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Short communication

δ-Subunit containing $GABA_A$ receptor knockout mice are less sensitive to the actions of 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol

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Abstract

The pharmacological profile of a γ-aminobutyric acid A ($GABA_A$) receptor depends upon subunit composition. Studies using recombinant expression systems suggest that δ-subunit containing $GABA_A$ receptors are particularly sensitive to the actions of the $GABA_A$ partial agonist, 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol (THIP, gaboxadol). Here we investigated the actions of THIP in mutant mice lacking the $GABA_A$ receptor δ-subunit gene. Using the chloride flux assay, we determined that the actions of THIP were reduced by 21% in the cortical, but not cerebellar, membranes of knockout mice. Similar results were seen with another $GABA_A$ agonist, muscimol. Moreover, δ-subunit knockout mice exhibited a 54% reduction in sensitivity to the hypnotic actions of THIP as assessed by the loss of righting reflex test. These data support the notion that δ-containing $GABA_A$ receptors are at least partially responsible for the actions of THIP, and contribute to the growing literature suggesting that the pharmacological specificity of $GABA_A$ receptors depends on which subunits are present or absent.

Index terms: $GABA_A$; δ-Subunit; Knockout mouse; THIP

1. Introduction

γ-Aminobutyric Acid A ($GABA_A$) receptor systems mediate fast synaptic inhibitory neurotransmission in the mammalian brain. These receptors are pentameric structures composed of five subunits arranged to form the ion channel pore. Although a number of $GABA_A$ receptor subunits have been discovered (α1-6, β1-3, γ1-3, δ, ε, θ1-3, π, ρ1-3), most native $GABA_A$ receptors are composed of two α, two β, and a γ subunit. Evidence suggests that subunit composition may determine $GABA_A$ receptor pharmacology (Ebert et al., 1997). The importance of subunit composition is reflected in recent evidence from the mutant mouse literature suggesting that different $GABA_A$ receptor subunits modulate different drug-induced behaviors (Boehm et al., 2004).

Recent interest has focused on δ-containing $GABA_A$ receptors. δ-Subunits are expressed in most brain regions, including the cerebellum, cortex, and hippocampus (Pirker et al., 2000). However, the expression of δ-subunits is almost exclusively extrasynaptic (Nusser et al., 1998). Interestingly, recent work has demonstrated that extrasynaptic δ-containing $GABA_A$ receptors are particularly sensitive to neurosteroids (Stell et al., 2003; Adkins et al., 2001) and although not all studies agree, to ethanol (Borghese et al., 2006; Wallner et al., 2003; Sundstrom-Poromaa et al., 2002). Additional evidence suggests that receptors possessing this subunit might also be particularly sensitive to the $GABA_A$ receptor partial agonist, 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol (THIP, gaboxadol). Compared with the $GABA_A$ agonists, GABA and muscimol, THIP had more robust actions at $GABA_A$ receptors possessing δ-subunits (Stórustovu and Ebert, 2006; Adkins et al., 2001), with greater than 10-fold lower EC_{50} values, and higher maximal efficacies, than similar recombinant receptors possessing γ2-subunits (Brown et al., 2002). Moreover, δ-subunit expression was up-regulated during diestrus, and this was associated with a heightened sensitivity to

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THIP's protective actions against seizures precipitated by kainic acid (Maguire et al., 2005).

δ-Subunit knockout mice were recently developed by Mihalek et al. (1999). Although these mice bred and developed normally, the knockouts displayed altered sensitivity in some ethanol behavioral tests (Mihalek et al., 2001), and were less sensitive to the behavioral and functional actions of some neurosteriods (Stell et al., 2003; Vicini et al., 2002; Mihalek et al., 1999). Given the work in recombinant expression systems suggesting that THIP may have greater actions at δ-containing $GABA_A$ receptors (Stórustovu and Ebert, 2006; Adkins et al., 2001; Brown et al., 2002), we propose that assessing behavioral sensitivity to THIP in δ-subunit knockout mice will help identify behaviors that are modulated by δ-subunit containing $GABA_A$ receptors. In the present work we first assess the *in vitro* functional actions of THIP (and muscimol) in δ-subunit knockout and wildtype mice using the chloride flux assay. We next examine sensitivity to THIP's hypnotic actions in these mice using the loss of righting reflex test.

2. Methods

2.1. Animals

δ-Knockout and wildtype mice were maintained on a mixed C57BL/6J X 129Sv/SvJ genetic background, and were derived from heterozygote matings. See Mihalek et al. (1999) for details on their production. Two-month old male and female mice (generation F8) were shipped to the University of Texas at Austin and allowed to acclimate for two weeks prior to behavioral testing. Mice were housed in same-sex groups, four to five to a cage, and food and water were available ad libitum. The vivarium was maintained on a 12:12-h light/dark cycle with lights on at 7:00 AM, with the temperature and humidity maintained at 20 °C and 50%, respectively. All experiments were performed during the light phase of the light/dark cycle. The procedures outlined herein were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and were approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

2.2. [$^{36}Cl^-$] flux

Isolated cortical or cerebellar membrane vesicles (microsacs) were prepared, and THIP-mediated $^{36}Cl^-$ uptake was assayed (Harris and Allan, 1985). Brain tissue was homogenized in 4.5 ml of ice-cold assay buffer (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM D-glucose, 10 mM Hepes, adjusted to pH 7.5 with Tris base). The final pellet was suspended in assay buffer and tissue aliquots (0.6–1.5 mg protein) were incubated in a 34 °C water bath for 5 min. Uptake was initiated by adding 200 μl of [$^{36}Cl^-$] solution (ICN, Costa Mesa, CA; 2 μCi/ml of assay buffer: 1 μCi=37 kBq) containing the drugs to be tested. Three seconds after the addition of [$^{36}Cl^-$], influx was terminated by adding 4 ml of ice-cold quench buffer (assay buffer with 100 μM picrotoxin) followed by rapid filtration through a GB100R filter (Advantec MFS, Dublin, CA), and subsequent washing with 8 ml quench buffer. Filters were incubated overnight in 4 ml Biosafe II scintillation liquid (Research Products International, Mount Prospect, IL) before analysis in a Beckman LS 6500 scintillation counter (Beckman Coulter, Fullerton, CA). The amount of [$^{36}Cl^-$] bound to the filters in the absence of membranes (no-tissue blank) was subtracted from all values. THIP-mediated flux was defined as the amount of [$^{36}Cl^-$] taken up when the receptor partial agonist was present in the assay medium (total uptake) minus the amount of [$^{36}Cl^-$] uptake when it was not present (THIP-independent or non-specific uptake). THIP was acquired from Sigma-Aldrich (St. Louis, MO), and dissolved in assay buffer.

For comparison, maximal muscimol-mediated [$^{36}Cl^-$] flux was assessed in both the cortical and cerebellar microsacs of δ-deficient and wildtype mice. The experimental procedures were similar to those described above for THIP. Muscimol was acquired from Sigma-Aldrich (St. Louis, MO), and dissolved in assay buffer. The muscimol concentration (30 μM) producing maximal [$^{36}Cl^-$] flux was determined from pilot studies using cortical and cerebellar membranes from wildtype mice of similar genetic background.

2.3. Loss of righting reflex

Animals were injected with THIP (30 mg/kg, s.c.), and the latency and duration of THIP-induced loss of righting reflex were measured by placing mice on their backs in a sleep trough (~90° angle). The 30 mg/kg dose was chosen based on preliminary studies showing that it produced robust loss of righting reflex for about 90 min in mice of similar genetic background. Loss of righting reflex was defined as the inability of a mouse to right itself within 30 s. Return of the righting response was defined as the ability of a mouse to right itself twice in 1 min. Duration of loss of righting reflex was defined as the time between loss of righting reflex and return of the righting response. THIP was dissolved in 0.9% saline.

2.4. Statistical analysis

Preliminary analyses did not indicate significant interactions of gender with any other factor. Thus, male and female data were combined for statistical analyses. Data were analyzed by two-tailed *t*-test or analysis of variance (ANOVA) using GraphPad Prism (version 4). Data are represented as mean±S.E.M.

3. Results

3.1. [$^{36}Cl^-$] flux

Basal [$^{36}Cl^-$] influx did not differ between the δ-knockout and wildtype mice, regardless of brain region assayed (cortex, 1.8±0.3 and 2.2±0.4 nmol/mg for knockout and wildtype, respectively; cerebellum, 3.2±0.5 and 3.0±1.2 nmol/mg for knockout and wildtype, respectively). THIP potentiated [$^{36}Cl^-$] influx in both the cortical and cerebellar membranes of δ-knockout and wildtype mice (n=4–10 per genotype). However, the cortical membranes of the δ-knockout mice were significantly less sensitive to this action of THIP (main effect of genotype, $F[1,78]=4.6$, $P<0.05$, Fig. 1A). EC_{50} and Hill Coefficients did not differ between the

genotypes, but THIP was less efficacious, with knockouts exhibiting a lower E_{max} (13.0±1.4 and 16.2±3.1 nmol/mg protein for δ-knockout and wildtype, respectively; $F[7,9]=3.9$, $P<0.05$). The actions of THIP did not differ in the cerebellar membranes of δ-knockout and wildtype mice (Fig. 1B).

For comparison, maximal muscimol-mediated [$^{36}Cl^-$] influx was also determined. Basal [$^{36}Cl^-$] influx did not differ between the genotypes or brain regions assayed (cortex, 1.8±0.3 and 2.2±0.4 nmol/mg for knockout and wildtype, respectively; cerebellum, 3.2±0.5 and 3.0±1.2 nmol/mg for knockout and wildtype, respectively). Maximal muscimol (30 μM) potentiated flux in both the cortical and cerebellar membranes of both genotypes ($n=4$–10 per genotype). However, similar to that which was reported for THIP, the response to maximal muscimol was reduced in the cortical membranes of δ-knockout mice (13.1±1.5 and 18.2±3.0 for knockouts and wildtypes, respectively; $t[17]=1.6$, $P=0.07$). The maximal actions of muscimol did not differ in the cerebellar membranes of δ-knockout and wildtype mice (9.8±0.9 and 8.9±1.2 for knockouts and wildtypes, respectively).

3.2. Loss of righting reflex

A subcutaneous injection of 30 mg/kg THIP produced loss of righting response in both δ-knockout and wildtype mice ($n=5$–6 per genotype). However, the latency to loss of righting response was significantly longer for the δ-knockouts ($t[9]=3.6$, $P<0.01$; Fig. 2A). Furthermore, the duration of loss of righting reflex was much shorter for the knockouts ($t[9]=5.6$, $P<0.001$; Fig. 2B).

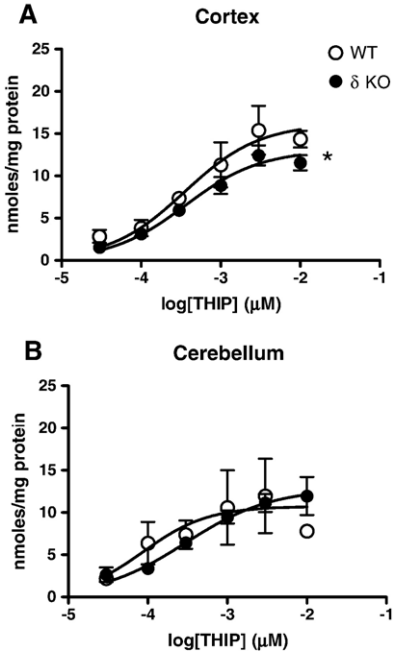

Fig. 1. Genetic deletion of the $GABA_A$ receptor δ-subunit gene in mice reduces the *in vitro* actions of THIP in cortical, but not cerebellar membranes. A. The cortical membranes of δ-knockout mice were less sensitive to THIP-mediated [$^{36}Cl^-$] flux. Although EC_{50} (362 and 351 μM, δ-knockout and wildtype, respectively) and Hill Coefficients (0.92 and 0.92 for δ-knockouts and wildtypes, respectively) did not differ between the genotypes, knockouts exhibited a reduced E_{max} (13.0 and 16.2 nmol/mg protein for δ-knockout and wildtype respectively). *$P<0.05$. B. The cerebellar membranes of δ-knockout and wildtype mice did not differ in sensitivity to THIP-mediated [$^{36}Cl^-$] flux (EC_{50}: 300 and 88 μM for δ-knockouts and wildtypes, respectively; Hill Coefficients: 0.80 and 1.10 for δ-knockout and wildtype, respectively; E_{max}: 12.9 and 10.7 nmol/mg protein for δ-knockout and wildtype, respectively).

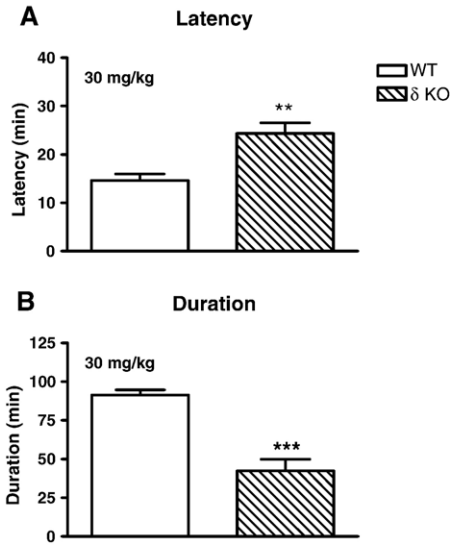

Fig. 2. Genetic deletion of the $GABA_A$ receptor δ-subunit gene in mice reduces the hypnotic actions of THIP. A. Subcutaneous administration of THIP (30 mg/kg) produced loss of righting reflex in both genotypes, but with a significantly longer latency in δ-knockout mice. **$P<0.01$. B. Subcutaneous administration of THIP (30 mg/kg) also produced a shorter duration of loss of righting reflex in δ-knockout mice. ***$P<0.001$.

4. Discussion

The pharmacological profile of $GABA_A$ receptors depends on which subunits are present or absent (Ebert et al., 1997). Previous work in recombinant expression systems has demonstrated that δ-containing receptors are more sensitive to the actions of drugs that enhance receptor function, including several neurosteriods (Brown et al., 2002), ethanol (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003), and the $GABA_A$ receptor partial agonist, THIP or gaboxadol (Stórustovu and Ebert, 2006; Adkins et al., 2001; Brown et al., 2002). Recently developed δ-subunit knockout mice exhibit impaired neuronal and behavioral sensitivity to neurosteriods (Stell et al., 2003; Vicini et al., 2002; Mihalek et al., 1999), and ethanol (Mihalek et al., 2001), but did not differ in sensitivity to a barbiturate, benzodiazepine, or an intravenous or volatile anesthetic (Mihalek et al., 1999). We speculated that if δ-containing $GABA_A$ receptors are particularly sensitive to the actions of THIP, δ-knockout mice should exhibit reduced sensitivity to the *in vitro* functional and behavioral actions of this drug. In the present work we show that THIP-mediated [$^{36}Cl^-$] flux is reduced in the cortical, but not cerebellar, membranes of the δ-knockouts, and this was paralleled by a similar change in the cortical actions of muscimol. We also show that the change in THIP's functional actions is accompanied by a change in THIP behavioral sensitivity; δ-knockout mice are less sensitive to the hypnotic actions of THIP as assessed by the loss of righting reflex test. These results support those from recombinant expression studies (Stórustovu and Ebert, 2006; Adkins et al., 2001; Brown et

al., 2002), and suggest that δ-containing $GABA_A$ receptors are sensitive to the actions of THIP.

Our results indicate a role for cortical circuits in the modulation of THIP-induced loss of righting reflex. Indeed, sensitivity to this behavior was reduced by 54% in δ-knockout mice, and this coincided with a reduction in the *in vitro* actions of THIP in cortical membranes. However, why were the actions of THIP not altered in cerebellum, a brain structure thought to have a key role in this behavior? One possibility is that our chloride flux assay was not sensitive enough to detect a reduction in $GABA_A$ receptor function in the cerebellar tissue of δ-knockout mice. Indeed, cerebellar granule cells are the only cell type known to possess δ-subunits in mouse cerebellum (Laurie et al., 1992). Given the far greater number of other cerebellar cell types that do not possess this subunit, the chloride flux assay may not have been sensitive enough to resolve δ-specific changes in THIP's actions on the smaller population of granule cells. However, yet another possibility is that developmental compensatory responses may have compensated for the otherwise deficient δ-knockout phenotype. For example, perhaps up or down-regulation of other $GABA_A$ receptor subunits in the cerebellar granule cells of the knockouts made up for the loss of the δ-subunit (i.e., producing $GABA_A$ receptors that maintain their sensitivity to THIP). At the present time we cannot say one way or the other. However, such developmental compensatory responses have been described in δ-knockouts; Tretter et al. (2001) demonstrated that α6β2/3 receptors co-expressing γ2 (instead of δ) are up-regulated in the granule cells of δ-deficient mice.

The presence of δ-subunits alone may not determine sensitivity to THIP. Indeed, the hypnotic and *in vitro* actions of THIP were not completely eliminated, nor was a biphasic concentration response curve seen, in the knockouts. These results suggest that THIP's actions are not exclusively mediated through δ-containing $GABA_A$ receptors. α4-Subunits are normally co-expressed with extrasynaptic δ-subunits in rodent forebrain. Interestingly, these subunits are down-regulated in the forebrains of δ-knockout mice (Peng et al., 2002), and the remaining α4-subunits appear to co-assemble with γ2-subunits (Korpi et al., 2002; Peng et al., 2002). That the δ-knockout mice exhibit a reduction in the cortical (and presumably forebrain) actions of THIP in the present work may suggest that THIP has preferential actions at $GABA_A$ receptors that possess both δ- and α4-subunits. Indeed, this might also explain why δ-gene deletion only resulted in a 21% reduction in cortical flux; α4-subunits are also important in conferring sensitivity to THIP. Future studies will be necessary to fully explore this possibility.

Interestingly, δ-gene deletion also resulted in a 28% reduction in muscimol-stimulated cortical flux. Although full concentration response curves were not generated, our goal was to assess the maximal actions of another GABAergic compound in δ-knockout mice. Muscimol was chosen because, like THIP, it is a $GABA_A$ receptor agonist, and therefore interacts with the GABA binding sites on the receptor channel. Based on the work of Stórustovu and Ebert (2006) and Adkins et al. (2001), our expectation was that muscimol-stimulated flux would also be reduced in the knockouts. However, we hypothesized that the reduction would be similar to or smaller than that seen using THIP. Our results support the notion that extrasynaptic δ-containing receptors are important targets of $GABA_A$ receptor agonists, and help explain why systemic administration of GABAergic compounds like THIP and muscimol appear to act as selective extrasynaptic $GABA_A$ receptor agonists (Stórustovu and Ebert, 2006). Moreover, considered along with the reduction in cortical flux seen with THIP, these results suggest that δ-containing extrasynaptic $GABA_A$ receptors may account for about 20–30% of the total $GABA_A$ receptor neurotransmission in cortical areas.

Induction of THIP-induced loss of righting reflex may depend as much on the amount of time it takes blood levels to equalize as it does nervous system sensitivity to the drug. Moreover, duration of loss of righting reflex may also be influenced by metabolism and elimination of THIP. Due to difficulties in its assessment, we did not assess blood levels of THIP in the δ-knockout and wild-type mice. Nevertheless, our flux data is consistent with a genotypic change in neuronal (cortical) sensitivity to THIP. Although we cannot be sure that this change in neuronal sensitivity underlies the change in hypnotic sensitivity to the drug, it does suggest that deletion of the δ-subunit gene can and does alter neuronal sensitivity to THIP.

δ-Containing $GABA_A$ receptors exhibit enhanced sensitivity to the actions of the $GABA_A$ receptor partial agonist, THIP (Stórustovu and Ebert, 2006; Maguire et al., 2005; Brown et al., 2002; Adkins et al., 2001). The current work adds to this literature by showing that the functional actions of THIP are reduced at cortical $GABA_A$ receptors in δ-subunit knockout mice, and that this change is paralleled by a reduced sensitivity to THIP's hypnotic actions. These results support the notion that the pharmacological profile of $GABA_A$ receptors depends upon which subunits come together to make up the receptor. Knowledge of which subunits confer heightened sensitivity to which $GABA_A$ receptor modulators, as well as the regional and cellular localization of specific $GABA_A$ receptor subunit combinations in brain, should lead to the development of better anxiolytic and anesthetic drugs with fewer unwanted side effects.

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References

Adkins, C.E., Pillai, G.V., Kerby, J., Bonnert, T.P., Haldon, C., McKernan, R.M., Gonzalez, J.E., Oades, K., Whiting, P.J., Simpson, P.B., 2001. α4β3δ $GABA_A$ receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. J. Biol. Chem. 276, 38934–38939.

Boehm II, S.L., Ponomarev, I., Jennings, A.W., Whiting, P.J., Rosahl, T.W., Garrett, E.M., Blednov, Y.A., Harris, R.A., 2004. γ-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions. Biochem. Pharmacol. 68, 1581–1602.

Borghese, C.M., Stórustovu, S.í., Ebert, B., Marshall, G., Wafford, K.A., Harris, R.A., 2006. The delta subunit of the γ-aminobutyric acid type A receptors

does not confer sensitivity to low concentrations of ethanol. J. Pharmacol. Exp. Ther. 316, 1360–1368.

Brown, N., Kerby, J., Bonnert, T.P., Whiting, P.J., Wafford, K.A., 2002. Pharmacological characterization of a novel cell line expressing human $\alpha4\beta3\delta$ $GABA_A$ receptors. Br. J. Pharmacol. 136, 965–974.

Ebert, B., Thompson, S.A., Saounatsou, K., McKernan, R., Krogsgaard-Larsen, P., Wafford, K.A., 1997. Differences in agonist/antagonist binding affinity and receptor transduction using recombinant human γ-aminobutyric acid type A receptors. Mol. Pharmacol. 52, 1150–1156.

Harris, R.A., Allan, A.M., 1985. Functional coupling of γ-aminobutyric acid receptors to chloride channels in brain membranes. Science 228, 1108–1110.

Korpi, E.R., Mihalek, R.M., Sinkkonen, S.T., Hauer, B., Hevers, W., Homanics, G.E., Sieghart, W., Luddens, H., 2002. Altered receptor subtypes in the forebrain of $GABA_A$ receptor δ subunit-deficient mice: recruitment of γ2 subunits. Neuroscience 109, 733–743.

Laurie, D.J., Seeburg, P.H., Wisden, W., 1992. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. J. Neurosci. 12, 1063–1076.

Maguire, J.L., Stell, B.M., Rafizadeh, M., Mody, I., 2005. Ovarian cycle-linked changes in GABAA receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat. Neurosci. Adv. (online publication).

Mihalek, R.M., Banerjee, P.K., Korpi, E.R., Quinlan, J.J., Firestone, L.L., Mi, Z.-P., Lagenaur, C., Tretter, V., Sieghart, W., Anagnostaras, S.G., Sage, J.R., Fanselow, M.S., Guidotti, A., Spigelman, I., Li, Z., DeLorey, T.M., Olsen, R.W., Homanics, G.E., 1999. Attenuated sensitivity to neurosteriods in gamma-aminobutyrate type A receptor delta subunit knockout mice. Proc. Natl. Acad. Sci. U. S. A. 96, 12905–12910.

Mihalek, R.M., Bowers, B.J., Wehner, J.M., Kralic, J.E., VanDoren, M.J., Morrow, A.L., Homanics, G.E., 2001. $GABA_A$-receptor δ-subunit knockout mice have multiple defects in behavioral sensitivity to ethanol. Alcohol Clin. Exp. Res. 25, 1708–1718.

Nusser, Z., Sieghart, W., Somogy, P., 1998. Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J. Neurosci. 18, 1693–1703.

Peng, Z., Hauer, B., Mihalek, R.M., Homancis, G.E., Sieghart, W., Olsen, R.W., Houser, C.R., 2002. $GABA_A$ receptor changes in δ subunit-deficient mice: altered expression of α4 and γ2 subunits in the forebrain. J. Comp. Neurol. 446, 179–197.

Pirker, S., Schwarzer, C., Wieselthaler, A., Sieghart, W., Sperk, G., 2000. $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101, 815–850.

Stell, B.M., Brickley, S.G., Tang, C.Y., Farrant, M., Mody, I., 2003. Neurosteriods reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing GABAA receptors. Proc. Natl. Acad. Sci. U. S. A. 100, 14439–14444.

Stórustovu, S.í., Ebert, B., 2006. Correlation between pharmacology at $\alpha_4\beta_3\delta$ containing receptors expressed in *Xenopus* oocytes and inhibition of spontaneous activity in the rat cortical wedge preparation. J. Pharmacol. Exp. Ther. 316, 1351–1359.

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 $\alpha4\beta2\delta$ $GABA_A$ receptors are a target for alcohol. Nat. Neurosci. 5, 721–722.

Tretter, V., Hauer, B., Nusser, Z., Mihalek, R.M., Höger, H., Homanics, G.E., Somogyi, P., Sieghart, W., 2001. Targeted disruption of the $GABA_A$ receptor δ subunit gene leads to up-regulation of γ2 subunit-containing receptors in cerebellar granule cells. J. Biol. Chem. 276, 10532–10538.

Vicini, S., Losi, G., Homanics, G.E., 2002. $GABA_A$ receptor δ subunit deletion prevents neurosteroid modulation of inhibitory synaptic currents in cerebellar neurons. Neuropharmacology 43, 646–650.

Wallner, M., Hanchar, H.J., Olsen, R.W., 2003. Ethanol enhances $\alpha4\beta3\delta$ and $\alpha6\beta3\delta$ γ-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc. Natl. Acad. Sci. 100, 15218–15223.