Published in final edited form as:
Psychoneuroendocrinology. 2009 December ; 34S1: S67–S73. doi:10.1016/j.psyneuen.2009.08.010.

Function and modulation of δ-containing $GABA_A$ receptors

Nadezhda N. Zheleznova[2,^], **Anna Sedelnikova**[1,^], and **David S. Weiss**[1,*]

[1]Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, 78229

[2]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin, 53226

Abstract

αβδ-containing $GABA_A$ receptors are 1) localized to extra- and peri-synaptic membranes, 2) exhibit a high sensitivity to GABA, 3) show little desensitization, and 4) are believed to be one of the primary mediators of tonic inhibition in the central nervous system. This type of signaling appears to play a key role in controlling cell excitability. This review article briefly summarizes recent knowledge on tonic GABA-mediated inhibition. We will also consider the mechanism of action of many clinically important drugs such as anxiolytics, anticonvulsants, and sedative/hypnotics and their effects on δ-containing GABA receptor activation. We will conclude that αβδ-containing $GABA_A$ receptors exhibit a relatively low efficacy that can be potentiated by endogenous modulators and anxiolytic agents. This scenario enables these particular GABA receptor combinations, upon neurosteroid exposure for example, to impart a profound effect on excitability in the central nervous system.

Keywords

αβδ $GABA_A$ Receptors; Tonic inhibition; Tracazolate; Neurosteroids

Introduction to $GABA_A$ receptors

GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the central nervous system (CNS). The inhibitory action of GABA is mediated by GABA receptors (GABARs), excluding early stages of development when GABA is excitatory (Valeyev *et al.*, 1993; Owens *et al.*, 1999; Demarque *et al.*, 2002), and results in a reduction in neuronal excitability. Three types of GABA receptors have been characterized: $GABA_A$, $GABA_B$ and $GABA_C$. $GABA_A$ and $GABA_C$ receptors are ligand-gated chloride channels (Barnard *et al.*, 1998; Sieghart and Sperk, 2002). $GABA_B$ receptors belong to the G-protein coupled receptor superfamily (Chen *et al.*, 2005). GABARs are widely distributed throughout the CNS. There are 19 different combinations of $GABA_A$ subunits that have been identified (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, θ and ρ_{1-3})(Fig.1)(Barnard *et al.*, 1998). Each subunit is comprised of a large extracellular *N*-terminus, four transmembrane domains (M1-M4), a large intracellular loop between M3 and M4, and a small extracellular *C*-terminus (Fig.2,A). There is general concordance that M2 from each of the five subunits lines the pore (Fig.2,B)(Leonard *et al.*, 1988). The prototypical

*Corresponding Author: David S. Weiss, Ph.D., Department of Physiology UTHSCSA, 7703 Floyd Curl Drive, San Antonio, Texas, 78229, Phone: 210.567.4325, Fax: 210.567.4326, weissd@uthscsa.edu
^Both authors contributed equally to this work.

$GABA_A$ receptor binds two GABA molecules at the interface between α and β subunits, thereby opening the gate and producing a chloride current across the membrane (Colquhoun and Sivilotti, 2004). Coexpression of only α and β subunits is sufficient to produce functional Cl^- channels. The fifth subunit typically provides for receptor modulation by a variety of pharmacologically and clinically relevant drugs which interact with distinct binding sites on the $GABA_A$ receptor.

A majority of native $GABA_A$ receptors are thought to be composed of two α, two β and one γ subunit (Chang *et al.*, 1996) in the arrangement γβαβα (Fig.2,C) (McKernan and Whiting, 1996; Sieghart and Sperk, 2002). However, it has been shown that the variability of native receptors might be significantly larger than expected. For instance, each individual receptor complex can contain two different α and two different β isoforms. One study, using quantitative immunoblotting, has suggested the presence of an $\alpha_1\alpha_6\beta_2\delta$ receptor combination in the rat cerebellum (Thompson *et al.*, 1996). It has also been shown that δ, like π and θ, is able to form four-subunit-type pentameric receptor complexes with α, β and γ (αβγδ) (Bonnert *et al.*, 1999). Different receptor combinations can vary in GABA sensitivity, kinetics, and modulation by different ligands. This review will primarily focus on δ-containing $GABA_A$ receptors.

Phasic and Tonic $GABA_A$-mediated inhibition

$GABA_A$-mediated synaptic transmission classically refers to the transient 'phasic' inhibitory postsynaptic current (IPSC) following activation of synaptic receptors by a high GABA concentration (mM range) (Maconochie *et al.*, 1994; Farrant and Nusser, 2005). A typical response of postsynaptic $GABA_A$ receptors lasts less than 50 ms (Cobb *et al.*, 1995; Hardie and Pearce, 2006). Synaptic $GABA_A$ receptors are believed to contain the γ subunit, have reduced GABA sensitivity, and are likely insensitive to the low ambient concentrations of GABA throughout the extracellular space (nM range). Synaptic inhibition plays a primary role in information transfer in the CNS, although tonic inhibition occurs before synapse formation in embryonic neurons (Valeyev *et al.*, 1993; Owens *et al.*, 1999; Demarque *et al.*, 2002). In the δ subunit-dense mature cerebellar granule cells, tonic inhibition contributes to nearly 90% of total inhibition (Hamann *et al.*, 2002). Thus, super-sensitive extrasynaptic $GABA_A$ receptor subtypes mediate "persistent" tonic inhibition (Walker and Semyanov, 2008).

Functional expression of δ-containing $GABA_A$ receptors and their role in tonic inhibition

The nM-μM range concentrations of GABA presumed to be present in the extratracellular space (Saxena and Macdonald, 1996; Wallner *et al.*, 2003), as well as spillover from synaptic GABA, activate extrasynaptic and perisynaptic GABA receptors. It is assumed that a majority of extrasynaptic receptors contain the δ subunit. High GABA sensitivity coupled with a low level of desensitization in the constant presence of ambient GABA concentrations make these receptors ideal for providing tonic inhibition. Less frequently, extrasynaptic receptors may be composed of αβ, $\alpha_5\beta_3\gamma_2$, $\alpha_1\beta_2\gamma_2$ or $\alpha_3\beta_3\gamma_2$ (Nusser *et al.*, 1998; Stell *et al.*, 2003; Belelli and Lambert, 2005; Jia *et al.*, 2005; Mortensen and Smart, 2006).

The δ subunit has been assumed to be predominately co-expressed with α_4 and/or α_6 subunits. The highest GABA affinity is exhibited by $\alpha_4\beta_{2,3}\delta$ or $\alpha_6\beta_{2,3}\delta$ combinations, with a GABA EC_{50} (concentration for half-maximal activation) in the nM range. The $\alpha_6\beta_{2,3}\delta$ combination is largely found in cerebellar granular cells (CGCs), which contain the highest CNS density of δ subunits at 30% (Jones *et al.*, 1997; Jechlinger *et al.*, 1998; Nusser *et al.*, 1999; Pirker *et al.*, 2000; Sassoe-Pognetto *et al.*, 2000). The $\alpha_6\beta_{2,3}\delta$ receptor complex is almost exclusively found at extrasynaptic locations (Nusser *et al.*, 1999; Sassoe-Pognetto *et al.*, 2000). The second highest level of δ subunit density has been found in granular cells of the dentate gyrus, where

most receptors appear to be the $\alpha_4\beta_{2,3}\delta$ combination. These receptors localize perisynaptically (Wei *et al.*, 2003) and have consistently been shown to mediate tonic inhibition in Dentate Gyrus Granule Cells (DGGCs) (Nusser and Mody, 2002; Stell *et al.*, 2003; Wei *et al.*, 2004; Maguire *et al.*, 2005; Herd *et al.*, 2008) and CGCs (Stell *et al.*, 2003). The $\alpha_4\beta_3\delta$ receptor subtype is mainly expressed in the ventro-basal nucleus of the thalamus and neocortex, mediating tonic inhibition in those brain regions (Chen *et al.*, 2005; Cope *et al.*, 2005; Glykys *et al.*, 2007).

It has also been determined that the α_5 and δ subunits are the principal $GABA_A$ receptor subunits responsible for mediation of tonic inhibition in hippocampal neurons (Glykys *et al.*, 2008). Glykys et al generated α_5 and δ double knock-out mice (Gabra5/Gabrd-/-) and recordings from CA1/CA3 Pyramidal Cells (PCs), DGGCs and Molecular Layer (ML) interneurons displayed an absence of tonic currents without compensatory changes in spontaneous inhibitory postsynaptic currents (sIPSCs), excitatory postsynaptic currents (sEPSCs), or membrane resistance.

It was previously shown that the α_4 subunit, the most common partner of δ, is not expressed in ML interneurons, but rather α_1 and δ subunits co-localize and most likely form functional receptors with β subunits and underlie the $GABA_AR$-mediated tonic inhibitory current in ML interneurons (Glykys *et al.*, 2007). There are additional reports that α_1, β_2 and δ subunits may form functional receptors when co-expressed in the same neurons (Mertens *et al.*, 1993; Pirker *et al.*, 2000; Mangan *et al.*, 2005). The α_1 subunit has been detected in the extrasynaptic region (Sun *et al.*, 2004; Baude *et al.*, 2007) consistent with the typical localization of δ-containing receptors (Nusser *et al.*, 1998; Farrant and Nusser, 2005). In addition, one recent report has indicated the importance of $\alpha_1\beta_x\delta$ receptors for tonic inhibition in humans. A significant correlation was illustrated between decreased RNA expression of δ and α_1 subunits (but not α_4 subunits) in human cortical pyramidal neurons in subjects with schizophrenia (Maldonado-Aviles *et al.*, 2009). Thus, a reduced number of $\alpha_1\beta_x\delta$ receptors could contribute to the deficient tonic inhibition involved in prefrontal cortical dysfunction that occurs in schizophrenia.

Pharmacological properties of δ-containing $GABA_A$ receptors

In addition to high potency and low desensitization, another pharmacological property of αβδ receptors is low efficacy. Although the ubiquitous $\alpha_{4,6}\beta_{2,3}\delta$ combinations exhibit the highest affinity to GABA, they have an I_{max} three-fold lower than corresponding $\alpha_{4,6}\beta_{2,3}$ receptors. For $\alpha_4\beta_3\delta$ receptors, agonists such as THIP or gabaxadol produced significantly larger chloride currents than GABA, but for $\alpha_6\beta_3\delta$-containing receptors, gaboxadol, muscimol, and isoguvacine exhibited even higher relative efficacy and potency levels (Adkins *et al.*, 2001; Brown *et al.*, 2002; Storustovu and Ebert, 2006; Wafford and Ebert, 2006). It appears that GABA is a partial agonist when δ is incorporated into $GABA_A$ receptors (Adkins *et al.*, 2001; Brown *et al.*, 2002). It was indicated in several studies that αβδ GABA receptors have a lower open channel probability and mean open time, as well as one less open state, than corresponding αβγ receptors (Fisher and Macdonald, 1997; Akk *et al.*, 2004).

$\alpha_1\beta_2\delta$ GABA receptors are low efficacy receptors that are nearly unresponsive to saturating concentrations of GABA. We observed a 50-fold decrease in maximum GABA activated currents for $\alpha_1\beta_2\delta$ receptors compared to $\alpha_1\beta_2$ receptors and a 126-fold decrease in currents compared to $\alpha_1\beta_2\gamma_2$ receptors (equal cRNA levels were injected) (Fig.3). At the same time, the GABA sensitivity for $\alpha_1\beta_2\delta$ receptors was not significantly lower than that of $\alpha_1\beta_2$ GABA receptors (Zheleznova *et al.*, 2008). Potentiation of these low efficacy, high potency receptors is likely achieved by increasing the efficacy rather than the affinity to GABA. Pharmacological compounds may bolster the efficacy by facilitating and stabilizing receptors in the open state.

Unlike most of γ subunit-containing GABA receptors, αβδ receptors have a very low sensitivity to benzodiazepines, but are highly sensitive to low concentrations of alcohol (3-30mM) (Mohler, 2006). It has been shown that a global deletion of the δ subunit in the $GABA_A$ receptor results in a decrease in the sensitivity of mice to the sedative/hypnotic, anxiolytic, and pro-absence effects of neuroactive steroids (Mihalek *et al.*, 1999; Spigelman *et al.*, 2002).

Neurosteroid regulation

Above all other pharmacological compounds, neurosteroids (including pregnanolone and allopregnanolone) are the most powerful modulators of αβδ-containing $GABA_A$ receptors (Adkins *et al.*, 2001; Brown *et al.*, 2002; Wohlfarth *et al.*, 2002). Various neurosteroids have been demonstrated to promote anxiolytic, analgesic, sedative, anticonvulsant and anesthetic effects. There are an extensive number of neurological and psychiatric diseases associated with steroid dysfunctions (Reddy, 2004; Belelli and Lambert, 2005; Eser *et al.*, 2006; Strous *et al.*, 2006; Mitchell *et al.*, 2008).

Neurosteroids are normally present in the intracellular space at the nanomolar concentration range, which is enough to affect δ-containing $GABA_A$ receptors, but insufficient to influence synaptic GABA receptors (Stell *et al.*, 2003). Only super-physiological neurosteroid concentrations can affect synaptic γ-containing receptors. Numerous data implicate neurosteroids in αβ receptor modulation. For instance, we have shown that the principal effect of neurosteroids on $\alpha_1\beta_2$ receptors is to increase their sensitivity to GABA (Zheleznova *et al.*, 2008). Wahlfarth et al obtained similar results for $\alpha_1\beta_3$ receptors (Wohlfarth *et al.*, 2002).

Interestingly, the δ subunit is unlikely to be involved in neurosteroid binding. Homology modeling of the neurosteroid binding site in $GABA_A$ receptors and site-directed mutagenesis of residues in other subunits (β and γ) suggests that this site is located solely in the α subunit (Hosie *et al.*, 2006). The previously identified residues of the α_1 subunit are Q241 (located near the intracellular portion of the TM1 domain), N407, and Y410 (located near the external end of TM4) (Hosie *et al.*, 2009). This binding site is highly conserved throughout the α subunit family (α_1-α_5). The homology model also accounts for a second putative neurosteroid binding site that is involved in the direct activation of the receptor. This site is activated by super-physiological concentrations of neurosteroids and appears to be located at the αβ interface.

Different endogenous steroids appear to modulate $GABA_A$ receptors in two divergent ways, potentiating or inhibiting receptor function depending on the presence of the 3α-hydroxi or 3β-sulfate group of sterol ring. For instance, nanomolar concentrations of THDOC, and allopregnanolone (3α,5α-tetrahydroprogesterone or THP) increased $GABA_A$ receptor currents, whereas low concentrations (micromolar) of pregnanolone sulfate and DHEAS antagonize the receptor (Majewska, 2007). The most efficacious endogenous potentiators of δ-containing GABA receptors are neurosteroids with the 3α-hydroxi ring A-reduced pregnane steroids. It has been proposed that such neurosteroids (THDOC) interact with both lipid membrane and receptor proteins at the protein-lipid interface, stabilizing the open state of the pore (Majewska, 2007). It has also been proposed that the plasma membrane is the most direct and relevant access pathway in neurosteroid interaction with the $GABA_A$ receptor (Akk *et al.*, 2005). Concordantly, on a single channel level, THDOC increases the efficacy of the $\alpha_1\beta_3\delta$ receptor by increasing the duration of channel opening and introducing a new open state (Wohlfarth *et al.*, 2002).

In opposition to this potentiation, THDOC and THP can also inhibit select δ-containing receptors by increasing desensitization in an apparently voltage-dependent manner. For these GABA receptor combinations, an especially strong inhibition was observed for outward chloride currents (Bianchi *et al.*, 2002; Shen *et al.*, 2007).

GABA efficacy and THDOC enhancement of αβδ receptors are critically dependent on the exact subunit composition. For $\alpha_{4,6}\beta_{2,3}\delta$ receptors, maximum GABA currents were amplified by THDOC ≈2.8-fold (Wohlfarth *et al.*, 2002; Meera *et al.*, 2009). It was discovered by comparing $\alpha_1\beta_3\delta$ and $\alpha_6\beta_3\delta$ GABA receptor currents that δ-containing GABA receptors can be potentiated by THDOC to a greater extent when δ is in combination with the α_1 subunit (Wohlfarth *et al.*, 2002). THDOC augmented maximum GABA currents of $\alpha_1\beta_2\delta$ receptors by 3.4 fold (Zheleznova *et al.*, 2008). The highest degree of potentiation was reported for the $\alpha_1\beta_3\delta$ receptor, which was potentiated by a factor of ≈10-17 fold by THDOC at saturating concentrations of GABA (Bianchi *et al.*, 2002; Wohlfarth *et al.*, 2002; Kaur *et al.*, 2009).

In addition to the increase in the maximum GABA-elicited current, there are noticeable changes in the kinetics of the αβδ $GABA_A$ receptor produced by THDOC. Normally, a mildly-desensitizing and rapidly-deactivating receptor becomes highly-desensitizing, slowly-deactivating. This effect could be explained if neurosteroids modify the gating behavior of the αβδ receptors by allowing entry into a typically unavailable desensitized state (Wohlfarth *et al.*, 2002).

Tracazolate potentiation

A unique group of anxiolytic and anticonvulsive agents, pyrazolopyridines, (i.e. tracazolate, etazolate and cartazolate) are powerful potentiators of δ-containing $GABA_A$ receptors (Young *et al.*, 1987; Thompson *et al.*, 2002), although the binding site of parazolopyridines has yet to be identified. The δ subunit is unlikely critical for pyrazolopyridine binding in $GABA_A$ receptors, although receptors containing the δ subunit are much more sensitive to pyrazolopyridines. Pyrazolopyridines are less potent than benzodiazepines in modulating αβγ receptors, generally producing a slight leftward shift in the GABA dose response curve. In contrast to αβδ receptors, high concentrations of pyrazolopyridines slightly inhibit chloride currents from αβ and αβγ receptors. Perhaps pyrazolopyridines have a higher affinity for the desensitized state in these receptors (Thompson *et al.*, 2002; Khom *et al.*, 2006).

Tracazolate potentiates GABA-induced currents for δ-containing receptors beyond the maximum for GABA alone. For example, tracazolate enhanced GABA maximum currents for $\alpha_2\beta_3\delta$ receptors nearly three-fold (Yang *et al.*, 2005). The $\alpha_1\beta_3\delta$ potentiation by tracazolate at an EC_{20} GABA concentration was nearly 14-fold higher than GABA alone and 3-fold higher than maximal GABA currents, yet no significant effect on the GABA dose-response relation (Thompson *et al.*, 2002).

Tracazolate enhanced the current amplitude for $\alpha_1\beta_2\delta$ in the presence of saturating GABA concentrations even more than THDOC (23-fold increase by tracazolate and 3.4-fold increase by THDOC, Fig.4). On the contrary, $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_3$ receptors exhibited no increase in the maximum current amplitude in the presence of tracazolate at saturating GABA concentrations. We observed an increase in the current amplitude for $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_2$ receptors by tracazolate only at low GABA concentrations due to a leftward displacement in the dose-response curve (Zheleznova *et al.*, 2008). Similar results have been obtained for $\alpha_1\beta_3\gamma_2$ (Thompson *et al.*, 2002) and $\alpha_1\beta_3\gamma_{1,2}$ receptors (Khom *et al.*, 2006).

The logical explanation of these findings is similar to that for THDOC; tracazolate restores the GABA efficacy of $\alpha_1\beta_2\delta$ receptors by increasing the open state probability of the channel. Indeed, tracazolate enhanced the GABA-induced maximum current amplitude nearly 25-fold, on level with the $\alpha_1\beta_2$ receptor maximum amplitude (Fig.4)(Zheleznova *et al.*, 2008). Tracazolate was not able to augment maximal GABA-activated current amplitudes for $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_2$ receptors, likely because these receptors were already at maximum efficacy. Mutation of the 9′ leucine residue (L286S) within the second transmembrane domain has been found to facilitate the receptor open state (Chang *et al.*, 1996;Bianchi and Macdonald, 2001).

When we introduced this mutation in the δ subunit, it restored the gating efficacy of $\alpha_1\beta_2\delta$ (L286S) receptors and profoundly increased GABA-mediated current amplitudes (Fig.3). As might be expected, tracazolate did not increase the efficacy of these mutant receptors (Zheleznova *et al.*, 2008).

Conclusion

$GABA_A$ receptors are formed by co-assembly of subunits from a large multigene family, and are differentially expressed throughout the brain. This heterogeneous expression provides a unique profile of physiological and pharmacological properties of GABA receptors. For the regions expressing extrasynaptic $\alpha_1\beta_2\delta$ receptors, the level of basal inhibition could be increased by multiple factors due to potentiation of these receptors by endogenous neurosteroids that increase their response to ambient GABA levels. This presents a unique mechanism for recruiting inhibition without the cost of receptor trafficking to the surface or the activation of intracellular signaling cascades and may play an important role in the modulation and potential silencing of certain neuronal circuits. Unlike the most common $\alpha_4\beta_{2/3}\delta$ or $\alpha_6\beta_{2/3}\delta$ combinations that are constantly active at nM GABA concentrations, the $\alpha_1\beta_2\delta$ receptor would be essentially silent even at saturating concentrations of GABA due to its very low efficacy. However, under the influence of THDOC or tracazolate, this receptor combination could exert a profound inhibitory influence on excitability in the CNS.

References

Adkins CE, Pillai GV, Kerby J, Bonnert TP, Haldon C, McKernan RM, Gonzalez JE, Oades K, Whiting PJ, Simpson PB. alpha4beta3delta GABA(A) receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. J Biol Chem 2001;276:38934–38939. [PubMed: 11495904]

Akk G, Bracamontes J, Steinbach JH. Activation of GABA(A) receptors containing the alpha4 subunit by GABA and pentobarbital. J Physiol 2004;556:387–399. [PubMed: 14966300]

Akk G, Shu HJ, Wang C, Steinbach JH, Zorumski CF, Covey DF, Mennerick S. Neurosteroid access to the GABAA receptor. J Neurosci 2005;25:11605–11613. [PubMed: 16354918]

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev 1998;50:291–313. [PubMed: 9647870]

Baude A, Bleasdale C, Dalezios Y, Somogyi P, Klausberger T. Immunoreactivity for the GABAA receptor alpha1 subunit, somatostatin and Connexin36 distinguishes axoaxonic, basket, and bistratified interneurons of the rat hippocampus. Cereb Cortex 2007;17:2094–2107. [PubMed: 17122364]

Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 2005;6:565–575. [PubMed: 15959466]

Bianchi MT, Haas KF, Macdonald RL. Alpha1 and alpha6 subunits specify distinct desensitization, deactivation and neurosteroid modulation of GABA(A) receptors containing the delta subunit. Neuropharmacology 2002;43:492–502. [PubMed: 12367596]

Bianchi MT, Macdonald RL. Mutation of the 9′ leucine in the GABA(A) receptor gamma2L subunit produces an apparent decrease in desensitization by stabilizing open states without altering desensitized states. Neuropharmacology 2001;41:737–744. [PubMed: 11640928]

Bonnert TP, McKernan RM, Farrar S, le Bourdelles B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Brown N, Wafford KA, Whiting PJ. theta, a novel gamma-aminobutyric acid type A receptor subunit. Proc Natl Acad Sci U S A 1999;96:9891–9896. [PubMed: 10449790]

Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. Br J Pharmacol 2002;136:965–974. [PubMed: 12145096]

Chang Y, Wang R, Barot S, Weiss DS. Stoichiometry of a recombinant GABAA receptor. J Neurosci 1996;16:5415–5424. [PubMed: 8757254]

Chen K, Li HZ, Ye N, Zhang J, Wang JJ. Role of GABAB receptors in GABA and baclofen-induced inhibition of adult rat cerebellar interpositus nucleus neurons in vitro. Brain Res Bull 2005;67:310–318. [PubMed: 16182939]

Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. Nature 1995;378:75–78. [PubMed: 7477292]

Colquhoun D, Sivilotti LG. Function and structure in glycine receptors and some of their relatives. Trends Neurosci 2004;27:337–344. [PubMed: 15165738]

Cope DW, Hughes SW, Crunelli V. GABAA receptor-mediated tonic inhibition in thalamic neurons. J Neurosci 2005;25:11553–11563. [PubMed: 16354913]

Demarque M, Represa A, Becq H, Khalilov I, Ben-Ari Y, Aniksztejn L. Paracrine intercellular communication by a Ca2+- and SNARE-independent release of GABA and glutamate prior to synapse formation. Neuron 2002;36:1051–1061. [PubMed: 12495621]

Eser D, Schule C, Baghai TC, Romeo E, Rupprecht R. Neuroactive steroids in depression and anxiety disorders: clinical studies. Neuroendocrinology 2006;84:244–254. [PubMed: 17159334]

Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 2005;6:215–229. [PubMed: 15738957]

Fisher JL, Macdonald RL. Single channel properties of recombinant GABAA receptors containing gamma 2 or delta subtypes expressed with alpha 1 and beta 3 subtypes in mouse L929 cells. J Physiol 1997;505(Pt 2):283–297. [PubMed: 9423172]

Glykys J, Mann EO, Mody I. Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci 2008;28:1421–1426. [PubMed: 18256262]

Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I. A new naturally occurring GABA(A) receptor subunit partnership with high sensitivity to ethanol. Nat Neurosci 2007;10:40–48. [PubMed: 17159992]

Hamann M, Rossi DJ, Attwell D. Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron 2002;33:625–633. [PubMed: 11856535]

Hardie JB, Pearce RA. Active and passive membrane properties and intrinsic kinetics shape synaptic inhibition in hippocampal CA1 pyramidal neurons. J Neurosci 2006;26:8559–8569. [PubMed: 16914682]

Herd MB, Haythornthwaite AR, Rosahl TW, Wafford KA, Homanics GE, Lambert JJ, Belelli D. The expression of GABAA beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. J Physiol 2008;586:989–1004. [PubMed: 18079158]

Hosie AM, Clarke L, da Silva H, Smart TG. Conserved site for neurosteroid modulation of GABA A receptors. Neuropharmacology 2009;56:149–154. [PubMed: 18762201]

Hosie AM, Wilkins ME, da Silva HM, Smart TG. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. Nature 2006;444:486–489. [PubMed: 17108970]

Jechlinger M, Pelz R, Tretter V, Klausberger T, Sieghart W. Subunit composition and quantitative importance of hetero-oligomeric receptors: GABAA receptors containing alpha6 subunits. J Neurosci 1998;18:2449–2457. [PubMed: 9502805]

Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA. An extrasynaptic GABAA receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 2005;94:4491–4501. [PubMed: 16162835]

Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, Mellor JR, Pollard S, Bahn S, Stephenson FA, Randall AD, Sieghart W, Somogyi P, Smith AJ, Wisden W. Ligand-gated ion channel subunit partnerships: GABAA receptor alpha6 subunit gene inactivation inhibits delta subunit expression. J Neurosci 1997;17:1350–1362. [PubMed: 9006978]

Kaur KH, Baur R, Sigel E. Unanticipated structural and functional properties of delta-subunit-containing GABAA receptors. J Biol Chem 2009;284:7889–7896. [PubMed: 19141615]

Khom S, Baburin I, Timin EN, Hohaus A, Sieghart W, Hering S. Pharmacological properties of GABAA receptors containing gamma1 subunits. Mol Pharmacol 2006;69:640–649. [PubMed: 16272224]

Leonard RJ, Labarca CG, Charnet P, Davidson N, Lester HA. Evidence that the M2 membrane-spanning region lines the ion channel pore of the nicotinic receptor. Science 1988;242:1578–1581. [PubMed: 2462281]

Maconochie DJ, Zempel JM, Steinbach JH. How quickly can GABAA receptors open? Neuron 1994;12:61–71. [PubMed: 8292360]

Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 2005;8:797–804. [PubMed: 15895085]

Majewska MD. Steroids and ion channels in evolution: from bacteria to synapses and mind. Evolutionary role of steroid regulation of GABA(A) receptors. Acta Neurobiol Exp (Wars) 2007;67:219–233. [PubMed: 17957902]

Maldonado-Aviles JG, Curley AA, Hashimoto T, Morrow AL, Ramsey AJ, O'Donnell P, Volk DW, Lewis DA. Altered markers of tonic inhibition in the dorsolateral prefrontal cortex of subjects with schizophrenia. Am J Psychiatry 2009;166:450–459. [PubMed: 19289452]

Mangan PS, Sun C, Carpenter M, Goodkin HP, Sieghart W, Kapur J. Cultured Hippocampal Pyramidal Neurons Express Two Kinds of GABAA Receptors. Mol Pharmacol 2005;67:775–788. [PubMed: 15613639]

McKernan RM, Whiting PJ. Which GABAA-receptor subtypes really occur in the brain? Trends Neurosci 1996;19:139–143. [PubMed: 8658597]

Meera P, Olsen RW, Otis TS, Wallner M. Etomidate, propofol and the neurosteroid THDOC increase the GABA efficacy of recombinant alpha4beta3delta and alpha4beta3 GABA A receptors expressed in HEK cells. Neuropharmacology 2009;56:155–160. [PubMed: 18778723]

Mertens S, Benke D, Mohler H. GABAA receptor populations with novel subunit combinations and drug binding profiles identified in brain by alpha 5- and delta-subunit-specific immunopurification. J Biol Chem 1993;268:5965–5973. [PubMed: 8383681]

Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. Proc Natl Acad Sci U S A 1999;96:12905–12910. [PubMed: 10536021]

Mitchell EA, Herd MB, Gunn BG, Lambert JJ, Belelli D. Neurosteroid modulation of GABAA receptors: molecular determinants and significance in health and disease. Neurochem Int 2008;52:588–595. [PubMed: 18055067]

Mohler H. GABA(A) receptor diversity and pharmacology. Cell Tissue Res 2006;326:505–516. [PubMed: 16937111]

Mortensen M, Smart TG. Extrasynaptic alphabeta subunit GABAA receptors on rat hippocampal pyramidal neurons. J Physiol 2006;577:841–856. [PubMed: 17023503]

Nusser Z, Ahmad Z, Tretter V, Fuchs K, Wisden W, Sieghart W, Somogyi P. Alterations in the expression of GABAA receptor subunits in cerebellar granule cells after the disruption of the alpha6 subunit gene. Eur J Neurosci 1999;11:1685–1697. [PubMed: 10215922]

Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J Neurophysiol 2002;87:2624–2628. [PubMed: 11976398]

Nusser Z, Sieghart W, Somogyi P. Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 1998;18:1693–1703. [PubMed: 9464994]

Owens DF, Liu X, Kriegstein AR. Changing properties of GABA(A) receptor-mediated signaling during early neocortical development. J Neurophysiol 1999;82:570–583. [PubMed: 10444657]

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 2000;101:815–850. [PubMed: 11113332]

Reddy DS. Pharmacology of catamenial epilepsy. Methods Find Exp Clin Pharmacol 2004;26:547–561. [PubMed: 15538544]

Sassoe-Pognetto M, Panzanelli P, Sieghart W, Fritschy JM. Colocalization of multiple GABA(A) receptor subtypes with gephyrin at postsynaptic sites. J Comp Neurol 2000;420:481–498. [PubMed: 10805922]

Saxena NC, Macdonald RL. Properties of putative cerebellar gamma-aminobutyric acid A receptor isoforms. Mol Pharmacol 1996;49:567–579. [PubMed: 8643098]

Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS. Reversal of neurosteroid effects at alpha4beta2delta GABAA receptors triggers anxiety at puberty. Nat Neurosci 2007;10:469–477. [PubMed: 17351635]

Sieghart W, Sperk G. Subunit composition, distribution and function of GABA(A) receptor subtypes. Curr Top Med Chem 2002;2:795–816. [PubMed: 12171572]

Spigelman I, Li Z, Banerjee PK, Mihalek RM, Homanics GE, Olsen RW. Behavior and physiology of mice lacking the GABAA-receptor delta subunit. Epilepsia 2002;43(Suppl 5):3–8. [PubMed: 12121286]

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors. Proc Natl Acad Sci U S A 2003;100:14439–14444. [PubMed: 14623958]

Storustovu SI, Ebert B. Pharmacological characterization of agonists at delta-containing GABAA receptors: Functional selectivity for extrasynaptic receptors is dependent on the absence of gamma2. J Pharmacol Exp Ther 2006;316:1351–1359. [PubMed: 16272218]

Strous RD, Kupchik M, Roitman S, Schwartz S, Gonen N, Mester R, Weizman A, Spivak B. Comparison between risperidone, olanzapine, and clozapine in the management of chronic schizophrenia: a naturalistic prospective 12-week observational study. Hum Psychopharmacol 2006;21:235–243. [PubMed: 16783815]

Sun C, Sieghart W, Kapur J. Distribution of alpha1, alpha4, gamma2, and delta subunits of GABAA receptors in hippocampal granule cells. Brain Res 2004;1029:207–216. [PubMed: 15542076]

Thompson CL, Pollard S, Stephenson FA. Developmental regulation of expression of GABAA receptor alpha 1 and alpha 6 subunits in cultured rat cerebellar granule cells. Neuropharmacology 1996;35:1337–1346. [PubMed: 9014150]

Thompson SA, Wingrove PB, Connelly L, Whiting PJ, Wafford KA. Tracazolate reveals a novel type of allosteric interaction with recombinant gamma-aminobutyric acid(A) receptors. Mol Pharmacol 2002;61:861–869. [PubMed: 11901225]

Valeyev AY, Cruciani RA, Lange GD, Smallwood VS, Barker JL. Cl- channels are randomly activated by continuous GABA secretion in cultured embryonic rat hippocampal neurons. Neurosci Lett 1993;155:199–203. [PubMed: 8397353]

Wafford KA, Ebert B. Gaboxadol--a new awakening in sleep. Curr Opin Pharmacol 2006;6:30–36. [PubMed: 16368265]

Walker MC, Semyanov A. Regulation of excitability by extrasynaptic GABA(A) receptors. Results Probl Cell Differ 2008;44:29–48. [PubMed: 17671772]

Wallner M, Hanchar HJ, Olsen RW. Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc Natl Acad Sci U S A 2003;100:15218–15223. [PubMed: 14625373]

Wei W, Faria LC, Mody I. Low ethanol concentrations selectively augment the tonic inhibition mediated by delta subunit-containing GABAA receptors in hippocampal neurons. J Neurosci 2004;24:8379–8382. [PubMed: 15385620]

Wei W, Zhang N, Peng Z, Houser CR, Mody I. Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. J Neurosci 2003;23:10650–10661. [PubMed: 14627650]

Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. J Neurosci 2002;22:1541–1549. [PubMed: 11880484]

Yang P, Jones BL, Henderson LP. Role of the alpha subunit in the modulation of GABA(A) receptors by anabolic androgenic steroids. Neuropharmacology 2005;49:300–316. [PubMed: 15993433]

Young R, Urbancic A, Emrey TA, Hall PC, Metcalf G. Behavioral effects of several new anxiolytics and putative anxiolytics. Eur J Pharmacol 1987;143:361–371. [PubMed: 2891554]

Zheleznova N, Sedelnikova A, Weiss DS. alpha1beta2delta, a silent GABAA receptor: recruitment by tracazolate and neurosteroids. Br J Pharmacol 2008;153:1062–1071. [PubMed: 18204487]

Receptors	Subunit composition							
$GABA_{A/C}$	α_1-α_6	β_1-β_3	γ_1-γ_3	δ	ε	π	θ	ρ_1- ρ_3

Fig.1.
Table showing the different $GABA_{A/C}$ receptor subunits.

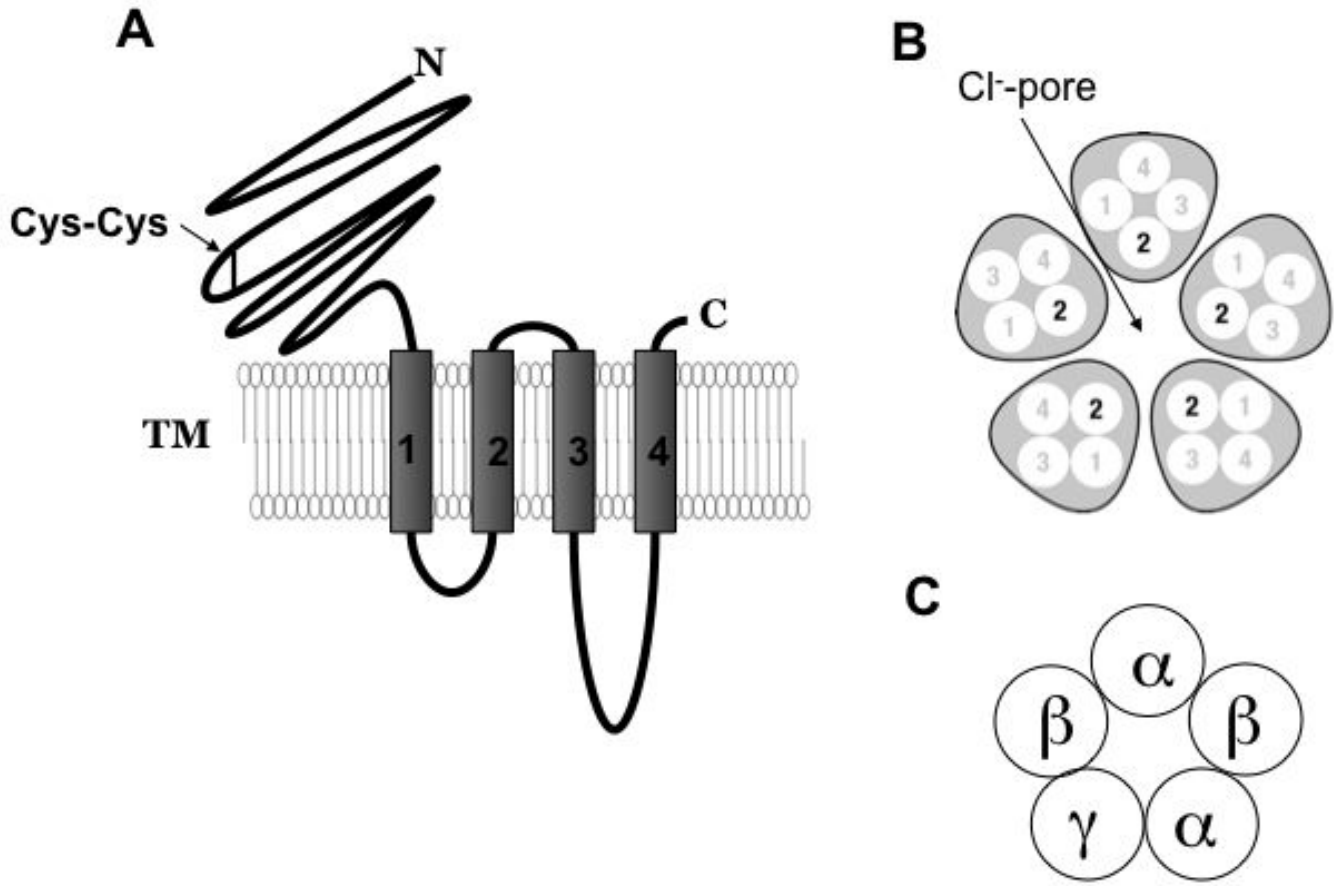

Fig.2.

Ligand-gated ionic channel (LGICs) structure. A. Each $GABA_A$ receptor subunit contains four transmembrane domains (TM). B. The TM2 domain forms the wall of the channel pore. C. The majority of native $GABA_A$ receptors are composed of 2α, 2β and 1γ subunit.

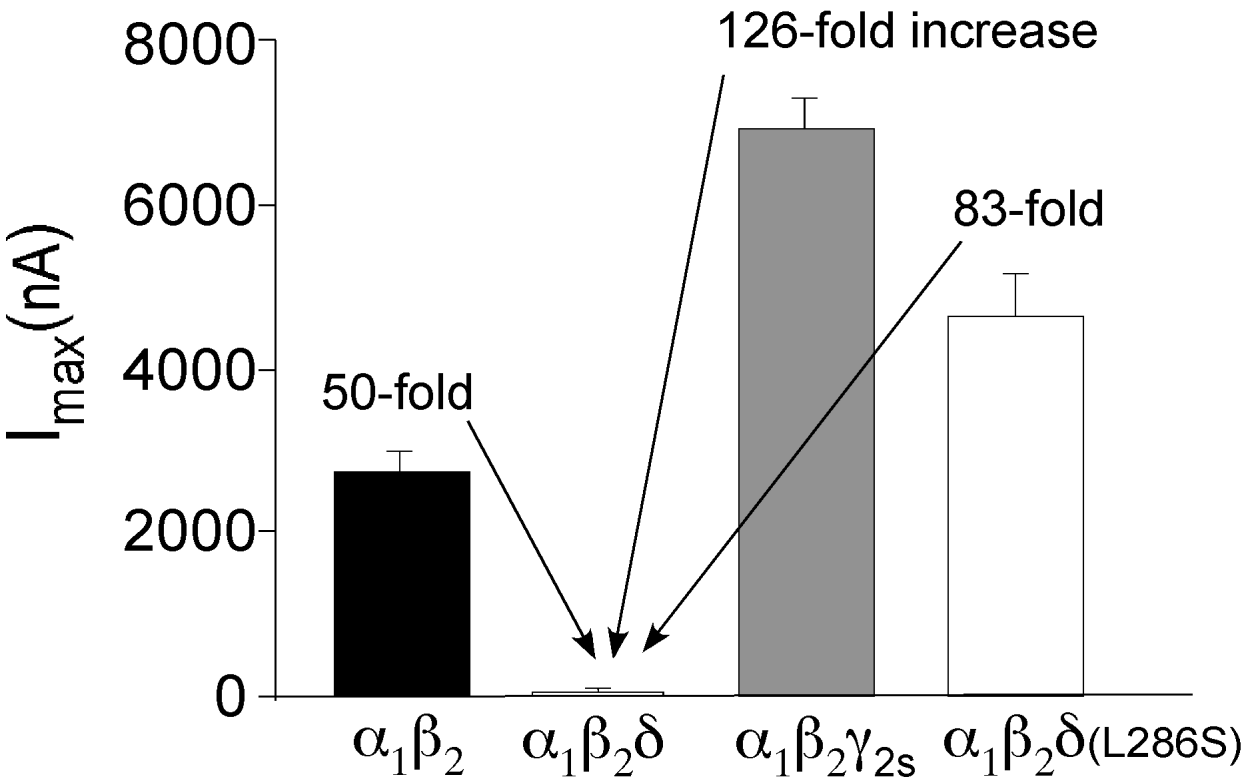

Fig.3.

Bar graphs plotting the maximum currents of $\alpha_1\beta_2$ (I_{max} =2736 ± 306, n=23; black bar), $\alpha_1\beta_2\delta$ (I_{max} =55 ± 5, n=39; open bar), $\alpha_1\beta_2\gamma_{2s}$ (I_{max} =6949±354, n=42; gray bar) and $\alpha_1\beta_2\delta$ (L9'S)(I_{max}=4565±372, n=8) $GABA_A$ receptors. Compared to $\alpha_1\beta_2\delta$, $\alpha_1\beta_2$, $\alpha_1\beta_2\gamma_{2s}$ and $\alpha_1\beta_2\delta$ (L9'S) show 50-fold, 126-fold and 83-fold increases in the maximum $GABA_A$-activated currents, respectively.

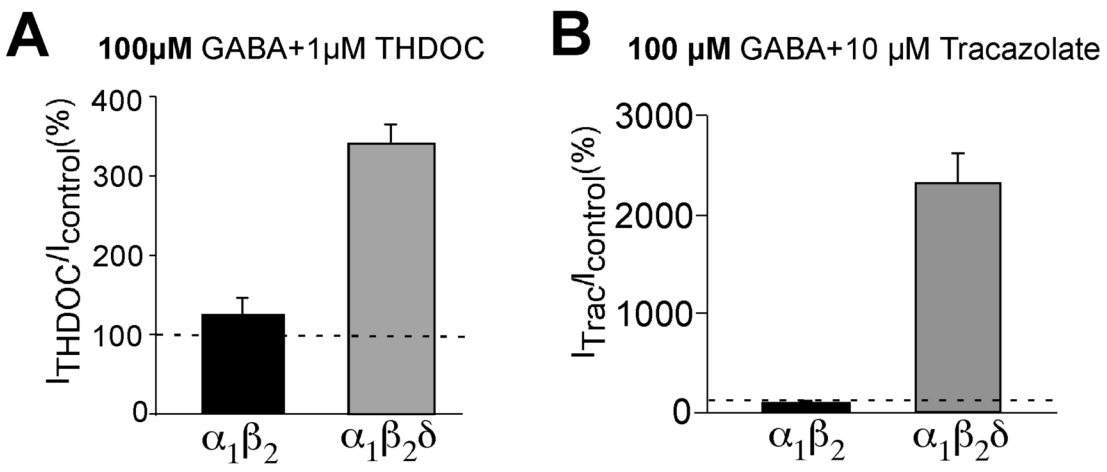

Fig.4.

The fold increase by THDOC (A) and tracazolate (B) is shown with a saturating concentration of GABA (100 μM) from oocytes expressing $\alpha_1\beta_2$ (black bar) and $\alpha_1\beta_2\delta$ (gray bar) $GABA_A$ receptors.