

Alcohol Modulation of Extra-synaptic Gamma-aminobutyric Acid Type A Receptors

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Abstract

Development of effective treatment agents for the alcohol use disorders requires the detailed understanding of molecular targets of alcohol in the brain. The gamma-aminobutyric acid type A receptors (GABA_ARs) are the major molecular targets of alcohol in the central nervous system. Mediating inhibitory neurotransmission upon GABA binding in the vertebrate central nervous system, GABA_ARs are heteropentameric chloride channels, assembled from a large subunit pool encoded by 19 distinct genes. It is the subunit composition that determines the receptor's biophysical properties, neurotransmitter affinity, the pharmacology, and the position on the cell i.e., synaptic or extra-synaptic. This review paper briefly presents the alcohol modulation of a specific GABA_AR subtype located at the extra-synaptic sites with a subunit composition of α , β and δ .

Keywords: Alcohol, GABA, extra-synaptic, GABA (A) receptors, δ (delta) subunit

1. Introduction

Affecting about 18 millions of adult Americans, alcohol abuse and alcohol dependence are classified as alcohol use disorders (AUD), which are not satisfactorily treatable. For example, Benzodiazepines (BZs), used for the treatment of symptoms of AUDs, cause addiction and Naltrexone, despite being an effective therapeutic agent, has severe side effects (Liang and Olsen, 2014). Thus, development of better treatment agents for the AUDs is essential which requires a detailed understanding of molecular targets of alcohol.

Accumulating evidence in the literature suggests that gamma-aminobutyric acid type A receptors (GABA_ARs) are the major target of alcohol in the brain (Mihic and Harris, 1997; Boehm et al., 2004; Kumar et al., 2009). GABA_ARs are the member of "Cys-loop receptors" together with nicotinic acetylcholine receptors (nAChR), the 5-hydroxytryptamine type 3 (5-HT₃) receptors, the zinc-activated ion channel (ZAC) and the glycine receptors (GlyR) (reviewed in Sine and Engel, 2006). They are GABA-gated heteropentameric chloride channels responsible for the fast inhibitory synaptic transmission in the vertebrate central nervous system (CNS) (reviewed by Sieghart and Sperk 2002). The GABA_ARs display a rich molecular and cellular diversity, which result in distinct functional roles. Assembled from a large subunit pool, receptor subunit composition affects the receptor gating, kinetics and the response to allosteric modulators (Haas and Macdonald, 1999; Lavoie, et al., 1997). Besides, subunit composition

is a determinant of the cellular and subcellular localization of the receptor, i.e., synaptic or extra-synaptic sites (Jones et al, 1997; Brickley et al., 2001; Goetz et al., 2007).

2. The Subunit Composition of GABA_ARs: Synaptic and Extra-synaptic Receptors

One of the most distinguishing features of GABA_ARs is the wide repertoire of subunits from which the receptor assembles (Seeburg et al., 1990). The GABA_ARs are assembled from a pool of 19 subunits ($\alpha 1$ – $\alpha 6$, $\beta 1$ – $\beta 3$, $\gamma 1$ – $\gamma 3$, δ , ϵ , θ , π , $\rho 1$ – $\rho 3$) (Rudolph and Mohler, 2006). The expression of the subunit genes is age- and region dependent (Wisden et al., 1992; Laurie et al., 1992a, b; Fritschy and Mohler, 1995; Schwarzer et al., 2001). The most abundant GABA_ARs in the mammalian brain seem to be the combination of $\alpha\beta\gamma 2$ subunits with a subunit ratio of $2\alpha/2\beta/1\gamma$ (Ernst et al., 2003). These $\gamma 2$ containing GABA_ARs ($\gamma 2$ -GABA_ARs) mediate classical fast synaptic inhibition (phasic inhibition) and massively clustered in the synapses. On the other hand δ subunit containing GABA_ARs receptors (δ -GABA_ARs), typically in combination with $\alpha 6$ and β subunits in the cerebellum (Jones et al, 1997; Brickley et al., 2001); and in combination with $\alpha 4$ and β subunits (Patel et al., 2014) in the forebrain, are located extra-synaptically or perisynaptically (Nusser et al., 1998; Wei et al., 2003). Activated by GABA diffusing out of the synaptic cleft, these receptors mediate a special form of inhibition called the tonic inhibition characterized by a higher affinity for GABA and with a slower

desensitization rate (Nusser and Mody, 2002). Tonic inhibition is critical for the threshold for the action potential generation. It shunts the excitatory synaptic signals controlling neuronal excitability (Hamann, et al., 2002, Semyanov et al., 2004). Thus the extra-synaptic GABA_ARs mediate a physiologically different form of GABAergic signaling than the synaptic receptors (Brickley and Mody 2012). The diversity of GABA_AR subunits with distinct physiological functions (Mody and Pearce, 2004) is also apparent at the level of its ligands. Benzodiazepines, barbiturates, alcohol and neurosteroids are the modulators of GABA_ARs with differences in efficacy, potency and binding sites in a subunit dependent manner (Goetz et al, 2007). In this study we will present the alcohol modulation of extra-synaptic receptors containing $\alpha 4\beta\delta$ or $\alpha 6\beta\delta$ subunits (δ -GABA_ARs).

3. The structure of the GABA_ARs

Until last year, the molecular structure of a GABA_AR subunit complex was based on the studies of the muscle nAChR from the electric organ of the torpedo ray and the snail acetylcholine receptor binding protein (AChBP) (Brejc et al., 2001; Cromer et al., 2002; Ernst et al., 2003; Unwin, 2003, 2005; Sine and Engel, 2006). However, the recent work done by Miller and Aricescu (2014) reports the crystallized structure of homomeric $\beta 3$ subunit containing GABA_ARs (GABA_AR- $\beta 3$ cryst) at 3Å resolution which provides a direct overview for the receptor structure for the first time. Together with the recent structural data from 5HT₃ receptors (Hassaine et al., 2014), these studies confirm the characteristics of eukaryotic Cys-loop receptors (reviewed by Lynagh and Pless, 2014).

GABA_ARs are pentamers consist of five subunits arranged counterclockwise (i.e., $\gamma, \alpha, \beta,$) around a central pore. Each subunit comprises a long N- terminus located at the extracellular domain (ECD), followed by four transmembrane domains (TM1–TM4), and a short extracellular C-terminal. There is a large intracellular loop between the third and fourth transmembrane domains. According to Miller and Aricescu (2014) the receptor looks like a cylinder with a height of 110Å and with a diameter of 60 to 80Å. From the extracellular side, the receptor is surrounded by 15 N-linked glycans. Each extracellular domain (ECD) comprises an amino-terminal α -helix ($\alpha 1$) followed by ten β -strands in parallel with the structure of other family members (reviewed in Lynagh and Pless, 2014). A second α -helix ($\alpha 2$), between β -strands 3 and 4, is located under the $\alpha 1$ helix. The pentameric transmembrane domain (TMD) is composed of four additional helices (M1–M4) from each subunit that come together to form a lining a pore with M2 segments. The subunit assembly is mediated by

hydrogen bonds, van der Waals forces and salt bridges in the subunit ECDs, which also involve the neurotransmitter binding pocket.

When subunits are assembled in to the heteropentameric receptor, the neurotransmitter binding pocket, i.e., the GABA binding site, is located at the interface between N terminus extracellular domains of the β and α subunits, which constitutes a “principal face” and a “complementary face”, respectively. As reported by Miller and Aricescu (2014), the principal face of human GABA_AR involves the $\beta 4$ strand and residues Asp95-Leu99, Glu155-Tyr159, Phe200-Tyr205 in the β subunit. The complementary face corresponds to the residues Tyr62-Gln64 and Leu125-Arg129 in the α subunit. On the other hand, agonist sensitivity seems to be affected also by the motifs, which are not located in the neurotransmitter binding pocket (Korpi and Luddens, 1993; Böhme et al., 2004). For example a domain (S238-V264) in the δ subunit might be important for the high agonist affinity of the extra-synaptic $\alpha 4\beta 3\delta$ receptors (You and Dunn, 2007) compared to synaptic $\gamma 2$ containing receptors. Thus, following the formation of receptor and agonist complex at the neurotransmitter binding site, the agonist affinity and efficacy might be affected by all subunits (see Unwin 2005).

4. Alcohol modulation GABA_ARs

Alcohol has profound effects in the brain. Interacting with multiple neurotransmitter systems (Valenzuela CF., 1997), its impact is characterized by intoxicating, sedative, anxiolytic and addictive features in the behavioral level (Bayard et al., 2004). Ethanol affects many ion channels, including the NMDA glutamate receptors (Hanchar et al., 2005). In the CNS, GABA_ARs are the major targets of alcohol (Mihic and Harris, 1997; Boehm et al., 2004; Hanchar et al., 2005; Kumar et al., 2009). In addition to direct allosteric effect of ethanol on GABA_ARs (Deitrich et al., 1989), there are also indirect effects on the receptor due to ethanol mediated increase in the levels of presynaptic release of GABA (Yang et al., 2000; Roberto et al., 2003; Ming et al., 2006; Theile et al., 2008; Mameli et al., 2008) and neuroactive steroids (Caldeira et al., 2004; Mameli and Valenzuela 2006; Izumi et al., 2007), which are the modulators of GABA receptors. Besides, ethanol affects the phosphorylation of GABA_ARs, which in turn lead to an increase in the GABA sensitivity (Hodge et al., 1999, 2002; Kumar S., 2009). For allosteric effects, ethanol sensitivity depends on the GABA_AR subtypes. In general $\gamma 2$ - GABA_AR subtypes are sensitive to ethanol at doses attained by severe intoxication (Kumar S., 2009). The extra-synaptic δ -GABA_ARs are thought to be most

sensitive to ethanol, which will be discussed in the following section.

5. Alcohol and extra-synaptic GABA_ARs

In general, 1-3 mM blood ethanol levels can result from drinking half a glass of wine or less (Goetz et al 2007). This is especially important as the extra-synaptic δ -GABA_ARs are thought to be most sensitive to ethanol at levels of social drinking, i.e., less than 30 mM (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003, 2006; Wei et al., 2003; Hanchar et al., 2005; 2006; Santhakumar et al., 2007; Glykys et al., 2007; Mody et al., 2007; Olsen et al., 2007). Studies of δ subunit knock out mice have shown the impact of extra-synaptic δ - GABA_ARs for mediating the effects of ethanol (Mihalek et al., 2001). These mice show less responsiveness to the anticonvulsant effects of ethanol, a decreased response of excitability to ethanol withdrawal, and a less preference for ethanol compared to wild-type mice. This phenomenon is dependent on β subunit with $\beta 3$ isoform providing maximal sensitivity to ethanol (Wallner et al., 2003).

A direct evidence of the effect of ethanol via δ -GABA_ARs on cerebellar granule cells has been shown by the R100Q mutation in the $\alpha 6$ subunits of the alcohol non-tolerant rats. Cerebellar granule cells express the extra-synaptic receptors with a specific partnership of $\alpha 6$ and δ subunits together with the β subunit (Jones et al., 1997; Brickley et al., 2001). Rats homozygous for the mutation ($\alpha 6$ -100QQ) have an increased alcohol-induced ataxia and they have an increased activity of $\alpha 6\beta\delta$ receptors enhanced by alcohol in cerebellar granule cells (Hanchar et al., 2005, 2006). On the other hand $\alpha 6$ knock-out mice do not show any distortion of alcohol sensitivity (Homanics et al., 1997). However, this may result from the adaptive responses of the cerebellar granule cells to the absence of $\alpha 6$ subunits, which could mask the relation of corresponding receptors ($\alpha 6$ and β subunits which has a specific partnership with δ subunit in the cerebellum) and ethanol actions. Indeed, the $\alpha 6$ subunit knock-out mice have increased expression of TASK-1 channel in these cells, which may impact on ethanol sensitivity. In line with this, TASK-1 knock-out mice are more sensitive to ethanol in behavioral level, which might explain the unchanged ethanol sensitivities of $\alpha 6$ knockout mice of GABA_AR (reviewed by Korpi et al., 2007). Other experiments done with the recombinant expression systems have shown that 3-30 mM alcohol is enough to activate $\delta\alpha 4\beta$ and $\delta\alpha 6\beta$ subunit containing receptors (Wallner et al., 2003; 2006; Sundstrom-Poromaa et al., 2002). This effect is shown to increase the tonic inhibition (Wei et al., 2003; Hanchar et al., 2005; Glykys et al., 2007; Santhakumar et al., 2007; Liang et

al., 2008), the specific form of inhibition mediated by the δ -GABA_ARs.

As a result, several lines of evidences from the studies of recombinant expression systems and electrophysiological recordings converge on the hypothesis that physiologically relevant, low dose (less than 50 mM) actions of ethanol is mediated by extra-synaptic δ - GABA_ARs. Thus, a glass of wine activating the extra-synaptic receptors could potentiate GABAergic tonic inhibition in the striatum and cerebellum via $\alpha 4\beta 3\delta$ and $\alpha 6\beta\delta$ receptors respectively (Hanchar et al., 2005, 2006; Olsen et al., 2007).

On the other hand, these results generated some controversy in the field as the findings regarding the high alcohol affinity of the extra-synaptic receptors have not been replicated by some groups and presynaptic mechanisms have been proposed for the alcohol potentiation of GABAergic system (Carta et al., 2004; Borghese et al., 2006; Botta et al., 2007a, b; Korpi et al., 2007; Baur 2009). Several experimental errors or methodological issues may cause this contradiction. For example, in one of the studies who fail to replicate the physiologically relevant alcohol potentiation of extra-synaptic receptors (Botta et al., 2007), the magnitude of GABAergic tonic currents has been described as 55 pA, which is much higher than tonic currents (i.e., less than 30 pA) described in many other studies for the comparative age and cell types of rodents (cited in Otis, 2008). δ subunit is a rare isoform of GABA receptor subunits by means of its distribution in the brain: Its expression is restricted to cerebellar granule cells (Jones et al., 1997), dentate gyrus granule cells in the hippocampus (Sun et al., 2004) and ventrobasal nucleus of the thalamus and neocortex (Cope et al., 2005; Glykys et al., 2007). Thus, it is reasonable to expect some experimental caveats for the *in vitro* ectopic expression of δ - GABA_ARs (Arslan et al., 2014). For example, *in vitro* expression of recombinant δ subunit is generated variable results by means of clustering on the cell membrane. Regarding this, one study reported that *in vitro* expression of recombinant δ subunit shows a diffusely distributed pattern on the cell membrane but *in vivo* studies show that δ subunit containing receptors form clusters (Sun et al., 2004). In parallel with the latter finding, Arslan et al., (2014) reported that recombinant δ subunit when expressed in the primary cultures of hippocampal neurons gave a punctate immunostaining on non-permeabilized cells. Here it is important to consider many factors that could contribute to this discrepancy. For example Arslan et al. (2014) used N-terminus GFP tagged version of δ subunit where as Christie et al (2006) used the

C-terminus GFP tagged version of this subunit. Also, low *in vitro* expression profile of recombinant δ subunit (Arslan et al., 2014) and its restricted ability to form functional receptors *in vitro* may produce experimental failures (Olsen et al., 2007; Santhakumar et al., 2007; Otis, 2008). Moreover there are some possible effect of species differences in alcohol and alcohol antagonist responses (Wallner, et al., 2014). For example, a recent study suggests that there are some significant differences in the pharmacology of murine and human $\alpha 4\beta 1\delta$ receptors (Villumsen et al., 2015). Therefore, it is clear that methodological issues should be carefully considered for studies with δ -GABA_ARs in general and for the effects of alcohol on these receptors in particular.

6. Alcohol binding site

Perhaps a direct evidence for the ethanol enhancement of δ -GABA_ARs would come from studies showing the alcohol binding site on the δ -GABA_ARs. For the synaptic receptors like $\alpha 1\beta 2\gamma 2$ containing ones, mutagenesis and labeling studies have led to the identification of several amino acid residues in transmembrane domain critical for alcohol modulation. For example, site directed mutagenesis studies identified S270 and A291 on the second and third transmembrane domain of α subunit of GABA_ARs critical for allosteric modulation by alcohol (and volatile anesthetics) (Mihic et al., 1997). Many of these residues are involved in the enhancement of receptor function by alcohol as positive allosteric modulator (Mihic et al., 1997; Jenkins et al., 2001; 2002; McCracken et al., 2010).

Regarding extra-synaptic δ -GABA_ARs several studies have reported that a competitive antagonist of ethanol, Ro15-4513, prevents many of the behavioral aspects of ethanol intoxication (Suzdak et al., 1986; Lüddens et al., 1990; Hancher et al., 2006, Wallner et al., 2006). Experiments utilizing the radiolabeled Ro15-4513 have shown that ethanol can displace Ro15-4513 on the δ subunit (Hancher et al., 2006, Wallner et al., 2006). Addressing this, a new extracellular alcohol/imidazobenzodiazepine (Ro15-4513) site has been identified for the δ -GABA_ARs (Wallner et al., 2014). By the use of site directed mutagenesis experiments and homology modeling, Wallner et al. (2014) have shown that this site, involving the residue Y76 in the $\beta 3$ subunit, is located at the interface between the $\alpha 4/\alpha 6$ and $\beta 3$ subunit of δ -GABA_ARs and matches with the residue ($\gamma 2$ T81) of benzodiazepine site of $\gamma 2$ - GABA_ARs. Thus the binding site of ethanol is likely located at a site on extra-synaptic δ -GABA_ARs

corresponding to benzodiazepine site of synaptic $\gamma 2$ - GABA_ARs.

7. Conclusion

The current pharmacotherapy for AUDs is not effective satisfactorily (Liang and Olsen 2014). Development of better treatment agents for the AUDs requires the detailed understanding of molecular targets of alcohol relevant to its effects in the brain. Accumulating evidences from the studies of recombinant expression systems, electrophysiological recordings from the neurons and labeling experiments converge on the hypothesis that physiologically relevant, low dose actions of ethanol is mediated by extra-synaptic δ -GABA_ARs. This action is likely to occur by an allosteric mechanism corresponding to a BZ site in the ECD of the δ -GABA_ARs (Wallner et al., 2014). But the ethanol action on GABA_ARs does not seem to be limited to one site. Probably there are multiple sites, some of which are physiologically critical while others not (Mihic et al., 1997; Jenkins et al., 2001; 2002; McCracken et al., 2010; Wallner et al., 2014). Current developments in our understanding of the structure of GABA_ARs (Miller and Aricescu, 2014) and related proteins from eukaryotic and prokaryotic organisms (reviewed by Lynagh and Pless, 2014) will likely answer these questions and initiate new opportunities addressing the alcohol actions on GABA_ARs. Based on the available X-ray data, molecular dynamics (MD) simulations have the potential to offer an atomic level dynamics of the conformational changes on the receptor during the process of signal transmission, and the effect of allosteric modulators on this process. These opportunities will not only lead to the verification of present data and clarification of inconsistencies in the literature described so far but also elucidation of allosteric interactions of ethanol besides to other GABA_AR modulators at level of atomic scales for better perspectives of drug design. Thus, a significant progress is expected in the field to address the mechanism of allosteric modulation of various ligands besides to ethanol on different subtypes of GABA_ARs, including extra-synaptic δ -GABA_ARs subtypes.

References

- Arslan A, von Engelhardt J, Wisden W. (2014) Cytoplasmic domain of δ subunit is important for the extra-synaptic targeting of GABAA receptor subtypes. *J Integr Neurosci*. Dec; 13(4):617-31.
- Bayard M, McIntyre J, Hill KR, Woodside J Jr. (2004) Alcohol withdrawal syndrome. *Am Fam Physician*. 2004 Mar 15; 69(6):1443-50.

- Boehm SL 2nd, Ponomarev I, Jennings AW, Whiting PJ, Rosahl TW, Garrett EM, Blednov YA, Harris RA. (2004) gamma-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions. *Biochem Pharmacol.* Oct 15;68(8):1581-602.
- Borghese CM, Stórustovu Sí, Ebert B, Herd MB, Belelli D, Lambert JJ, Marshall G, Wafford KA, Harris RA. (2006) The delta subunit of gamma-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. *J Pharmacol Exp Ther.* Mar; 316(3):1360-8.
- Botta P, Mameli M, Floyd KL, Radcliffe RA, Valenzuela CF. (2007a) Ethanol sensitivity of GABAergic currents in cerebellar granule neurons is not increased by a single amino acid change (R100Q) in the alpha6 GABAA receptor subunit. *J Pharmacol Exp Ther.* Nov; 323(2):684-91.
- Botta P, Radcliffe RA, Carta M, Mameli M, Daly E, Floyd KL, Deitrich RA, Valenzuela CF. (2007b) Modulation of GABAA receptors in cerebellar granule neurons by ethanol: a review of genetic and electrophysiological studies. *Alcohol.* 2007 May; 41(3):187-99.
- Bonnert, T.P., McKernan, R.M., Farrar, S., le Bourdelles, B., Heavens, R.P., Smith, D.W., Hewson, L., Rigby, M.R., Sirinathsinghji, D.J., Brown, N., Wafford, K.A., Whiting, P.J. (1999) A novel g-aminobutyric acid type A receptor subunit. *Proc Natl Acad Sci U S A*, 96:9891-9896.
- Böhme I, Rabe H, Lüddens H. (2004) Four amino acids in the alpha subunits determine the gamma-aminobutyric acid sensitivities of GABAA receptor subtypes. *J Biol Chem.* 2004 Aug 20; 279(34):35193-200.
- Brejc, K., van Dijk, W.J., Klaassen, R.V., Schuurmans, M., van Der Oost, J., Smit, A.B., Sixma, T.K. (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature*, 411:269-276.
- Brickley, S.G., Revilla, V., Cull-Candy, S.G., Wisden, W., Farrant, M. (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature*, 409:88-92.
- Brickley SG, Mody I. (2012) Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. *Neuron.* 2012 Jan 12;73(1):23-34.
- Cromer BA, Morton CJ, Parker MW. Anxiety over GABA(A) receptor structure relieved by AChBP. *Trends Biochem Sci.* 2002 Jun; 27(6):280-7.
- Caldeira JC, Wu Y, Mameli M, Purdy RH, Li PK, Akwa Y, Savage DD, Engen JR, Valenzuela CF. (2004) Fetal alcohol exposure alters neurosteroid levels in the developing rat brain. *J Neurochem.* Sep; 90(6):1530-9.
- Carta M, Mameli M, Valenzuela CF. (2004) Alcohol enhances GABAergic transmission to cerebellar granule cells via an increase in Golgi cell excitability. *J Neurosci.* Apr 14; 24(15):3746-51.
- Cope, D.W., Hughes, S.W., Crunelli, V. (2005) GABAA receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci.*, 25:11553-11563.
- Deitrich RA, Dunwiddie TV, Harris RA, Erwin VG. Mechanism of action of ethanol: initial central nervous system actions. *Pharmacol Rev.* 1989 Dec;41(4):489-537.
- Ernst, M., Brauchart, D., Boresch, S., Sieghart, W. (2003) Comparative modeling of GABAA receptors: limits, insights, future developments. *Neuroscience*, 119:933-943.
- Fritschy, J.M., Mohler, H. (1995) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol.*, 359:154-194.
- Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I. (2007) A new naturally occurring GABA(A) receptor subunit partnership with high sensitivity to ethanol. *Nat Neurosci.* Jan;10(1):40-8.
- Goetz, T., Arslan, A., Wisden, W., Wulff, P., (2007) GABAA receptor structure and function in the basal ganglia. *Prog. Brain Res.* 160: 21-41.
- Haas KF, Macdonald RL. (1999) GABAA receptor subunit gamma2 and delta subtypes confer unique kinetic properties on recombinant GABAA receptor currents in mouse fibroblasts. *J Physiol.* 1; 514 (Pt 1): 27-45.
- Hamann, M., Rossi, D.J., Attwell, D., (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* 33(4): 625-33.
- Hanchar, H.J., Wallner, M., Olsen, R.W. (2004) Alcohol effects on g-aminobutyric acid type A receptors: are extra-synaptic receptors the answer? *Life Sciences*, 76:1-8.
- Hanchar, H.J., Dodson, P.D., Olsen, R.W., Otis, T.S., Wallner, M. (2005) Alcohol-induced motor impairment caused by increased extra-synaptic GABAA receptor activity. *Nat Neurosci.*, 8:339-345.

- Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M, Olsen RW. (2006) Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to alpha4/6beta3delta GABAA receptors. *Proc Natl Acad Sci U S A*. 103(22):8546-51.
- Hassaine G, Deluz C, Grasso L, Wyss R, Tol MB, Hovius R, Graff A, Stahlberg H, Tomizaki T, Desmyter A, Moreau C, Li XD, Poitevin F, Vogel H, Nury H. (2014) X-ray structure of the mouse serotonin 5-HT₃ receptor. *Nature*, 21; 512(7514):276-81. doi: 10.1038/nature13552.
- Hausser, M. and Clark, B.A. (1997) Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration *Neuron* 19 (3) 665-78.
- Hodge, C.W., Mehmert, K.K., Kelley, S.P., McMahon, T., Haywood, A., Olive, M.F., Wang, D., Sanchez-Perez, A.M., Messing, R.O. (1999) Supersensitivity to allosteric GABAA receptor modulators and alcohol in mice lacking PKC epsilon. *Nat Neurosci.*, 2:997-1002.
- Homanics GE, Ferguson C, Quinlan JJ, Daggett J, Snyder K, Lagenaur C, Mi ZP, Wang XH, Grayson DR, Firestone LL. Gene knockout of the alpha6 subunit of the gamma-aminobutyric acid type A receptor: lack of effect on responses to ethanol, pentobarbital, and general anesthetics. *Mol Pharmacol*. 1997 Apr; 51(4):588-96.
- Homanics GE, Le NQ, Kist F, Mihalek R, Hart AR, Quinlan JJ. (1998) Ethanol tolerance and withdrawal responses in GABA(A) receptor alpha 6 subunit null allele mice and in inbred C57BL/6J and strain 129/SvJ mice. *Alcohol Clin Exp Res*. Feb; 22(1):259-65.
- Izumi Y, Murayama K, Tokuda K, Krishnan K, Covey DF, Zorumski CF. (2007) GABAergic neurosteroids mediate the effects of ethanol on long-term potentiation in rat hippocampal slices. *Eur J Neurosci*. Oct; 26(7):1881-8.
- Jones, A., Korpi, E.R., McKernan, R.M., Pelz, R., Nusser, Z., Makela, R., Mellor, J.R., Pollard, S., Bahn, S., Stephenson, F.A., Randall, A.D., Sieghart, W., Somogyi, P., Smith, A.J., Wisden, W. (1997) Ligand-gated ion channel subunit partnerships: GABAA receptor α 6 subunit gene inactivation inhibits delta subunit expression. *J Neurosci.*, 17:1350-1362
- Korpi ER, Lüddens H. (1993) Regional gamma-aminobutyric acid sensitivity of t-butylbicyclopheosphoro[35S]thionate binding depends on gamma-aminobutyric acid A receptor alpha subunit. *Mol Pharmacol*. Jul; 44(1):87-92.
- Korpi ER, Debus F, Linden AM, Malécot C, Leppä E, Vekovischeva O, Rabe H, Böhme I, Aller MI, Wisden W, Lüddens H. (2007) Does ethanol act preferentially via selected brain GABAA receptor subtypes? the current evidence is ambiguous. *Alcohol*. 2007 May;41(3):163-76.
- Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, Morrow AL. (2009) The role of GABA(A) receptors in the acute and chronic effects of ethanol: a decade of progress. *Psychopharmacology (Berl)*. Sep; 205(4):529-64.
- Laurie, D.J., Seeburg, P.H., Wisden, W. (1992a) The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J Neurosci.*, 12:1063-1076.
- Laurie DJ, Wisden W, Seeburg PH. (1992b) The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci.*, 12:4151-4172.
- Lavoie AM, Tingey JJ, Harrison NL, Pritchett DB, Twyman RE (1997) Activation and deactivation rates of recombinant GABA(A) receptor channels are dependent on alpha-subunit isoform. *Biophys J*. 1997 Nov;73(5):2518-26.
- Liang J, Suryanarayanan A, Chandra D, Homanics GE, Olsen RW, Spigelman I. (2008) Functional consequences of GABAA receptor alpha 4 subunit deletion on synaptic and extrasynaptic currents in mouse dentate granule cells. *Alcohol Clin Exp Res*. Jan;32(1):19-26.
- Liang J, Olsen RW. (2014) Alcohol use disorders and current pharmacological therapies: the role of GABA(A) receptors. *Acta Pharmacol Sin*. 2014 Aug;35(8):981-93. doi: 10.1038/aps.2014.50.
- Lüddens, H., Pritchett, D.B., Kohler, M., Killisch, I., Keinanen, K., Monyer, H., Sprengel, R., Seeburg, P.H. (1990) Cerebellar GABAA receptor selective for a behavioural alcohol antagonist. *Nature*, 346:648-651.
- Lynagh T, Pless, S.A., Principles of agonist recognition in Cys-loop receptors *Front Physiol*. 2014 Apr 24;5:160. doi: 10.3389/fphys.2014.00160. eCollection 2014.
- Macdonald, R.L., Haas, K.F. (2000) Kinetic Properties of GABAA Receptor Channels. In Martin, D.L., Olsen, R.W. (Eds.), *GABA in the Nervous System: The View at Fifty Years*, Lippincott Williams and Wilkins, Philadelphia, pp. 141-165.

- Mameli M, Valenzuela CF. (2006) Alcohol increases efficacy of immature synapses in a neurosteroid-dependent manner. *Eur J Neurosci.* Feb;23(3):835-9.
- Mameli M, Botta P, Zamudio PA, Zucca S, Valenzuela CF. Ethanol decreases Purkinje neuron excitability by increasing GABA release in rat cerebellar slices. *J Pharmacol Exp Ther.* 2008 Dec; 327(3):910-7.
- Meera P, Olsen RW, Otis TS, Wallner M. (2010) Alcohol- and alcohol antagonist-sensitive human GABAA receptors: tracking δ subunit incorporation into functional receptors. *Mol Pharmacol.* Nov; 78(5):918-24.
- Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL, Homanics GE. (2001) GABA(A)-receptor delta subunit knockout mice have multiple defects in behavioral responses to ethanol. *Alcohol Clin Exp Res.* 2001 Dec; 25(12):1708-18.
- Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA,
- Harrison NL. (1997) Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature*, 389(6649):385-9.
- Mihic SJ, Harris RA. (1997) GABA and the GABAA receptor. *Alcohol Health Res World.* 21(2):127-31.
- Miller PS, Aricescu AR. (2014) Crystal structure of a human GABAA receptor. *Nature.* 2014 Aug 21;512(7514):270-5. doi: 10.1038/nature13293. Epub 2014 Jun 8.
- Ming Z, Criswell HE, Yu G, Breese GR. (2006) Competing presynaptic and postsynaptic effects of ethanol on cerebellar purkinje neurons. *Alcohol Clin Exp Res.* 2006 Aug;30(8):1400-7.
- Mirheydari P, Ramerstorfer J, Varagic Z, Scholze P, Wimmer L, Mihovilovic MM, Sieghart W, Ernst M. (2014) Unexpected Properties of δ -Containing GABAA Receptors in Response to Ligands Interacting with the α + β - Site. *Neurochem Res.* Jun; 39(6):1057-67.
- Mody I, Pearce, R.A. (2004) Diversity of inhibitory neurotransmission through GABAA receptors. *Trends Neurosci.*, 27:569-575.
- Mody I, Glykys J, Wei W (2007) A new meaning for "Gin & Tonic": tonic inhibition as the target for ethanol action in the brain. *Alcohol* (41)145-53.
- Nusser, Z., Sieghart, W., Benke, D., Fritschy, J.M., Somogyi, P. (1996) Differential synaptic localization of two major g-aminobutyric acid type A receptor subunits on hippocampal pyramidal cells. *Proc Natl Acad Sci U S A.* Oct 15;93(21):11939-44.
- Nusser Z, Mody I. (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol.* 87(5):2624-8.
- Nusser, Z., Sieghart, W., Somogyi, P. (1998) Segregation of different GABAA receptors to synaptic and extra-synaptic membranes of cerebellar granule cells. *J Neurosci.*, 18:1693-1703.
- Olsen, R.W., Chang, C.S., Li, G., Hanchar, H.J., Wallner, M. (2004) Fishing for allosteric sites on GABAA receptors. *Biochem Pharmacol.*, 68:1675-1684.
- Olsen RW, Hanchar HJ, Meera P, Wallner M. GABAA receptor subtypes: the "one glass of wine" receptors. *Alcohol.* 2007 May; 41(3):201-9.
- Otis TS. (2008) Comments on "Ethanol sensitivity of GABAergic currents in cerebellar granule neurons is not increased by a single amino acid change (R100Q) in the alpha6 GABA(A) receptor subunit". *J Pharmacol Exp Ther.* 2008 Jan; 324(1): 399-400; author reply 401-3.
- Patel B, Mortensen M, Smart TG. (2014) Stoichiometry of δ subunit containing GABA(A) receptors. *Br J Pharmacol.* 2014 Feb;171(4):985-94.
- Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. (2003) Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc Natl Acad Sci U S A.* 2003 Feb 18; 100(4):2053-8.
- Rudolph, U., Moehler, H. (2006) GABA-based therapeutic approaches: GABAA receptor subtype functions. *Current Opinion in Pharmacology*, 6:18–23.
- Santhakumar V, Wallner M, Otis TS. (2007) Ethanol acts directly on extra-synaptic subtypes of GABAA receptors to increase tonic inhibition. *Alcohol.* May;41(3):211-21.
- Schwarzer, C., Berresheim, U., Pirker, S., Wieselthaler, A., Fuchs, K., Sieghart, W., Sperk, G. (2001) Distribution of the major gamma-aminobutyric acid(A) receptor subunits in the basal ganglia and associated limbic brain areas of the adult rat. *J Comp Neurol.*, 433:526-549.

- Seeburg, P.H., Wisden, W., Verdoorn, T.A., Pritchett, D.B., Werner, P., Herb, A., Luddens, H., Sprengel, R., Sakmann, B. (1990) The GABAA receptor family: molecular and functional diversity. *Cold Spring Harb Symp Quant Biol.*, 55:29-40.
- Semyanov, A., Walker, M.C., Kullmann, D.M., Silver, R.A. (2004) Tonicity active GABAA receptors: modulating gain and maintaining the tone. *Trends Neurosci.*, 27:262-269.7.
- Sieghart, W., Sperk, G. (2002) Subunit composition, distribution and function of GABAA receptor subtypes. *Curr Top Med Chem.*, 2:795-816.
- Sine, S.M., Engel, A.G. (2006) Recent advances in Cys-loop receptor structure and function. *Nature* 440: 448-455
- Sine, S.M., Engel, A.G. (2006) Recent advances in Cys-loop receptor structure and function. *Nature* 440: 448-455
- Sun C, Sieghart W, Kapur J. (2004) Distribution of alpha1, alpha4, gamma2, and delta subunits of GABAA receptors in hippocampal granule cells. *Brain Res.* 17;1029(2):207-16.
- Sundstrom-Poromaa, I., Smith, D.H., Gong, Q.H., Sabado, T.N., Li, X., Light, A., Wiedmann, M., Williams, K., Smith, S.S. (2002) Hormonally regulated alpha(4)beta(2)delta GABAA receptors are a target for alcohol. *Nat Neurosci.*, 5:721-722.
- Suzdak PD, Glowa JR, Crawley JN, Schwartz RD, Skolnick P, Paul SM. (1986) A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science.* Dec 5;234(4781):1243-7.
- Theile JW, Morikawa H, Gonzales RA, Morrisett RA. (2008) Ethanol enhances GABAergic transmission onto dopamine neurons in the ventral tegmental area of the rat. *Alcohol Clin Exp Res.* Jun;32(6):1040-8.
- Unwin, N. (2003) Structure and action of the nicotinic acetylcholine receptor explored by electron microscopy. *FEBS Lett.*, 555:91-95.
- Unwin, N. (2005) Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J. Mol. Biol.*, 346:967-989.7.
- Valenzuela CF. (1997) Alcohol and neurotransmitter interactions. *Alcohol Health Res World.* 21(2):144-8.
- Villumsen IS, Wellendorph P, Smart TG. (2015) Pharmacological characterisation of murine $\alpha 4\beta 1\delta$ GABAA receptors expressed in *Xenopus* oocytes. *BMC Neurosci.* 2015 Mar 5;16(1):8.
- Wallner, M., Hanchar, H.J., Olsen, R.W. (2003) Ethanol enhances $\alpha 4\beta 3\delta$ and $\alpha 6\beta 3\delta$ gammaaminobutyric acid type A (GABAA) receptors at low concentrations known to affect humans. *Proc Natl Acad Sci U S A.*, 100:15218-15223.
- Wallner M, Hanchar HJ, Olsen RW. (2006) Low-dose alcohol actions on $\alpha 4\beta 3\delta$ GABAA receptors are reversed by the behavioral alcohol antagonist Ro15-4513. *Proc Natl Acad Sci U S A.* 103(22):8540-5.
- Wallner M, Hanchar HJ, Olsen RW. (2014) Alcohol selectivity of $\beta 3$ -containing GABAA receptors: evidence for a unique extracellular alcohol/imidazobenzodiazepine Ro15-4513 binding site at the $\alpha +\beta$ - subunit interface in $\alpha\beta 3\delta$ GABAA receptors. *Neurochem Res.* 2014 Jun;39(6):1118-26.
- Wei, W., Zhang, N., Peng, Z., Houser, C.R., Mody, I. (2003) Perisynaptic localization of delta subunit-containing GABAA receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci.*, 23: 10650-10661.
- Wisden, W., Laurie, D.J., Monyer, H., Seeburg, P.H. (1992) The distribution of 13 GABAA receptor subunit mRNAs in the rat brain I: Telencephalon, diencephalon, mesencephalon. *J Neurosci.*, 12:1040-1062.
- Yang X, Criswell HE, Breese GR. Ethanol modulation of gamma-aminobutyric acid (GABA)-mediated inhibition of cerebellar Purkinje neurons: relationship to GABA_A receptor input. *Alcohol Clin Exp Res.* 2000 May;24(5):682-90.
- You H, Dunn SM. (2007) Identification of a domain in the delta subunit (S238-V264) of the $\alpha 4\beta 3\delta$ GABAA receptor that confers high agonist sensitivity. *J Neurochem.* Nov; 103(3):1092-101.