α_5-subunits was regulated by ethanol in a region-specific and time-dependent manner. In most regions a decrease in subunit content was detected. Hippocampal α_1-subunit mRNA content was significantly reduced (to 53% of control) after 12 weeks of the treatment. In contrast, α_5-subunit mRNA content was increased in this brain region by 64%. Protein content of α_1- and α_5-subunits changed in similar manner: there was 32% decrease in α_1-subunit content and increase in α_5-subunit by 64% [62].

Decrease of α_1-subunit mRNA level in various cerebellar neurons was also recognized after chronic ethanol consumption in mice for 14 days (ethanol represented 5-

Table 2. Effect of ethanol on the levels of mRNA and corresponding subunits of $GABA_A$ receptor

No.	Research object	mRNA content	Subunit protein content	References
1	2	3	4	5
1	postmortem prefrontal cortex of alcoholics	increase of α_1-, β_2-, and β_3-subunit mRNAs; lack of changes in α_4-subunit mRNA	lack of changes in the content of α_1-, α_4-, β_2-, and β_3-subunits	[12]
2	cerebellum and cerebral cortex of male Sprague–Dawley rats	not determined	1 and 48 h after alcohol withdrawal no changes were detected in α_1-subunit content	[17]
3	cerebral cortex of male Sprague–Dawley rats	not determined	decrease in α_1-subunit content, increase of α_4-, β_2-, β_3-, and γ_1-subunit content; lack of changes in γ_2-subunit	[19]
4	cerebellum of chronically alcoholized female mice C57 BL/6J	reduction in α_1-subunit mRNA, increase of α_6- and γ_2-subunit mRNAs; lack of changes in β_2- and β_3-subunit mRNAs	changes in subunit content corresponded to changes in respective mRNAs	[44]
5	cerebellum of female mice C57 BL/6J subjected to a single dose ethanol administration	increase of α_1- and β_3-subunit mRNAs; lack of changes in α_6-, β_2-, and γ_2-subunit mRNAs	changes in subunit content corresponded to changes in respective mRNAs	[44]
6	cerebral cortex, hippocampus, amygdala, and other brain structures of male rats preferring ethanol in comparison with Wistar male rats (control)	decrease of α_1-subunit mRNA in substantia nigra, increase of α_1-subunit mRNA in some other brain regions of rats preferring ethanol; lack of changes in α_2-subunit mRNA in all brain regions	not determined	[56]
7	cerebral cortex of male and female Sprague–Dawley rats	not determined	increase in β_2- and β_3-subunits and lack of changes in α_1-, α_4-, and γ_2-subunit content in female rats; increase of β_2-, β_3-, and α_4-subunits, decrease of α_1- and lack of changes in γ_2-subunit content in male rats	[57]
8	brain of ethanol sensitive mice	decrease in α_1-subunit mRNA, increase of γ_3-subunit mRNA, lack of changes in α_3-, α_6-, and γ_{2L}-subunit mRNAs	not determined	[59]
9	brain of ethanol resistant mice	decrease in α_6-subunit mRNA, increase of γ_3-subunit mRNA, lack of changes in α_1-, α_3-, and γ_{2L}-subunit mRNAs	not determined	[59]
10	cerebral cortex of male Sprague–Dawley rats	short term reduction of α_1-subunit mRNA after termination of alcoholization	long term decrease in α_1-subunit after termination of alcoholization	[60]
11	cerebral cortex of male Sprague–Dawley rats	short term increase in α_4-subunit mRNA after termination of alcoholization	long term increase in α_4-subunit after termination of alcoholization	[60]
12	cerebral cortex of female Sprague–Dawley rats	long term increase in α_1-subunit mRNA after termination of alcoholization	long term increase in α_1-subunit after termination of alcoholization	[60]

Table 2. (Contd.)

1	2	3	4	5
13	cerebral cortex of female Sprague–Dawley rats	short term increase in α_4-subunit mRNA after termination of alcoholization	long term increase in α_4-subunit after termination of alcoholization	[60]
14	cerebral cortex of male Sprague–Dawley rats	not examined	reduction of α_1- and α_5-subunits after two and four weeks of alcoholization, lack of changes in α_1- and α_5-subunit content after 12 weeks of alcoholization	[62]
15	cerebellum of male Sprague–Dawley rats	not examined	reduction in α_1- and α_5-subunits after 4 weeks of alcoholization	[62]
16	oblongatal ventral tegmental region of male Sprague–Dawley rats	not examined	reduction in α_1-subunit content after 12 weeks of alcoholization	[62]
17	substantia nigra of male Sprague–Dawley rats	not examined	lack of changes in α_1-subunit content after 1, 2, 4, and 12 weeks of alcoholization	[62]
18	hippocampus of male Sprague–Dawley rats	not examined	reduction in α_1-subunit and increase of α_5-subunit content after 12 weeks of alcoholization	[62]
19	hippocampus, cerebral cortex, and thalamus of male Sprague–Dawley rats	increase in α_4-subunit mRNA was more pronounced in hippocampus, lack of changes in α_5-subunit mRNA in all structures studied	not determined	[63]
20	cerebellum, cerebral cortex, and hippocampus of male Sprague–Dawley rats	lack of changes in α_1-, α_4-, α_5-, α_6-, γ_1-, γ_2-, γ_{2L}-, and γ_{2S}-subunit mRNAs in all brain structures except cerebellum (where increase of α_6-subunit mRNA is found); reduction of γ_{2L}-mRNA/γ_{2S}-mRNA ratio in hippocampus	lack of changes in α_1-, α_6-, γ_{2L}-, and γ_{2S}-subunit content in all structures except cerebellum (where increased α_6-subunit level was detected) after termination of alcoholization; normalization of α_6-subunit level after 6 days of withdrawal	[64]
21	cerebral cortex of male Sprague–Dawley rats	reduction in α_1-subunit mRNA, increase of α_4-, γ_1-, and γ_{2S}-subunit mRNAs; lack of changes of α_5-, β_1-, β_2-, β_3-, δ-, and γ_{2L}-subunit mRNAs	not determined	[65]
22	cerebral cortex and cerebellum of male Sprague–Dawley rats	reduction in α_1-, α_2-, and α_5-subunit mRNAs in cerebral cortex, reduction of α_1- and increase of α_6-subunit mRNAs in cerebellum	reduction in α_1-, α_2-, and α_3-subunit levels in cerebral cortex and increase of α_6-subunit content in cerebellum	[66]
23	cerebral cortex of male Sprague–Dawley rats	increase in β_1-, β_2-, and β_3-subunit mRNAs at the moment of termination of chronic alcoholization; increase of β_2- and β_3-subunit mRNAs 24 h after ethanol withdrawal	increase in β_2- and β_3-subunit levels at the moment of termination of chronic alcoholization	[67]

Note: In all studies persistent chronic alcoholization of animals was used. In [44] animals were treated with single and chronic administrations of ethanol; in [12] post-mortum preparations of the prefrontal cortex of alcoholics were used.

7% of daily liquid consumption). However, such treatment caused an increase in mRNA levels for α_6- and γ_2-subunits. No changes were found for in levels of β_2- and β_3-subunit mRNAs. There was a correlation between changes in mRNA levels and subunit content which was evaluated by specific binding of [^{3}H]flunitrazepam and [^{3}H]muscimol [44].

There were several postmortem studies of $GABA_A$ receptor subunits and their corresponding levels in brains of alcoholics. In prefrontal cortex increased levels of mRNA of α_1-, β_2-, and β_3-subunits were found (level of α_4-subunit mRNA remained unchanged) [12]. No changes were detected in content of these subunits. Genome analysis of alcohol abusers revealed changes in allele frequencies of genes encoding $GABA_A$ receptor subunits, particularly α_3-subunit gene located on X-chromosome [10].

Attempts of interpretations of some changes morpho-functional characteristics of $GABA_A$ receptors in terms of manifestations of chronic alcoholization or abstinent syndrome meet certain difficulties [15, 38]. It is clear that a particular role of some subunit cannot account for the appearance of particular signs. For example, involvement of α_6-subunit in some neurotoxic effects of ethanol was firmly recognized. However, in experiments on mice lacking α_6-subunit mRNA it was convincingly demonstrated that this subunit is not the unique element underlying the neurotoxic effect of ethanol [9].

Increased excitement and decreased seizure threshold seen in chronic ethanol consumption and abstinence are determined by inhibition of $GABA_A$ receptor functioning [54]. The important role of GABA-ergic insufficiency during alcohol consumption and abstinence is supported by the following observations.

First of all functional insufficiency of $GABA_A$ receptors was found in experiments employing chloride currents. For example, one day after ethanol withdrawal (ethanol was given for 60 days at a dose 6 g per kg) significant reduction of muscimol-induced uptake of radioactive chloride was observed in hippocampal slices. (In some other brain regions this parameter did not differ from control values.) Parallel electrophysiological experiments revealed attenuation of GABA-ergic inhibitory control of spike activity in CA1 pyramidal neurons. This phenomenon remained by the 40th day after the beginning of abstinent syndrome. These results are quite important if we take into consideration unique role of hippocampus in formation of proconvulsant and initiation and maintenance of convulsant activity [54].

Secondly, some indirect evidence exists that chronic alcohol treatment is accompanied by suppression of GABA-dependent inhibition and GABA-positive preparations attenuate seizure activity in abstinent period whereas GABA negative ones increase it [7, 54].

Genetic mechanisms responsible for the development of abstinent syndrome are now intensively studied. Five loci have been investigated in the mouse genome. For example, the locus on 11 chromosome is responsible for ~12% genetic differences in the development of abstinence. It is closely related to genes encoding α_1-, α_6-, and γ_2-subunits of $GABA_A$ receptors [11].

Thus, $GABA_A$ receptor is a supramolecular complex; it is sensitive to toxic effects of ethanol. Acute treatment with ethanol is accompanied by increased functioning of $GABA_A$ receptors, whereas chronic ethanol intoxications have an opposite effect. Changes in $GABA_A$ receptor functioning may be attributed to altered expression of subunits constituting this receptor complex. Impairments of the morpho-functional status of $GABA_A$ receptors may be a basis for some manifestations of ethanol intoxication. They should be taken into consideration during medical treatment of alcoholism.

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