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Regulation of $GABA_A$ Receptor Subunit Expression by Pharmacological Agents

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Abstract*——The γ-aminobutyric acid (GABA) type A receptor system, the main fast-acting inhibitory neurotransmitter system in the brain, is the pharmacological target for many drugs used clinically to treat, for example, anxiety disorders and epilepsy, and to induce and maintain sedation, sleep, and anesthesia. These drugs facilitate the function of pentameric $GABA_A$ receptors that exhibit widespread expression in all brain regions and large structural and pharmacological heterogeneity as a result of composition from a repertoire of 19 subunit variants. One of the main problems in clinical use of $GABA_A$ receptor agonists is the development of tolerance. Most drugs, in long-term use and during withdrawal, have been associated with important modulations of the receptor subunit expression in brain-region-specific manner, participating in the mechanisms of tolerance and dependence. In most cases, the molecular mechanisms of regulation of subunit expression are poorly known, partly as a result of neurobiological adaptation to altered neuronal function. More knowledge has been obtained on the mechanisms of $GABA_A$ receptor trafficking and cell surface expression and the processes that may contribute to tolerance, although their possible pharmacological regulation is not known. Drug development for neuropsychiatric disorders, including epilepsy, alcoholism, schizophrenia, and anxiety, has been ongoing for several years. One key step to extend drug development related to $GABA_A$ receptors is likely to require deeper understanding of the adaptational mechanisms of neurons, receptors themselves with interacting proteins, and finally receptor subunits during drug action and in neuropsychiatric disease processes.

I. Introduction

Chemical balance in the brain systems for neuronal excitation, inhibition, rhythmic activity, and scaling of neuronal discharge probabilities is a very important basic mechanism that has been used in pharmacological modulation of behavior, including therapies of emotional and affective disturbances, cognitive impairment, and motor disturbances resulting from neurodegeneration and genetic abnormalities. In simple terms, the chemical balance is primarily set by the activities of the most widely distributed neurotransmitter systems in the CNS,[1] namely the excitatory glutamate system and the inhibitory GABA system, although the balance is also modulated by a large number of other slower acting transmitters, modulators, and ion channels. The GABA system is widely used in the treatment of anxiety disorders, insomnia, epilepsy, restlessness, and aggressive behaviors, and it is the target for many intravenous and inhalational anesthetics and drugs of abuse, such as alcohol. Several severe problems are related to the long-term therapeutic use of GABA system-affecting drugs, most significantly the loss of efficacy, tolerance development, dependence development, and finally addiction to at least some of these drugs.

The GABA system is a ubiquitous system regulated by a number of different genes, affecting synthesis of various receptor subunits, interacting proteins, and associated transporters and synthetic and catabolic enzymes. Figure 1 illustrates a GABAergic synapse with its main pharmacological components. Pharmacology has focused on the modification of receptors and transmitters of the system: most of the clinically used drugs target the GABA type A receptors ($GABA_A$), the GABA type B receptors (e.g., agonist baclofen), GABA transporters in neurons and glial cells (e.g., inhibitor tiagabine), and the catabolic enzyme GABA-transaminase (e.g., inhibitor vigabatrin) to increase the level of GABA.

This review will focus on one part of the GABA system: the $GABA_A$ receptors (Fig. 2). $GABA_A$ receptors are pentameric complexes of subunits, and they form an integral anion channel, permeable to chloride and bicarbonate ions. These receptors are also molecular targets for various

[1] Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; BZ, benzodiazepine; CA, cornu ammonis; CE, continuous ethanol; CGC, cerebellar granule cell; CIE, continuous intermittent ethanol; CNS, central nervous system; CRE, cAMP response element; CREB, cAMP response element binding protein; FG 7142, *N*-methyl-β-carboline-3-carboxamide; GABA, γ-aminobutyric acid; GABARAP, $GABA_A$ receptor-associated protein; ICER, inducible cAMP early repressor; JAK, Janus kinase; L, long splice variant; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A; Ro 15-4513, 8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid ethyl ester; S, short splice variant; STAT, signal transducer and activator of transcription; wdr-CE, withdrawal from CE; wdr-CIE, withdrawal from CIE.

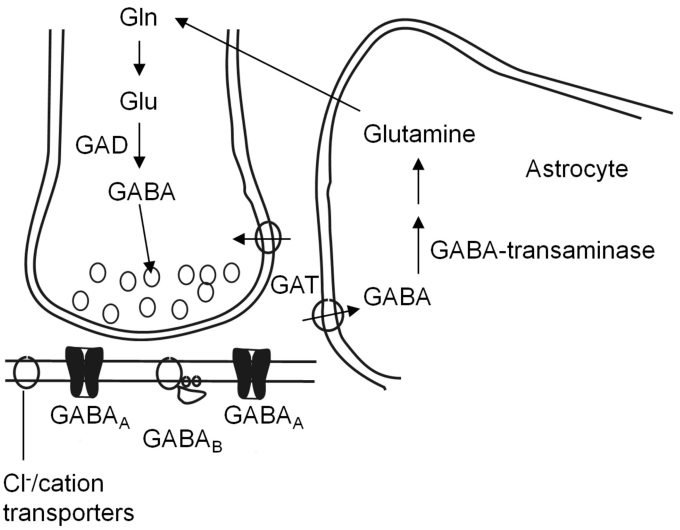

FIG. 1. GABAergic synapse with synthetic and catabolic enzymes, receptors, and transporters. GAD, glutamate decarboxylase, the GABA-synthesizing enzyme that exists in two forms (GAD65) and GAD67; GABA transaminase, GABA metabolizing enzyme; GAT, a GABA transporter existing at least in four subtypes; $GABA_B$ receptor is a G-protein-coupled heptahelix receptor. The most important of the Cl^-/cation transporters is KCC2, which provides the chloride gradient for the hyperpolarizing action of $GABA_A$ receptor activation in mature neurons.

classes of benzodiazepine-site (BZ-site) ligands, barbiturates, neurosteroids, intravenous anesthetics propofol and etomidate, inhalation anesthetics, and alcohol (Fig. 3). The molecular actions of various drugs on the $GABA_A$ receptors have been well reviewed (Lüddens et al., 1995; Rabow et al., 1995; Sieghart, 1995; Sigel and Buhr, 1997; Korpi et al., 2002; Lambert et al., 2003). In mature neurons, under normal conditions, the activation of $GABA_A$ receptors leads to hyperpolarization of cell membrane potential and inhibition of neuronal activity. However, in immature neurons, in which the ion gradient-forming anion transporters are not yet fully operational, $GABA_A$ receptor activation produces depolarization (Ben-Ari et al., 1997). The opposite process may take place in such pathological conditions as neuropathic pain (see, Kahle et al., 2008). Prolonged activation of the system leads to loss of function, tolerance, the mechanisms of which are still poorly known. This review does not deal with mechanisms of acute tolerance (i.e., the reduced sensitivity of the system due to changes within one use period of a drug) or those of innate tolerance (i.e., inherited mechanisms regulating the acute sensitivity of the system). Drugs acting via $GABA_A$ receptors often affect the regulation of various $GABA_A$ receptor subunits, which might provide the simplest mechanism for tolerance. Therefore, the present review is focused on the data how the expressions of different subunits are affected by specific pharmacological manipulations.

II. Expression of $GABA_A$ Receptors

A. $GABA_A$ Receptor Genes

A total of 19 mammalian genes coding for $GABA_A$ receptor subunits, belonging to eight subunit classes, have been cloned: $\alpha1$–$\alpha6$, $\beta1$–$\beta3$, $\gamma1$–$\gamma3$, δ, ε, θ, π, $\rho1$–$\rho3$ (Olsen and Sieghart, 2008). Subsequent gene mapping showed that most $GABA_A$ receptor genes are clustered in vertebrate genomes (Wilcox et al., 1992; Russek and Farb, 1994; Bailey et al., 1999; Russek, 1999). Fourteen of the 19 human $GABA_A$ receptor genes are clustered on four chromosomes, 4p12-p13, 5q31-q35, 15q11-q13, and Xq28 (Russek, 1999). Two clusters of four genes each encode two α subunits, one β subunit, and one γ subunit (*GABRA2*, *GABRA4*, *GABRB1*, and *GABRG1* on chromosome 4, and *GABRA1*, *GABRA6*, *GABRB2*, and *GABRG2* on chromosome 5). The two other clusters each contains three genes: the cluster in chromosome 15 comprises one α subunit gene (*GABRA5*), one β subunit gene (*GABRB3*), and one γ subunit gene (*GABRG3*), and the cluster in X chromosome consists of one α subunit gene (*GABRA3*), the θ subunit gene (*GABRQ*), and the ε subunit gene (*GABRE*). The θ and ε polypeptides exhibit about 50% identity to β and γ subunits, respectively. Therefore, the θ and ε subunits are considered "β-like"

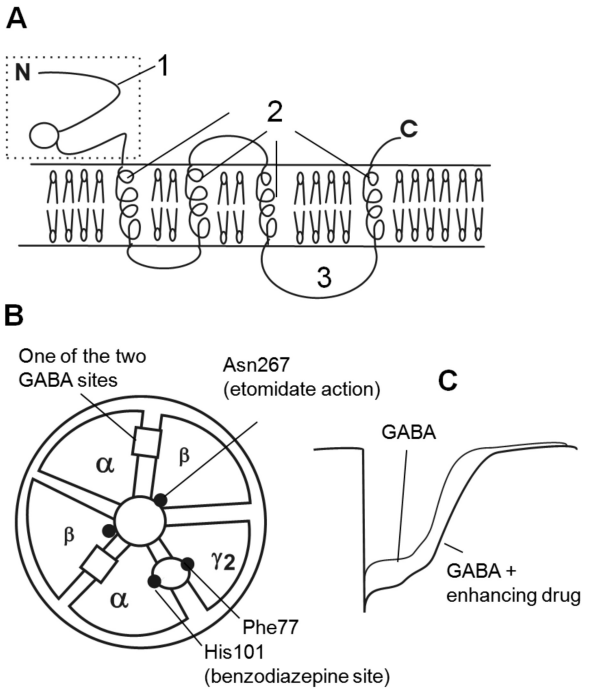

FIG. 2. Schematics of $GABA_A$ receptor structure and function. A, topography of a $GABA_A$ receptor subunit partially embedded in the lipid bilayer. 1, N-terminal extracellular domain responsible for transmitter and ligand binding and coupling of the binding sites with ion channel. This part is also important for the allosteric effects and for the assembly of various receptor subunits into functional receptors. 2, four transmembrane regions forming the anion channel are responsible for binding of hydrophobic ligands, ion selectivity, and channel binding sites. 3, intracellular loop between transmembrane helices 3 and 4 forms the motif for regulatory phosphorylation sites and for the intracellular factors anchoring the receptors in appropriate locations (e.g., on the postsynaptic thickening) using interactions with auxiliary and cell structural proteins. B, hypothetical binding sites for GABA and allosteric modulators such as benzodiazepine ligands and a domain essential for the functions of various ligands such as etomidate, loreclezole, and methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate in a pentameric receptor complex. C, allosteric activation of $GABA_A$ receptor may increase the peak height (amplitude) of the response and/or prolong the response as compared with the response by GABA alone.

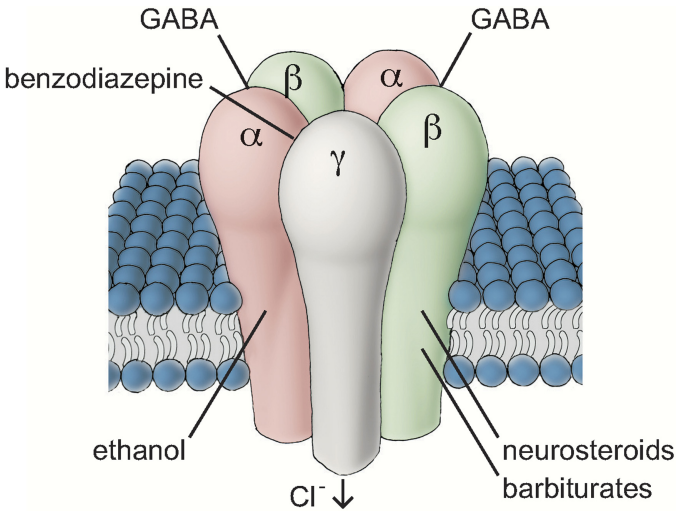

FIG. 3. Schematic illustration of the $GABA_A$ receptor and its associated binding sites. The receptor is pentameric, being composed of two α, two β, and one γ subunit. $GABA_A$ receptors contain recognition sites for a variety of clinically relevant drugs. The binding of GABA in two GABA binding sites at the interface between α and β subunits open the receptor-associated chloride (Cl^-) channel. The benzodiazepine binding site is located at the interface between α and $\gamma 2$ subunits. Barbiturates, ethanol, and neurosteroids bind to sites in the membrane-spanning transmembrane regions of the subunits.

and "γ-like," respectively. Thus, each cluster contains one or two α, one β (or "β-like"), and one γ (or "γ-like") subunit gene. The rat and mouse $GABA_A$ receptor genes are clustered similarly to human genes (Olsen and Sieghart, 2008).

It has been proposed that the mammalian $GABA_A$ receptor genes evolved from a common ancestral gene cluster, which is presumed to have comprised an "α-like," a "β-like," and a "γ-like" subunit gene, as a consequence of two whole-genome duplication events (Russek and Farb, 1994; Bailey et al., 1999; Russek, 1999). It is assumed that a tandem duplication of an α subunit gene took place in one of the clusters after the first but before the second duplication. The clusters on human chromosomes 4 and 5 evolved from a common ancestral cluster, and the clusters on chromosome 15 and X arose from a second common ancestral cluster. The model is supported by the fact that genes having the same location within the clusters on chromosomes 4 and 5 (i.e., $\alpha 1$ and $\alpha 2$ or $\alpha 4$ and $\alpha 6$, respectively) are more closely related to one another than to any other α subunit genes.

B. Brain Regional Expression of Subunit mRNAs

Each of the $GABA_A$ receptor subunit mRNAs displays a unique distribution (Laurie et al., 1992a; Persohn et al., 1992; Wisden et al., 1992). Some subunits are ubiquitously expressed almost throughout the brain, whereas the localization of most subunits is more narrowly confined. Some neuronal populations coexpress large number of subunit mRNA isoforms, whereas others express only a few $GABA_A$ receptor subunits (Laurie et al., 1992a; Persohn et al., 1992; Wisden et al., 1992). Neocortex, hippocampus, and caudate-putamen display complex expression patterns of large diversity of subunit combinations. In contrast, essentially one of each isoform of the α, β, and γ subunit classes is expressed in the inferior colliculus, substantia nigra pars reticulata, and cerebellar stellate/basket cells (Laurie et al., 1992a; Wisden et al., 1992). The brain regional expression profiles of $GABA_A$ receptor subunits are presented in Table 1.

The expression of the two major α subunit isoforms, $\alpha 1$ and $\alpha 2$, is both widespread in the brain. The most abundant subunit, $\alpha 1$, is expressed almost ubiquitously in the brain (Wisden et al., 1992). Its expression is especially rich in the olfactory bulb tufted and mitral cells, pyriform cortex, globus pallidus, endopeduncular nucleus, medial septum and diagonal band, parafascicular nucleus of the thalamus, red nucleus, inferior colliculus, cerebellar molecular and granule cell layers, and Purkinje cells (Laurie et al., 1992a: Persohn et al., 1992; Wisden et al., 1992). Despite widespread expression of the $\alpha 2$ subunit, there is a negative correlation between the brain regional expression patterns of $\alpha 1$ and $\alpha 2$ subunits. The expression of $\alpha 2$ mRNA is high in granule cells of the olfactory bulb, hippocampus, amygdala, lateral septum and in the medial preoptic area of the hypothalamus (Persohn et al., 1992; Wisden et al., 1992). In the cerebellum $\alpha 2$ mRNA is solely expressed in Bergmann glial cells (Laurie et al., 1992a; Persohn et al., 1992). The expression of $\alpha 3$ mRNA is localized in the olfactory bulb, cerebral cortex and brain stem nuclei (Persohn et al., 1992; Wisden et al., 1992). The expression of $\alpha 4$ mRNA is confined to the hippocampus, thalamus, caudate-putamen, nucleus accumbens, and neocortex (Wisden et al., 1992). The $\alpha 5$ mRNA is highly expressed in the hippocampus. It is also present at lower levels in the olfactory bulb granule cells, neocortex, and hypothalamus (Laurie et al., 1992a; Persohn et al., 1992). The $\alpha 6$ mRNA expression pattern is the most restricted of the $GABA_A$ receptor subunits: $\alpha 6$ mRNA is expressed in the cerebellar granule cells and cochlear nucleus granule cells (Laurie et al., 1992a, Varecka et al., 1994).

The expression of $\beta 1$ mRNA is strong in the olfactory bulb mitral cells and in the hippocampus. It is also expressed at lower levels in the cerebral cortex, substantia nigra, superior colliculus, and cerebellum (Persohn et al., 1992; Wisden et al., 1992). The expression of $\beta 2$ is more widespread and strongly correlates with that of $\alpha 1$ mRNA. This suggests that the expression of genes coding for $\alpha 1$ and $\beta 2$ subunits, members of the same gene cluster, might be coordinately regulated (Steiger and Russek, 2004). In addition, it suggests that the two subunits assemble within the same receptor complexes with high probability. The mRNA expression pattern of $\beta 3$ is also widespread and strongly correlates with that of $\alpha 2$ mRNA. The genes for these subunits reside in different chromosomes/gene clusters. In any case, their similar expression patterns suggest that the subunits coassemble to form receptors.

TABLE 1

Quantitative estimates for brain regional distribution of $GABA_A$ receptor subunits in adult rat brain

The table is based on the published mRNA in situ hybridization data (Laurie et al., 1992; Persohn et al., 1992; Wisden et al., 1992) and subunit polypeptide immunohistochemical data (Pirker et al., 2000; Schwarzer et al., 2001). The table as such should not be used to compare the absolute levels of various subunits in any brain region. Thus, the brain regional profile for each subunit is given using the same scale, even if the absolute concentrations of subunit mRNAs or peptides are different. The regional profile of each subunit is presented so that 3 denotes the highest expression of the subunit in question; 2, strong expression; 1, low expression; and no grading (empty fields), very low or undetectable expression. The γ3 subunit probes have so faint and even staining in many brain regions both in situ hybridization and immunohistochemical assays that we decided to mark its expression with 1.

Brain Region	$GABA_A$ Receptor Subunit mRNA and Polypeptide Expression												
	α1	α2	α3	α4	α5	α6	β1	β2	β3	γ1	γ2	γ3	δ
Olfactory areas													
External plexiform layer of olfactory bulb	3	1	1		1		2	3	3		3	1	
Glomerular layer	3	1	1				1	3	3		3		1
Internal granular layer		3	3	1	2								1
Olfactory tubercle		2		2			1	1	3		1	1	1
Islands of Calleja	3							3				1	
Primary olfactory cortex	3	3	3	2			1	3	3	1	3	1	1
Cerebral cortex													
Layers 1–4	2	2	1	2	1		2	2	3		2	1	2
Layers 5–6	3	1	3	1	2		3	2	3		2	1	1
Anterior cingulate cortex	2	3	1	1			2	1	3		2		
Limbic regions, hippocampus, amygdala													
Entorhinal cortex	2	2	1	1	1		2	2	2		2	1	1
Subiculum	2	2	1	1	1		2	2	2		2	1	1
Hippocampus, CA1	2	3		2	3		3	1	3		3		
Hippocampus, CA3	1	3		1	3		3	1	3		3		
Hippocampus, dentate gyrus	1	3		2	1		2	2	3		3		1
Bed nucleus stria terminalis, medial	1	3	2	1	1		2	1	2	3	1	1	1
Nucleus of horizontal limb of diagonal band	3		1		1			3	1		2	1	1
Septohippocampal nucleus/taenia tecta	1	3	2	2	1		2		3		1		
Lateral septal nuclei	1	3	2	1			2	1	1	3	2	1	
Triangular septal nucleus	1	2						1	1		1		
Bed nucleus of anterior commissural	1	3	1	1						1			
Anterior amygdaloid area	1	1	1		1		1	2	1	1	2	1	
Amygdala	1	3	1	1			1	2	2	3	1	1	
Posteromedial cortical amygdaloid nucleus	1	3	1		1		1	2	2		1	1	1
Basal ganglia/striatum													
Nucleus accumbens	1	3	1	2	1		1	1	3		1	1	2
Caudate/putamen	1	3	1	2	1		1	1	3		1	1	2
Globus pallidus	3	1			1			3		3	1	1	1
Claustrum	2	2	3	1	1		1	2	2		1	1	1
Ventral pallidum/substantia innominata	3		1	1	1		1	3		3	1	1	
Thalamus, epithalamus													
Paraventricular thalamic nucleus	1	2	1	3			2	2	2		2		1
Anterodorsal thalamic nucleus	3			3				3	1		1		3
Centrolateral/medial thalamic nucleus	2	1	1	2			1	3	1	2	1		1
Intermediodorsal thalamic nucleus	2			3				3					1
Lateral posterior/laterodorsal thalamic nucleus	2			3				3					3
Ventroposterior thalamic nucleus	1			3				3					2
Zona incerta/subthalamic nucleus	2		1					2			1		1
Medial habenular nucleus		2							2		2	1	
Medial geniculate nucleus	2			3				3			1	1	3
Hypothalamus													
Medial preoptic area/periventricular nucleus	1	3	1		1		1		2	2	1		1
Lateral preoptic area	1	1	1					1			1	1	
Lateral hypothalamic area	1						1	1	1		1		1
Anterior hypothalamic area	1						3	1	1		2		1
Paraventricular hypothalamic nucleus	1	1					2	1	2		1		1
Ventromedial hypothalamic nucleus	1	2	1		1		3		2	1	2		1
Mesencephalon, pons, medulla													
Substantia nigra, pars reticulata	3		1				1	2		1	1	1	
Substantia nigra, pars compacta			2	1			1				1	1	1
Ventral tegmental area	1	1	1				2				2	1	1
Interpeduncular nucleus	2	1		2	1				2		1	1	1
Red nucleus	3							2			2	1	1
Superior colliculus, superior gray layer	2	2		1	2			2	2	1	1		
Superior colliculus, intermediate gray layer	2						1	1	2		1	1	
Central gray	3	1	1				2	1	2		1	1	2
Inferior colliculus	3	1						2	1		1	1	1
Raphe nuclei	2	1	2		1		1	2	2		2	1	1
Locus ceruleus			2										
Cerebellum													
Granule cell layer	3					3	1	3	3		1		3
Molecular layer	3	1			1		2	1			2		

The expression of γ1 mRNA is almost undetectable in most brain regions. The expression is highest in the hippocampus, globus pallidus, amygdala, septum, and medial preoptic area of the hypothalamus. The expression pattern of γ1 mRNA correlates with that of α2 mRNA. The mRNA of the major γ subunit, γ2, is expressed throughout the brain. The expression of γ3 mRNA, although low, is most clearly expressed in the neocortex and thalamus (Wisden et al., 1992).

The expression of δ mRNA is high in the neocortex, hippocampal dentate granule cells, thalamus, and cerebellar granule cells (Laurie et al., 1992a; Persohn et al., 1992; Wisden et al., 1992). Its likely partners are α4 in the forebrain and α6 in the cerebellum (Jones et al., 1997; Korpi et al., 2002; Peng et al., 2002) and, at least in some cortical interneurons, the α1 subunit as well (Glykys et al., 2007). The ε and θ mRNAs are strongly concentrated in the monoaminergic nuclei of the brainstem, such as the noradrenergic locus ceruleus (Sinkkonen et al., 2000; Moragues et al., 2002). The π mRNA has not been detected in the brain, but it is abundant in female peripheral organs such the uterus (Hedblom and Kirkness, 1997). The ρ subunits are mostly expressed in the retina, colliculi, and cerebellum (Boue-Grabot et al., 1998; Wegelius et al., 1998).

C. Brain Regional Expression of Subunit Polypeptides

Brain regional localizations of $GABA_A$ receptor subunit polypeptides strongly correlate with those of the subunit mRNAs. The α1 subunit displays the most widely distributed expression, being present in practically all brain regions (Fritschy and Mohler, 1995; Pirker et al., 2000). Expression of α2 subunit is highest in the accessory olfactory bulb, hippocampus, amygdala, septum, striatum, accumbens, and hypothalamus (Fritschy and Mohler, 1995; Pirker et al., 2000). The α3 subunit is expressed in the olfactory bulb, inner layers of the cerebral cortex, endopiriform cortex, amygdala, lateral septum, claustrum, and superior colliculus (Pirker et al., 2000). It is usually expressed in monoaminergic neurons (Gao et al., 1993, 1995; Gao and Fritschy, 1994). The expression of α4 subunit is strongest in the thalamus, caudate-putamen, nucleus accumbens, olfactory tubercle, and hippocampus (Pirker et al., 2000). The α5 subunit is highly expressed in the olfactory bulb, inner layers of the cerebral cortex, endopiriform nucleus, subiculum, and hippocampus (Fritschy and Mohler, 1995; Pirker et al., 2000). The expression of α6 subunit is restricted to the granule cells of the cerebellum and cochlear nuclei (Gutiérrez et al., 1996; Pirker et al., 2000).

All three β subunits are widely distributed in the brain (Pirker et al., 2000). They are strongly expressed in the cerebral cortex. In many brain regions, their distribution is more or less complementary (Pirker et al., 2000). In the pallidum and thalamus, β2 is the main β subunit, whereas in the striatum, β3 is the most abundant β subunit (Moreno et al., 1994; Miralles et al., 1999; Pirker et al., 2000). The most abundant γ subunit, γ2, is expressed throughout the brain. In the thalamus, however, γ2 displays low expression level (Gutiérrez et al., 1994; Fritschy and Mohler, 1995; Pirker et al., 2000). The expression of γ1 subunit is located in the pallidum, substantia nigra, septum, and amygdala (Pirker et al., 2000). The γ3 subunit is diffusely distributed throughout the brain (Pirker et al., 2000). The δ subunit is expressed in the cerebellar granule cells, thalamus, dentate molecular layer, subiculum, cerebral cortex, and striatum (Fritschy and Mohler, 1995; Pirker et al., 2000).

D. Brain Regional Expression of $GABA_A$ Receptor Subtypes

The most abundant $GABA_A$ receptor subtype, α1β2γ2, is present in most brain regions. In immunostaining, the subunits display striking similarity of location in the internal granular layer of the olfactory bulb, polymorph cell layer, and CA3 region of the hippocampus, cerebral cortical interneurons, the globus pallidus and several thalamic nuclei (Benke et al., 1994; Pirker et al., 2000).

Subunits of the most prominent α2-containing subtype, α2β3γ2, have been most clearly colocalized in the accessory olfactory bulb, striatum, septum, molecular layer of the dentate gyrus, and hypothalamus (Benke et al., 1994; Pirker et al., 2000). Of the other α2-containing subtypes, α2βγ1 is a likely possibility. This subtype is suggested to be expressed in Bergmann glia and nuclei of the limbic systems (McKernan and Whiting, 1996).

The α3βγ2 subtype is chiefly expressed in several monoaminergic cells in various brain nuclei. The α3θε subunit combination is also likely, because their mRNAs are all highly enriched in rat locus ceruleus (Sinkkonen et al., 2000). The α4βγ2 is expressed in thalamus, caudate-putamen, and dentate gyrus (Pirker et al., 2000). The thalamic relay nuclei express α4β2δ subtype (Chandra et al., 2006), whereas the dentate granule cells express the α4β3δ subtype (Liang et al., 2006). Both α4βδ subtypes are obviously expressed in the cerebral cortex, whereas α4β3δ subtype predominates in the striatum. The α5β3γ2 subtype is expressed in CA1 pyramidal neurons (Sur et al., 1998; Caraiscos et al., 2004; Olsen and Sieghart, 2008). The α6-containing α6βγ2, α6β2δ, and α6β3δ subtypes are expressed in the cerebellar granule cells and cochlear nuclei.

E. In Vitro Models to Study Regulation of $GABA_A$ Receptor Expression

1. Mouse Cortical Neurons. The procedure of preparation of primary cultures of mouse cortical neurons is based on techniques described in detail by Yavin and Yavin (1980) and Yu et al. (1984). The cultures are highly enriched in well differentiated GABAergic cortical neurons. More than 90% of the cells in the cultures are neurons (Kuriyama et al., 1987). Cerebrum of 15-

day-old mouse embryos is used for the preparation. At that stage of development, large numbers of neurons have just entered their postmitotic stage of differentiation, and only a few proliferative glial precursors are present. The cerebral hemispheres are dissected and minced. The cells are dissociated by trituration and seeded in poly-L-lysine–coated culture dishes (Mehta and Ticku, 1992). The cultures are virtually homogeneous. The cells grow neurite extensions, migrate, and form clumps (Yu et al., 1984). Prenatally and during early postnatal days, cortical neurons express predominantly α2, α3, α4, α5, β2, β3, and γ2 subunits (Laurie et al., 1992b). Cultured cortical neurons express α1–α5, β2, β3, γ2, and δ subunits (Sheela Rani and Ticku, 2006).

2. Cerebellar Granule Cells. Cerebellar granule cells (CGCs) constitute the vast majority of neurons in the cerebellum. They undergo cell division until postnatal days 7 to 9, at which time their differentiation process is initiated. Because granule cells outnumber the other types of neurons in the cerebellum by a 1000-fold, it is quite easy to establish an in vitro cell culture system to study the development of granule cells (Messer, 1977). Cultured CGCs have been widely used as a model system to study regulation of gene expression during development both in vivo and in vitro (Savill et al., 2005). The expression of $GABA_A$ receptor subunits in CGCs undergoes maturational developmental changes during the first 2 postnatal weeks (Laurie et al., 1992a). Expression of the major $GABA_A$ receptor subunits in adult cerebellum, α1, α6, β2, β3, γ2, and δ, is strongly increased, and that of α2, α3, and β1 subunits is decreased during the second postnatal week both in vivo and in vitro (Laurie et al., 1992a, 1992b; Carlson et al., 1998; Grayson et al., 1998). Cerebella from decapitated 5- to 8-day-old rats or mice are dissected out, cut in small cubes, and subjected to acute trypsin dissociation. After trituration the dissociated cells are suspended in culture medium. The cells are seeded in poly-L-lysine–coated culture dishes. The cultures are homogeneous and consist of at least 90% granule cells (Messer, 1977). Cultured CGCs express α1–α6, β1–β3, γ1–γ3, and δ subunits (Bovolin et al., 1992).

3. Primary Cultures of Hippocampal Neurons. Primary cultures of hippocampal neurons are usually prepared from embryonic day 18 rat fetuses (Banker and Cowan, 1977). The cultures are enriched in pyramidal cells and the number of non-neuronal cells present is minimal. Meninges-free hippocampi are microdissected and trypsinized. The cells are then triturated and plated in culture dishes coated with poly-L-lysine and collagen (Banker and Cowan, 1977). Embryonic hippocampal neuronal cultures include a heterogeneous mixture of pyramidal and nonpyramidal neurons of multiple different morphologies (Rothman and Cowan, 1981). The cultured cells express $GABA_AR$ subunits α1-α5, β1–β3, γ1–γ3 and δ (Brooks-Kayal et al., 1998; Maric et al., 1999).

F. Mechanisms Regulating $GABA_A$ Receptor Subunit Expression

The large number of $GABA_A$ receptor genes and the various types of neurons and glial cells in the brain with different patterns of subunit expression suggest a complex system regulating their transcription (Laurie et al., 1992a; Wisden et al., 1992; Olsen and Sieghart, 2008). Major changes occur during development in the subunit expression patterns (Laurie et al., 1992b). However, several studies indicate changes in $GABA_A$ receptor subunit expression also in adult brain (Huntsman et al., 1994; Kamphuis et al., 1995; Loup et al., 2000). The changes are often suggested to reflect a change in neuronal activity. In cultured neurons in vitro, the expression of several $GABA_A$ receptor subunit mRNAs and polypeptides is up-regulated by depolarization with glutamate receptor agonists (Harris et al., 1995; Gault and Siegel, 1998; Salonen et al., 2006). However, these effects obviously correspond to developmental induction and up-regulation of the expression occurring in vivo. It has been shown that stimulation of *N*-methyl-D-aspartate (NMDA) receptors increases the transcription rate of α1 mRNA in cultured rat CGCs (Harris et al., 1995). On the other hand, the presence of $GABA_A$ agonists in medium of cultured neurons down-regulates $GABA_A$ receptor subunits (Montpied et al., 1991a; Mehta and Ticku, 1992; Baumgartner et al., 1994). Prolonged presence of GABA has been shown to reduce the transcription rate of α1 mRNA (Lyons et al., 2000). This use-dependent regulation of $GABA_A$ receptors has been suggested to include (in putative temporal order): 1) desensitization, 2) endocytosis of subunit polypeptides, 3) subunit polypeptide degradation, and 4) repression of subunit gene expression (Barnes, 1996).

Activity-dependent signaling pathways modulate the function of both transcriptional activators and repressors (West et al., 2002). Calcium is a crucial second messenger in the transduction of synaptic activity into gene expression (Carafoli et al., 2001), and it is involved in the mechanisms of $GABA_A$ receptor up- and down-regulation (Gault and Siegel, 1998; Lyons et al., 2001). The transcription factor cAMP response element (CRE) binding protein (CREB) is induced in response to neurotransmitters, neuromodulators, and neurotrophic factors (Lonze and Ginty, 2002). It was recently shown that the activation of protein kinase C in primary rat neocortical cultures increases transcription of α1 mRNA via phosphorylation of CREB that is bound to the *GABRA1* promoter (Hu et al., 2008). In contrast, activation of protein kinase A (PKA) represses α1 mRNA transcription via inducible cAMP early repressor (ICER) that forms inactive heterodimers with CREB (Hu et al., 2008). Brain-derived neurotrophic factor (BDNF) decreases α1 transcription via activation of the Janus kinase/signal transducer and activator of transcription (STAT) pathway (Lund et al., 2008). BDNF-dependent

phosphorylation of STAT3 induces the synthesis of ICER that binds with phosphorylated CREB at the *GABRA1* promoter CRE site, thereby repressing transcription (Lund et al., 2008).

Expression of $GABA_A$ receptor subunits is regulated in cultured CGCs by both cAMP- and BDNF-mediated signaling mechanisms. Activation of adenylate cyclase or the presence of cAMP analogs in cultured CGCs up-regulate $\beta 2$ and down-regulate $\alpha 6$ mRNA expression but have no effect on $\alpha 1$ and $\beta 3$ mRNA (Thompson et al., 2000). In addition, $\alpha 6$ and $\beta 3$ polypeptides are down-regulated, whereas $\alpha 1$ and $\beta 2$ are up-regulated, indicating subunit-specific regulation of expression. The transcriptional and/or translational mechanisms mediating these effects are only partially mediated by PKA (Thompson et al., 1996, 2000). Addition of BDNF in cultured rat visual cortex cells induces a rapid increase in the total number of functional cell surface $GABA_A$ receptors (Mizoguchi et al., 2003). In cultured CGCs, BDNF induces $\alpha 6$ mRNA expression and enhances the expression of $\alpha 1$ and $\gamma 2$ mRNA (Bulleit and Hsieh, 2000). These enhancements are mediated via mitogen-activated protein kinase pathway (Bulleit and Hsieh, 2000). In contrast, in cultured hippocampal pyramidal cells, BDNF reduced cell surface expression of $\alpha 2$, $\beta 2/3$, and $\gamma 2$ subunits (Brünig et al., 2001). The results suggest that BDNF affects $GABA_A$ receptor expression in a brain region- and cell-specific manner.

The transcription factors responsible for developmental and brain region/cell-specific expression of $GABA_A$ receptor subunits are presently unknown. Both coordinated and independent expression of the genes in a $GABA_A$ receptor gene cluster is suggested by the $\beta 2$-$\alpha 6$-$\alpha 1$-$\gamma 2$ cluster: the expression patterns of $\alpha 1$ and $\beta 2$ are almost identical, whereas $\gamma 2$ is expressed virtually throughout the brain, and $\alpha 6$ expression is restricted to cerebellar granule cells (Laurie et al., 1992a; Wisden et al., 1992). Coordinated expression is strongly suggested for the subunits in the θ-$\alpha 3$-ε cluster: all subunits colocalize in monoaminergic neurons (Fritschy et al., 1992; Sinkkonen et al., 2000; Moragues et al., 2002). But it is clear nonetheless that although coordinated expression of genes in a cluster seems to apply to some subunits, the brain regional and temporal expression of genes in most clusters is independent of other genes of the cluster.

The findings of protein kinase C/CREB mediated initiation of $\alpha 1$ mRNA transcription that is repressed by PKA/ICER, and BDNF/Janus kinase/STAT/ICER-mediated repression of $\alpha 1$ mRNA transcription are new promising examples of approaches that produce information on how expression of $GABA_A$ receptor genes is regulated.

G. Mechanisms Regulating $GABA_A$ Receptor Cell Surface Expression

More than 20 intracellular or transmembrane proteins can be considered $GABA_A$ receptor accessory proteins because they intimately interact with various sites of the large TM3–TM4 intracellular loops to regulate the surface expression of receptors. They regulate receptor trafficking from intracellular machinery to cell membranes and back, modify vesicular trafficking of receptors along the neurites, link receptors to cytoskeleton, affect inhibitory postsynaptic structures, and phosphorylate/dephosphorylate specific subunits (for review, see Lüscher and Keller, 2004; Birnir and Korpi, 2007; Chen and Olsen, 2007; Kneussel and Loebrich, 2007; Jacob et al., 2008). Most of the accessory proteins interact with the β or γ subunits, often very specifically with a certain subunit. This means that the interactions may participate in the regulation of functional activities of selected receptor subtypes rather than that of the whole $GABA_A$ receptor population. All these processes contribute to the dynamic nature of $GABA_A$ receptor-mediated inhibition, but in most cases the significance of the interactions on pharmacology of intact nervous systems remains to be assessed.

One of the interacting proteins is $GABA_A$ receptor-associated protein (GABARAP) (Wang et al., 1999), which interacts with the $\gamma 2$ subunit intracellularly, for the most part in the Golgi apparatus. GABARAP affects the receptor function in a heterologous expression system (Everitt et al., 2004), but it has not been found in synapses (Kittler et al., 2001). The actions of GABARAP are regulated by its post-translational lipid modifications (Chen et al., 2007).

Another interesting interacting protein is gephyrin (Prior et al., 1992) found originally to associate with strychnine-sensitive glycine receptors. It is needed for synaptic clustering and anchoring of $\alpha 2$ or $\gamma 2$ subunit-containing $GABA_A$ receptors (Essrich et al., 1998; Jacob et al., 2005), thus stabilizing the inhibitory receptors. A 10-amino acid part of the $\alpha 2$ subunit has been identified as the critical domain for gephyrin interaction (Tretter et al., 2008).

Various kinases (e.g., protein kinase A, protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II) and phosphatases (e.g., protein phosphatases PP1α and PP2A) target mostly the $GABA_A$ receptor β subunits (Jacob et al., 2008; Houston et al., 2009; Vithlani and Moss, 2009) and affect, for example, pharmacological sensitivity of the receptors to neurosteroids in a subunit-dependent manner (Koksma et al., 2003; Harney et al., 2003). Some ancillary proteins interact only with a selected $GABA_A$ receptor subtype. For example, radixin, a member of the ezrin, radixin, and moesin family of proteins that link the actin cytoskeleton to the cell plasma membrane, binds to $\alpha 5$ subunits that form extrasynaptic $\alpha 5\beta 3\gamma 2/3$ receptor clusters on dendrites of hippocampal principal neurons (Loebrich et al., 2006). Quantum dot labeling of single receptor molecules have shown that individual receptor molecules can move laterally in the surface membrane (Bouzigues and Dahan, 2007) and

move in and out of synapses apparently independently of endocytosis (Bogdanov et al., 2006).

It is a major challenge to translate the novel findings on protein-protein interactions and processes of $GABA_A$ receptors to possible mechanisms affecting pharmacological properties, such as drug sensitivity and tolerance development.

III. Regulation of $GABA_A$ Receptor Expression By Pharmacological Agents

A. Benzodiazepines

BZs were developed in the mid-1950s and 1960s in response to a need for safe and effective anxiolytics (Sternbach, 1978; Shader and Greenblatt, 1993). Classic 1,4-benzodiazepines such as diazepam display a wide variety of behavioral effects, and they are clinically used as anticonvulsants, sedatives/hypnotics, anxiolytics, muscle relaxants, and preanesthetics (Ashton, 1994). They are especially effective in short-term treatment. However, therapy with BZs is often prolonged, which is associated with the most serious problems of BZ usage: development of tolerance and dependence (Ashton, 1991; Pétursson, 1994). Tolerance to sedative and ataxic effects of BZs usually develops at a faster rate than tolerance to their anxiolytic effects (File, 1985; Hutchinson et al., 1996). Physical dependence in preclinical experiments can be measured as the emergence of characteristic withdrawal signs upon cessation of the drug and/or administration of a BZ antagonist such as flumazenil (Licata and Rowlett, 2008). BZs are also addictive drugs; i.e., their use is associated with acute rewarding effects in animals (Heikkinen et al., 2009; Straub et al., 2010). In some humans, these effects may turn into BZ abuse and finally to compulsive drug-seeking behavior. The treatment of BZ addiction is very difficult, especially in patients with multiple, complicated drug addictions, and even a reduction of BZ dosing/usage is often a good treatment outcome (Vorma et al., 2002, 2004).

BZs exert their action by interacting with several $GABA_A$ receptor subtypes with different pharmacological characteristics (Olsen and Sieghart, 2008). The majority of the pentameric $GABA_A$ receptors are believed to be composed of α, β, and γ subunits in the ratio of 2:2:1, respectively (Sieghart and Sperk, 2002; Ernst et al., 2003). The BZ binding site is located at the interface between an α and a γ subunit, and its pharmacology is thus influenced by both α and γ subunits (Fig. 4) (Ernst et al., 2003, Ogris et al., 2004). Most classic BZs bind to $\alpha\beta\gamma2$ receptors containing $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunits with approximately the same affinity. In contrast, several non-BZs, such as zolpidem, zaleplon, and abecarnil, have high affinity (low nanomolar) to $\alpha1\beta\gamma2$ receptors and intermediate affinity (high nanomolar) to $\alpha2$- and $\alpha3$-containing receptors, the affinity of zolpidem to $\alpha5\beta\gamma2$ receptors being very low (Korpi et al., 2002; Olsen and Sieghart, 2008).

Insensitivity of $\alpha4$- and $\alpha6$ subunit-containing receptors to BZs is based on the presence of an arginine residue instead of a histidine at a conserved position in BZ binding site (residue 101 in Fig. 1) (Wieland et al., 1992). The need for the particular His residue has been used to generate knockin mutant mouse lines [$\alpha1$(H101R), $\alpha2$(H101R), $\alpha3$(H126R), $\alpha5$(H105R] in which the particular arginine-containing receptor subtype is insensitive to classic BZs (for review, see Rudolph and Möhler, 2004). These knockin mouse lines have been used to demonstrate which subtypes of $GABA_A$ receptors mediate specific behavioral actions of diazepam. The $\alpha1$-containing receptors seem to mediate sedative, anterograde amnesic, and antimyoclonic actions of diazepam (Rudolph et al., 1999). Anxiolytic activity of BZs is mediated by $\alpha2$-containing $\alpha\beta\gamma2$ receptors, especially in the amygdala and hippocampus, whereas some anxiolytic activity is probably mediated by $\alpha3$-containing receptors (Löw et al., 2000; Crestani et al., 2001). Muscle relaxant activity of BZs is mediated partially by each of the $\alpha\beta\gamma2$ receptor subtypes containing $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunits (Löw et al., 2000; Crestani et al., 2001, 2002). In addition, hippocampal extrasynaptic $\alpha5$-containing receptors are involved in learning and memory processes, such as trace fear conditioning (Crestani et al., 2002). It remains to be seen whether the roles of various $GABA_A$ receptor subtypes in humans are similar to the roles the above rodent models suggest. For example, the BZ-site ligand ocinaplon exhibits $\alpha1$-preferring full agonist profile in human recombinant $GABA_A$ receptors and anxiolytic activity without sedative effects in patients suffering from generalized anxiety disorder (Lippa et al., 2005). Anxioselective partial agonists in rodents may have adverse sedative effects in man arising from the differences in $\alpha1$ subunit-containing BZ binding sites. This may explain the difficulties found in translating the promising preclinical rodent-based results into clinical benefit in man (for example, see Atack, 2003).

Development of tolerance and dependence to BZs upon long-term administration suggests that continuous exposure of $GABA_A$ receptors to BZs might affect receptor function by desensitizing the receptors and/or affecting receptor subunit expression and thereby changing the number and/or subtype of cell surface receptors. Decreased number of BZ binding sites after long-term BZ treatment has been observed in some studies (Rosenberg and Chiu, 1979; Miller et al., 1988, 1989), whereas no change has been found in most studies (Gallager et al., 1984; Heninger and Gallager, 1988; Ramsey-Williams et al., 1994). Long-term treatment of rats with BZs results in so-called "uncoupling," a decrease in the ability of BZs to potentiate the action of GABA on $GABA_A$ receptors and in a decrease in the ability of GABA to potentiate BZ binding (Gallager et al., 1984; Marley and Gallager, 1989; Tietz et al., 1989). This uncoupling might be due to changes from BZ-sensitive to -insensitive receptor subtypes (changes in receptor subunit com-

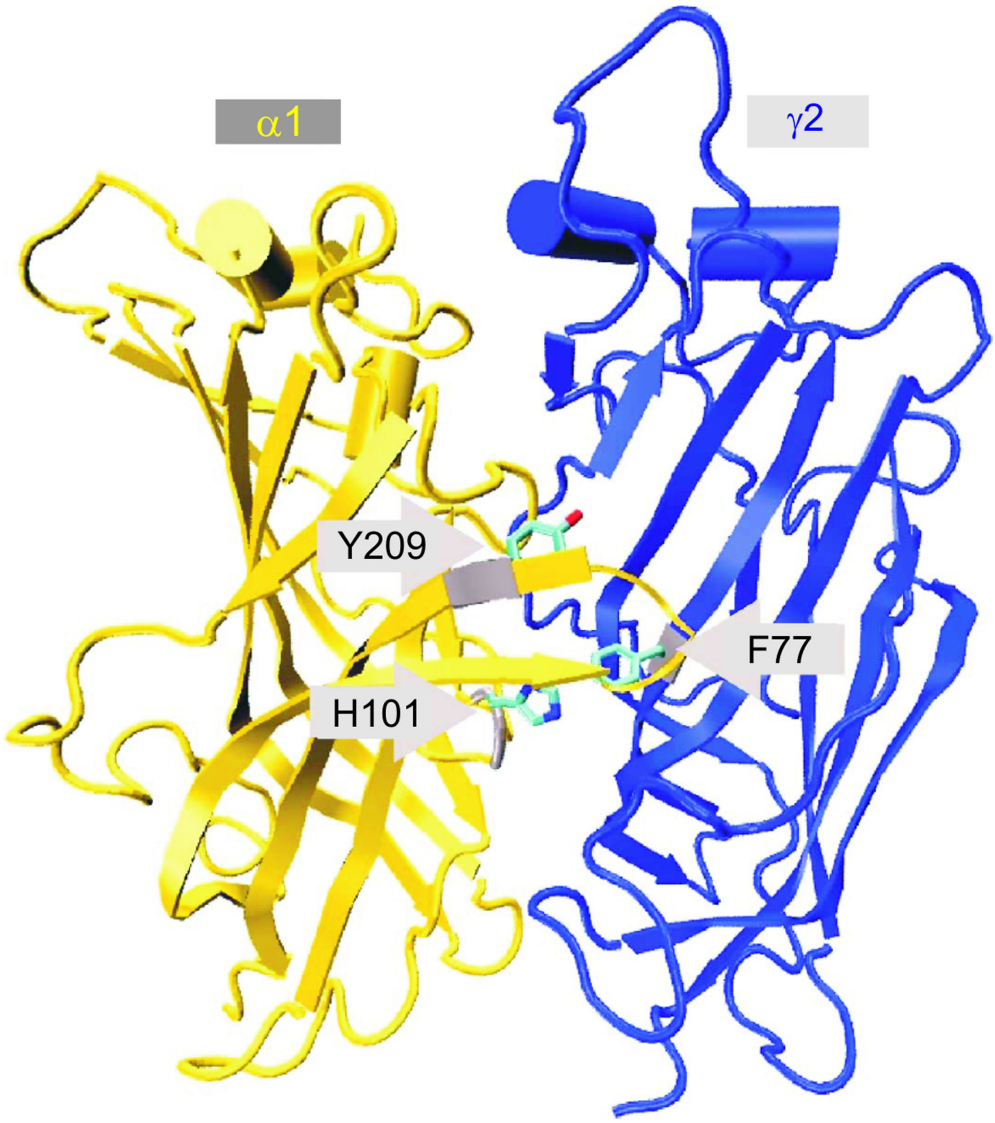

FIG. 4. Three-dimensional homology model of the extracellular domains of the α1 and γ2 subunits, indicating the interface between the subunits where the BZ binding pocket is located. The view is approximally perpendicular to the pore mouth, the bottom end of the figure corresponding to the C-terminal ends of the extracellular domains. The α1 subunit is depicted in yellow and the γ2 subunit in blue. Residues α1His101, α1Tyr209, and γ2Phe77, indicated with gray arrows, reside in the so-called loops A, C, and D, respectively. The residues line the BZ binding pocket and interact with the binding BZ ligands. [Reproduced from Ogris W, Pöltl A, Hauer B, Ernst M, Oberto A, Wulff P, Höger H, Wisden W, and Sieghart W (2004) Affinity of various benzodiazepine site ligands in mice with a point mutation in the $GABA_A$ receptor γ2 subunit. *Biochem Pharmacol* **68:**1621–1629. Copyright © 2004 Elsevier Science. Used with permission.]

bination) and/or changes in receptor function without changes in receptor subtype. Administration of the BZ antagonist flumazenil blocks the uncoupling, indicating that the changes are mediated via $GABA_A$ receptor-associated BZ binding sites (Roca et al., 1990; Primus et al., 1996). Uncoupling, however, occurs too rapidly to be the mechanism responsible for BZ tolerance and dependence (for review, see Bateson, 2002).

The effects of long-term BZ administration on $GABA_A$ receptor subunit expression have been studied using various BZ site compounds from several structural classes and with different pharmacological efficacy and receptor subtype selectivity. Treatment durations (7–32 days) and doses (0.25–150 mg/kg/day, the dose usually inversely correlating with efficacy) used have often differed, which is apparently reflected as variability in the results between the studies. Most of the studies have been performed using Sprague-Dawley rats. Mice have been used in only a few studies. The use of mice instead of rats has been noted in the text and tables of this review. There are only a few studies using cultured neuronal cells. Cultured rat CGCs and hippocampal cells have been used to study the effects of long-term BZ treatment and withdrawal from the treatment on the expression of $GABA_A$ receptor subunit mRNAs and polypeptides. Details for animals, treatment times, and doses used in the studies are listed in Tables 2 to 11.

1. α1 Subunit. The expression of the most abundant α subunit, the α1 subunit, has been studied most often.

Sedative doses of the compounds have been used in most in vivo studies. In the cerebral cortex, α1 mRNA expression was significantly down-regulated by diazepam, the general agonist for α1/2/3/5$\beta\gamma$2 receptors in several (Heninger et al., 1990; Impagnatiello et al., 1996; Longone et al., 1996) but not all (Wu et al., 1994; Holt et al., 1996) studies (Table 2). Long-term treatment of rats with the general agonist lorazepam and the α1-preferring agonist zolpidem down-regulated cerebral cortical α1 mRNA by 50 and 27%, respectively (Kang and Miller, 1991; Holt et al., 1997a). In contrast, long-term treatment with other full or partial agonists (e.g., abecarnil, alprazolam, flurazepam, imidazenil, and triazolam) were ineffective (Zhao et al., 1994a,b; Holt et al., 1996; Impagnatiello et al., 1996; Ramsey-Williams and Carter, 1996; Fahey et al., 1999; Tietz et al., 1999a). Long-term treatment with the inverse agonist FG 7142 slightly up-regulated α1 mRNA (Primus and Gallager, 1992). Withdrawal (2 days) from long-term flurazepam treatment reduced α1 mRNA in the cerebral cortical layers II to III and IV (Tietz et al., 1993), whereas on withdrawal day 7, α1 mRNA had returned to the control level (Tietz et al., 1999a) (Table 4). Long-term treatment with diazepam and flurazepam (but not imidazenil) reduced α1 polypeptide in the cerebral cortex (Impagnatiello et al., 1996; Pesold et al., 1997; Chen et al., 1999) (Table 6). This reduction was not detected on day 2 of withdrawal from long-term flurazepam treatment (Tietz et al., 1999b) (Table 7).

In the cerebellum, none of the above-mentioned BZ-site compounds affected α1 mRNA expression except for a 21% up-regulation by diazepam (Holt et al., 1999). Treatment of cultured rat CGCs with diazepam reduced α1 mRNA expression slightly (Follesa et al., 2001a, 2002), but treatments with imidazenil, zaleplon, or zolpidem were ineffective (Table 8). Withdrawal (6 h) of CGCs from diazepam, imidazenil, zaleplon, and zolpidem decreased α1 mRNA by 20 to 37% (Table 9). Long-term treatment of CGCs with diazepam, flunitrazepam, and bretazenil reduced the level of α1 polypeptide (Brown and Bristow, 1996; Brown et al., 1998; Johnston and Bristow, 1998; Follesa et al., 2001a), whereas imidazenil had no effect on it (Johnston and Bristow, 1998) (Table 10). Withdrawal (6 h) from long-term diazepam treatment down-regulated α1 polypeptide in CGCs by 75% (Follesa et al., 2001a) (Table 11).

Effects of long-term BZ treatments on $GABA_A$ receptor subunit expression have been extensively studied in the hippocampus. Long-term treatment with diazepam either down-regulated (Wu et al., 1994; Impagnatiello et al., 1996) or had no effect (Heninger et al., 1990) on hippocampal α1 mRNA expression. Long-term treatment with flurazepam, imidazenil, or lorazepam had no effect on the expression (Kang and Miller, 1991; Zhao et al., 1994a,b; Impagnatiello et al., 1996), whereas FG 7142 up-regulated it (Primus and Gallager, 1992). The use of in situ hybridization revealed down-regulation of α1 mRNA in the hippocampal CA1 region and in dentate gyrus granule cells in brain sections from rats continuously treated with flurazepam (Tietz et al., 1999a). Long-term treatment of mice with alprazolam or rats with triazolam had no effect on α1 mRNA expression in any hippocampal subregion (Ramsey-Williams and Carter, 1996; Fahey et al., 1999). Withdrawal (2 days) from long-term flurazepam treatment slightly reduced α1 mRNA in the CA1 region, the expression returning to control level in 7 days (Tietz et al., 1993, 1999a). Long-term treatment with flurazepam down-regulated the α1 polypeptide in hippocampal CA1 and CA3 regions and dentate gyrus (Chen et al., 1999). On day 2 of withdrawal from long-term treatment with flurazepam, down-regulation of α1 polypeptide persisted in the stratum oriens region of the CA1 (Tietz et al., 1999b). Long-term treatment of cultured rat hippocampal cells with lorazepam decreased α1 mRNA by 19%, which returned to control level during a withdrawal of 6 h. Long-term treatment of cultured primary hippocampal cells with lorazepam reduced α1 polypeptide by 36% (Sanna et al., 2005), remaining reduced by 26% after 6-h withdrawal. Treatment of cultured hippocampal cells with etizolam or withdrawal from it had no effect on α1 mRNA or polypeptide (Sanna et al., 2005). Triazolam reduced α1 mRNA in the diagonal band and up-regulated it in the nucleus basalis, substantia nigra pars reticulata, and inferior colliculus (Ramsey-Williams and Carter, 1996).

Overall, there are controversies in results of studies on long-term BZ treatments on expression of α1 subunit. Many of the studies indicate down-regulation of α1 mRNA and polypeptide after long-term treatment and especially on withdrawal in the cerebral cortex and in hippocampus. There is a tight control between α1 mRNA and α1 polypeptide expression that was particularly clearly manifested in the $GABA_A$ receptor α6 subunit knockout mice, where an alteration in the structure of α6 gene within the β2-α6-α1-γ2 gene cluster altered the expression of the other genes in the cluster (Uusi-Oukari et al., 2000). Down-regulation of α1 mRNA transcription in the forebrain of α6 knockout mice was accompanied with similar down-regulation of forebrain α1 polypeptide (Uusi-Oukari et al., 2000).

2. α2 Subunit. Long-term treatment with abecarnil, diazepam, imidazenil, and zolpidem in vivo had no effect on α2 mRNA expression in any brain region quantified (Wu et al., 1994; Holt et al., 1996; Impagnatiello et al., 1996) (Table 2). Long-term treatment with alprazolam up-regulated α2 mRNA in the brainstem by 76% (Tanay et al., 2001). Of all brain regions studied, the caudate-putamen was the only one in which flurazepam down-regulated α2 mRNA (Tietz et al., 1999a). Triazolam down-regulated α2 mRNA in the prefrontal, olfactory, and cingulate cortices and in several other brain regions, up-regulated it in the hippocampal CA3 region, dentate gyrus, and some other brain regions, but had no effect in many of the regions studied (Ramsey-Williams and

TABLE 2
Effect of long-term benzodiazepine treatment in vivo on $GABA_A$ receptor subunit mRNA expression

Compound, Species, Dose, Duration, Brain Region	Subunit mRNA						Reference
	α1	α2	α3	α4	α5	α6	
	% change						
Abecarnil							
Rat, 6 mg/kg/d, 14 d							Holt et al., 1996
Cerebral cortex	N.S.	N.S.	N.S.	N.S.	N.S.	—	
Alprazolam							
Mouse, 28 d							Fahey et al., 1999
Cerebral cortex							
Layers II–IV	N.S.						
Layer V	N.S.						
Layer VI	N.S.						
Hippocampus							
CA1	N.S.						
CA3	N.S.						
Dentate gyrus	N.S.						
Rat, 10 mg/kg/d, 21 d							Tanay et al., 2001
Brainstem	N.S.	+76	N.S.	N.S.	N.S.	—	
Diazepam							
Rat, 0.25 μg/g tissue,[a] 21 d							Heninger et al., 1990
Cerebral cortex	−25						
Cerebellum	N.S.						
Hippocampus	N.S.						
Rat, 0.25 μg/g tissue,[a] 21 d							Wu et al., 1994
Cerebral cortex	N.S.	N.S.	N.S.	N.S.	−28		
Cerebellum	N.S.						
Hippocampus	−40	N.S.		N.S.	−15		
Rat, 15 mg/kg/d, 14 d							
Cerebral cortex	N.S.	N.S.	+36	+50	+42	—	Holt et al., 1996
Cerebellum	+21						Holt et al., 1999
Rat, 3×5 to 3×20 mg/kg/d, 14 d							Impagnatiello et al., 1996
Frontoparietal motor cortex	−42	N.S.	N.S.		+30	—	
Frontoparietal somatosensory cortex	N.S.	N.S.	N.S.		N.S.	—	
Cerebellum	N.S.	N.S.	N.S.		N.S.	—	
Hippocampus	−20	N.S.	N.S.		N.S.	—	
Olfactory bulb	N.S.						
Striatum	N.S.						
Rat, 3×5 to 3×20 mg/kg/d, 14 d							Longone et al., 1996
Frontoparietal motor cortex	−30				+37		
Rat, 15 mg/kg/d, 14 d							Arnot et al., 2001
Cerebral cortex							
Minipump infusion	N.S.	N.S.	N.S.	−18	N.S.		
One daily injection	N.S.	N.S.	N.S.	+19	N.S.		
FG 7142							
Rat, 0.25 g/g, 8 d							Primus and Gallager, 1992
Cerebral cortex	+17						
Cerebellum	N.S.						
Hippocampus	+34						
Flurazepam							
Rat, 40 mg/kg/d, 32 d							O'Donovan et al., 1992b
Whole brain	N.S.	N.S.	+50		−37	+50	
Rat, 150 mg/kg/d, 14d							Zhao et al., 1994b
Cerebral cortex	N.S.				−50		
Hippocampus	N.S.				−50		
Rat, 150 mg/kg/d, 28 d							Zhao et al., 1994b
Cerebral cortex	N.S.				N.S.		
Cerebellum	N.S.						
Hippocampus	N.S.				N.S.		
Rat, 100 mg/kg/day, 7 d							Tietz et al., 1999a
Hippocampus							
CA1	−14	N.S.		N.S.	N.S.	—	
CA2	N.S.	N.S.		N.S.	N.S.	—	
CA3	N.S.	N.S.		N.S.	N.S.	—	
Dentate polymorphic cells	N.S.	N.S.		N.S.	N.S.	—	
Granule cells	−20	N.S.		N.S.	N.S.	—	
Cerebral cortex							
Frontal	N.S.	N.S.		N.S.	N.S.	—	
Parieto-occipital	N.S.	N.S.		N.S.	—	—	
Caudate-putamen	—	−32		—	—	—	
Thalamus	N.S.	N.S.		N.S.	—	—	
Cerebellum, granule cell layer	N.S.	N.S.		N.S.	—	N.S.	
Imidazenil							
Rat, 3×1 to 3×4 mg/kg/d, 14 d							Impagnatiello et al., 1996
Frontoparietal motor cortex	N.S.	N.S.	N.S.		N.S.	—	

Continued

TABLE 2—*Continued*

Compound, Species, Dose, Duration, Brain Region	Subunit mRNA						Reference
	α1	α2	α3	α4	α5	α6	
Frontoparietal somatosensory cortex	N.S.	N.S.	N.S.		N.S.	—	
Cerebellum	N.S.	N.S.	N.S.		N.S.	—	
Hippocampus	N.S.	N.S.	N.S.		N.S.	—	
Striatum	N.S.						
Lorazepam							
Mouse, 2 mg/kg/d, 14–28 d							Kang and Miller, 1991
Cerebral cortex	−50						
Cerebellum	N.S.						
Hippocampus	N.S.						
Triazolam							
Rats, 6 mg/kg/d, 28 d							Ramsey-Williams and Carter, 1996
Prefrontal cortex	N.S.	−41	N.S.				
Olfactory cortex	N.S.	−28	N.S.				
Diagonal band	−20	N.S.	N.S.				
Cingulate cortex	N.S.	−24	N.S.				
Sensory cortex	N.S.	N.S.	N.S.				
Fornix	N.S.	N.S.	+43				
Nucleus basalis	+32	N.S.	N.S.				
Dorsal raphe	N.S.	N.S.	N.S.				
Anteroventral thalamus	N.S.	N.S.	N.S.	+30			
Central amygdala	N.S.	N.S.	N.S.				
Hippocampus							
CA1	N.S.	N.S.	N.S.		N.S.		
CA3	N.S.	+30	N.S.		N.S.		
Dentate gyrus	N.S.	+20	N.S.		N.S.		
Subthalamus	N.S.						
Lateral geniculate	N.S.	−21	−39				
Mammillary body	N.S.	−46	N.S.				
Substantia nigra reticulata	+32	N.S.	N.S.				
Substantia nigra compacta	N.S.	−28	N.S.				
Entorhinal cortex	N.S.	N.S.	−30				
Inferior colliculus	+26	−34	N.S.				
Periaqueductal grey	N.S.	−27	N.S.				
Zolpidem							
Rats, 15 mg/kg/d, 7 and 14 d							Holt et al., 1997a
Cerebral cortex	-27^{14d}	N.S.	N.S.	$+71^{7d}$	N.S.	—	

d, day(s); N.S., not significantly different from the control value; —, not detected.
[a] Continuous concentration in brain tissue.

Carter, 1996). Flurazepam withdrawal had no effect on α2 mRNA expression (Tietz et al., 1999a). Treatment of cultured rat hippocampal cells with 10 μM lorazepam or etizolam had no effect on the expression of α2 mRNA, but withdrawal (6 h) from them slightly increased the expression (Sanna et al., 2005). The α2 polypeptide was not affected by long-term treatment with diazepam or flurazepam, but imidazenil down-regulated it in the frontoparietal cortex (Pesold et al., 1997; Chen et al., 1999).

In contrast to α1, studies with most BZs suggest no effect of long-term BZ treatment or withdrawal from the treatment on α2 subunit expression. Triazolam might be an exception in having heterogeneous and brain region-specific effects on α2 expression.

3. α3 Subunit. Holt et al. (1996) found that long-term treatment with diazepam up-regulated α3 mRNA expression in the cerebral cortex, but other studies (Wu et al., 1994; Impagnatiello et al., 1996) found no such effect. Long-term treatment with abecarnil, imidazenil, triazolam, and zolpidem had no effect on cerebral cortical α3 mRNA expression (Wu et al., 1994; Holt et al., 1996, 1997a; Impagnatiello et al., 1996; Ramsey-Williams and Carter, 1996). Triazolam up-regulated α3 mRNA in the fornix and down-regulated it in the lateral geniculate nucleus and entorhinal cortex (Ramsey-Williams and Carter, 1996). Treatment of cultured rat hippocampal cells with lorazepam increased α3 mRNA by 59%, whereas a similar treatment with etizolam had no effect on the expression (Sanna et al., 2005). Withdrawal from lorazepam and etizolam (6 h) increased α3 mRNA by 38 and 30%, respectively. The α3 polypeptide was up-regulated in frontoparietal somatosensory cortex by long-term treatment with diazepam but not imidazenil (Pesold et al., 1997).

In conclusion, studies in vivo indicate that α3 expression, although not affected by most BZs, is regulated by long-term treatment with some BZs. The effects are brain region-specific and, in contrast to α1, α3 expression is usually up-regulated.

4. α4 Subunit. Expression of α4 mRNA was up-regulated in the cerebral cortex of rats by long-term treatment with diazepam or zolpidem (Holt et al., 1996, 1997a) and in the anteroventral thalamus by long-term treatment with triazolam (Ramsey-Williams and Carter, 1996). Long-term treatment of rats with diazepam continuously infused with minipumps down-regulated cortical α4 mRNA, whereas the same dose given as subcu-

TABLE 3
Effect of long-term benzodiazepine treatment in vivo on $GABA_A$ receptor subunit β, γ and δ mRNA expression

Compound, Species, Dose, Duration, Brain Region	Subunit mRNA							Reference
	β1	β2	β3	γ1	γ2	γ3	δ	
	% change							
Abecarnil								
Rat, 6 mg/kg/d, 14 d								Holt et al., 1996
Cerebral cortex	N.S.	−27	N.S.	N.S.	−41		N.S.	
Alprazolam								
Rat, 10 mg/kg/d, 21 d								Tanay et al., 2001
Brainstem	N.S.	−24	N.S.	N.S.	N.S.		N.S.	
Mouse, 2 mg/kg/d, 28 d								Fahey et al., 1999
Cerebral cortex								
Layers II–IV					N.S.			
Layer V					N.S.			
Layer VI					N.S.			
Hippocampus								
CA1					N.S.			
CA3					N.S.			
Dentate gyrus					+27			
Diazepam								
Rat, 0.25 μg/g tissue,[a] 21 d								Heninger et al., 1990
Cerebral cortex	N.S.							
Cerebellum	N.S.							
Hippocampus	N.S.							
Rat, 0.25 μg/g tissue,[a] 21 d								Primus and Gallager, 1992
Cerebral cortex					−10			
Cerebellum					N.S.			
Hippocampus					N.S.			
Rat, 0.25 μg/g tissue,[a] 21 d								Wu et al., 1994
Cerebral cortex		N.S.	N.S.		−40			
Cerebellum		N.S.	N.S.		N.S.			
Hippocampus		N.S.	N.S.		N.S.			
Rat, 15 mg/kg/d, 14 d								
Cerebral cortex	+32	N.S.	N.S.	N.S.	−26	+57		Holt et al., 1996
Cerebellum		N.S.			+58			Holt et al., 1999
Rat, 3×5 to 3×20 mg/kg/d, 14 d								Impagnatiello et al., 1996
Frontoparietal motor cortex		N.S.		N.S.	−30,−50[b]		N.S.	
Frontoparietal somatosensory cortex		N.S.		N.S.	N.S.		N.S.	
Cerebellum		N.S.		N.S.	N.S.		N.S.	
Hippocampus		N.S.		N.S.	N.S.		N.S.	
Rat, 3×5 to 3×20 mg/kg/d, 14 d								Longone et al., 1996
Frontoparietal motor cortex					−30,−50[b]			
Rat, 15 mg/kg/d, 14 d								Arnot et al., 2001
Cerebral cortex,								
Minipump infusion	N.S.	N.S.	N.S.	+37	N.S.		N.S.	
One daily injection	N.S.	N.S.	+21	N.S.	N.S.		N.S.	
FG 7142								
Rat, 0.25 mg/kg tissue,[a] 8 d								Primus and Gallager, 1992
Cerebral cortex	N.S.				+27			
Cerebellum	N.S.				N.S.			
Hippocampus	—				N.S.			
Flurazepam								
Rat, 40 mg/kg/d, 32 d								O'Donovan et al., 1992a
Whole brain	N.S.	N.S.	N.S.		N.S.			
Rat, 150 mg/kg/d, 14 d								Zhao et al., 1994b
Cerebral cortex					N.S.			
Hippocampus					N.S.			
Rat, 150 mg/kg/d, 28 d								Zhao et al., 1994b
Cerebral cortex					−31			
Cerebellum					N.S.			
Hippocampus					−39			
Rat, 150 mg/kg/d, 28 d								Zhao et al., 1994a
Cerebral cortex		−27	N.S.					
Cerebellum		−49	−35					
Hippocampus		−48	−30					
Rat, 100 mg/kg/day, 7 d								Tietz et al., 1999a
Hippocampus								
CA1	N.S.	N.S.	−24		N.S.			
CA2	N.S.	N.S.	−15		N.S.			
CA3	N.S.	N.S.	−18		N.S.			
Dentate cells	N.S.	N.S.	N.S.		N.S.			
Granule cells	N.S.	N.S.	−21		N.S.			
Cerebral cortex frontal	N.S.	N.S.	−37		N.S.			
Parieto-occipital	N.S.	N.S.	N.S.		N.S.			
Caudate-putamen	—	—	−29		N.S.			

Continued

TABLE 3—*Continued*

Compound, Species, Dose, Duration, Brain Region	Subunit mRNA							Reference
	β1	β2	β3	γ1	γ2	γ3	δ	
Thalamus	—	N.S.	—		N.S.			
Cerebellum, granule cell layer	N.S.	N.S.	N.S.		N.S.			
Imidazenil								
Rat, 3×1 to 3×4 mg/kg/d, 14 d								Impagnatiello et al., 1996
Frontoparietal motor cortex		N.S.		N.S.	N.S.		N.S.	
Frontoparietal somatosensory cortex		N.S.		N.S.	N.S.		N.S.	
Cerebellum		N.S.		N.S.	N.S.		N.S.	
Hippocampus		N.S.		N.S.	N.S.		N.S.	
Lorazepam								
Mouse, 2 mg/kg/d, 14–28 d								Kang and Miller, 1991
Cerebral cortex					−50			
Cerebellum					N.S.			
Hippocampus					N.S.			
Triazolam								
Rat, 6 mg/kg/d, 28 d								Ramsey-Williams and Carter, 1996
Olfactory cortex			−43					
Cingulate cortex	+62							
Sensory cortex			−46					
Dorsal raphe							−46	
Anteroventral thalamus							−47	
Central amygdala			−42					
Hippocampus CA1	+32							
Subthalamus							−58	
Lateral geniculate			−48				−39	
Substantia nigra compacta							−51	
Interpeduncular nucleus	−45	−49						
Periaqueductal grey							−51	
Zolpidem								
Rat, 15 mg/kg/d, 7 and14 d								Holt et al., 1997a
Cerebral cortex	+49[7d]	N.S.	N.S.	N.S.	N.S.		N.S.	

d, day(s); N.S., not significantly different from the control value; —, not detected.
[a] Continuous concentration in brain tissue.
[b] Two values represent S and L splice variants, respectively.

taneous injections up-regulated it (Arnot et al., 2001). No effect on α4 mRNA expression was found for long-term treatment with abecarnil, alprazolam, or flurazepam (Table 2). Flurazepam withdrawal did not affect α4 subunit expression (Tietz et al., 1999a). Treatment of cultured rat CGCs with diazepam, imidazenil, zaleplon, or zolpidem had no effect on α4 mRNA expression (Follesa et al., 2001a, 2002), but 6-h withdrawal from them increased the expression by 32 to 59%. Long-term treatment of rat CGC cultures with diazepam had no effect on α4 polypeptide (Follesa et al., 2001a), but the withdrawal from the treatment increased it by 45%. Long-term treatment of cultured rat hippocampal cells with lorazepam or etizolam had no effect on α4 mRNA or polypeptide expression (Sanna et al., 2005), but the withdrawal from lorazepam (6 h) increased the mRNA and polypeptide expression by 23 and 81%, respectively. Etizolam withdrawal had no effects on α4 mRNA or polypeptide expression (Sanna et al., 2005).

Overall, studies on the effects of long-term treatment with BZ on α4 expression suggest strong up-regulation of the subunit by some BZs. In cultured CGCs and hippocampal neurons, α4 was up-regulated on BZ withdrawal. These findings are especially interesting because the ligands tested do not bind to α4 subunit-containing $GABA_A$ receptors.

5. α5 Subunit. Long-term treatment of rats with diazepam either increased (Holt et al., 1996; Impagnatiello et al., 1996; Longone et al., 1996) or reduced (Wu et al., 1994) cerebral cortical α5 mRNA expression. A 2-week treatment with flurazepam down-regulated cerebral cortical and hippocampal α5 mRNA expression for 50%, whereas after a 4-week treatment, the expressions returned to same level as in control rat cerebral cortex (Zhao et al., 1994b). Long-term treatment with abecarnil, imidazenil, triazolam and zolpidem in vivo had no effect on cortical α5 mRNA expression (Holt et al., 1996, 1997a; Impagnatiello et al., 1996; Ramsey-Williams and Carter., 1996). Hippocampal α5 mRNA was slightly down-regulated by long-term treatment with diazepam in the study by Wu et al. (1994) but not in that of Impagnatiello et al. (1996). Long-term treatment with imidazenil and triazolam did not affect hippocampal α5 mRNA expression (Impagnatiello et al., 1996; Ramsey-Williams and Carter, 1996). Treatment with diazepam, but not imidazenil, produced a drastic up-regulation of α5 polypeptide in frontoparietal motor and somatosensory cortices (Impagnatiello et al., 1996; Pesold et al., 1997). Treatment of cultured rat hippocampal cells with etizolam slightly reduced α5 mRNA expression, whereas lorazepam had no effect (Sanna et al., 2005). Etizolam or lorazepam withdrawal had no effect on α5 mRNA expression (Sanna et al., 2005).

In summary, long-term BZ treatment and withdrawal do not much influence α5 mRNA expression. Up-regulation of α5 polypeptide in the frontoparietal motor and

TABLE 4
Effect of withdrawal from long-term benzodiazepine treatment in vivo on $GABA_A$ receptor α subunit mRNA expression

Compound, Species, Dose, Duration, Withdrawal Time, Brain Region	Subunit mRNA					Reference
	α1	α2	α4	α5	α6	
	% change					
Diazepam						
Rat, 0.25 μg/g tissue, 21 d, 2 d						Wu et al., 1994
Cerebral cortex				N.S.		
Hippocampus	N.S.			N.S.		
Rat, 3×5 to 3×20 mg/kg/d, 14 d, 18 h						Longone et al., 1996
Frontoparietal motor cortex	−31			+31		
Hippocampus	−24			N.S.		
Frontoparietal somatosensory cortex	N.S.					
Olfactory bulb	N.S.					
Striatum	N.S.					
Cerebellum	N.S.					
Rat, 3×5 to 3×20 mg/kg/d, 14 d, 3 d						Longone et al., 1996
Frontoparietal motor cortex	N.S.			N.S.		
Hippocampus	N.S.			N.S.		
Flurazepam						
Rat, 150 mg/kg/d, 7 d, 2 d						Tietz et al., 1993
Cerebral cortex	−50[Layers II-III, IV]					
Hippocampus	−35[CA1 region]			N.S.		
Rat, 100 mg/kg/d, 7 d, 2 d						Tietz et al., 1999a
Hippocampus						
CA1	−11	N.S.	N.S.	N.S.	—	
CA2	N.S.	N.S.	N.S.	N.S.	—	
CA3	N.S.	N.S.	N.S.	N.S.	—	
Dentate polymorphic cells	N.S.	N.S.	N.S.	N.S.	—	
Granule cells	N.S.	N.S.	N.S.	N.S.	—	
Cerebral cortex						
Frontal	N.S.	N.S.	N.S.	N.S.	—	
Parieto-occipital	N.S.	N.S.	N.S.	—	—	
Caudate-putamen	—	N.S.	—	—	—	
Thalamus	N.S.	N.S.	N.S.	—	—	
Cerebellum, granule cell layer	N.S.	N.S.	N.S.	—	N.S.	
Rat, 100 mg/kg/d, 7 d, 7 d						Tietz et al., 1999a
Hippocampus						
CA1	N.S.	N.S.	N.S.	N.S.	—	
CA2	N.S.	N.S.	N.S.	N.S.	—	
CA3	N.S.	N.S.	N.S.	N.S.	—	
Dentate polymorphic cells	N.S.	N.S.	N.S.	N.S.	—	
Granule cells	N.S.	N.S.	N.S.	N.S.	—	
Cerebral cortex						
Frontal	N.S.	N.S.	N.S.	N.S.	—	
Parieto-occipital	N.S.	N.S.	N.S.	—	—	
Caudate-putamen	—	N.S.	—	—	—	
Thalamus	N.S.	N.S.	N.S.	—	—	
Cerebellum, granule cell layer	N.S.	N.S.	N.S.	—	N.S.	
Imidazenil						
Rat, 3×5 to 3×20 mg/kg/d, 14 d, 18 h						Longone et al., 1996
Frontoparietal motor cortex	N.S.			N.S.		

d, day(s); N.S., not significantly different from the control value; —, not detected.
[a] Continuous concentration in brain tissue.

somatosensory cortices by diazepam seems to be an exception (Impagnatiello et al., 1996). However, using mutant α5(H105R) mice possessing diazepam-insensitive $\alpha5\beta\gamma2$ receptors, it has been suggested that α5-containing receptors are required for development of tolerance to the sedative action of diazepam (van Rijnsoever et al., 2004). After long-term diazepam treatment, the mutant α5(H105R) mice developed no sedative tolerance to diazepam (van Rijnsoever et al., 2004).

6. α6 Subunit. Increased α6 mRNA expression in whole brain has been found after long-term flurazepam treatment by O'Donovan et al. (1992a), whereas no effect of flurazepam was found in cerebellar α6 mRNA (Tietz et al., 1999a). Withdrawal from flurazepam did not affect α6 mRNA expression (Tietz et al., 1999a). Long-term treatment with flunitrazepam had no effect on α6 mRNA expression in cultured GCGs (Brown and Bristow, 1996). The results do not suggest significant regulation of cerebellar α6 subunit expression by long-term BZ administration.

7. β1 Subunit. Holt et al. (1996) found that long-term treatment with diazepam up-regulated cerebral cortical β1 mRNA expression, but Heninger et al. (1990) found it to be ineffective. Abecarnil, FG 7142, and flurazepam had no effect on cortical β1 mRNA (Primus and Gallager, 1992; Holt et al., 1996; Tietz et al., 1999a), whereas zolpidem up-regulated it (Holt et al., 1997a). Long-term treatment with diazepam, FG 7142, and flurazepam had no effect on cerebellar β1 mRNA (Heninger et al., 1990; Primus and Gallager, 1992; Tietz et al., 1999a). Hippocampal β1

TABLE 5
Effect of withdrawal from long-term benzodiazepine treatment in vivo on $GABA_A$ receptor subunit β and γ mRNA expression

Compound, Species, Dose, Duration, Withdrawal Time, Brain Region	Subunit mRNA				Reference
	β1	β2	β3	γ2	
			% change		
Diazepam					
Rat, 0.25 μg/g tissue, 21 d, 2 d					Wu et al., 1994
Cerebral cortex				N.S.	
Rat, 3×5 to 3×20 mg/kg/d, 14 d, 18 h					Longone et al., 1996
Frontoparietal motor cortex				−38, −52[a]	
Hippocampus				N.S., N.S.	
Rat, 3×5 to 3×20 mg/kg/d, 14 d, 3 d					Longone et al., 1996
Frontoparietal motor cortex				N.S., N.S.[a]	
Hippocampus				N.S., N.S.[a]	
Flurazepam					
Rat, 150 mg/kg/d, 7 d, 2 d					Tietz et al., 1993
Hippocampus				N.S.	
Rat, 150 mg/kg/d, 28 d, 2 d					Zhao et al., 1994b
Cerebral cortex				N.S.	
Cerebellum				N.S.	
Hippocampus				N.S.	
Rat, 150 mg/kg/d, 28 d, 2 d					Zhao et al., 1994a
Cerebral cortex		N.S.	N.S.		
Cerebellum		N.S.	N.S.		
Hippocampus		N.S.	−11		
Rat, 100 mg/kg/d, 7 d, 2 d					Tietz et al., 1999a
Hippocampus					
CA1	N.S.	+31	−17	N.S.	
CA2	N.S.	N.S.	−18	N.S.	
CA3	N.S.	+37	−14	N.S.	
Dentate polymorphic cells	N.S.	N.S.	−17	N.S.	
Granule cells	N.S.	+29	−15	N.S.	
Cerebral cortex					
Frontal	N.S.	N.S.	N.S.	N.S.	
Parieto-occipital	N.S.	N.S.	N.S.	N.S.	
Caudate-putamen	—	—	N.S.	N.S.	
Thalamus	—	N.S.	—	N.S.	
Cerebellum, granule cell layer	N.S.	N.S.	−20	N.S.	
Rat, 100 mg/kg/d, 7 d, 7 d					Tietz et al., 1999a
Hippocampus					
CA1	N.S.	N.S.	N.S.	N.S.	
CA2	N.S.	N.S.	N.S.	N.S.	
CA3	N.S.	N.S.	+18	N.S.	
Dentate polymorphic cells	N.S.	N.S.	N.S.	N.S.	
Granule cells	N.S.	N.S.	N.S.	N.S.	
Cerebral cortex					
Frontal	N.S.	N.S.	+40	+34	
Parieto-occipital	N.S.	N.S.	N.S.	N.S.	
Caudate-putamen	—	—	N.S.	N.S.	
Thalamus	—	N.S.	—	N.S.	
Cerebellum, granule cell layer	N.S.	N.S.	N.S.	N.S.	
Imidazenil					
Rat, 3×5 to 3×20 mg/kg/d, 14 d, 18 h					Longone et al., 1996
Frontoparietal motor cortex				N.S., N.S.[a]	

d, day(s); N.S., not significantly different from the control value; —, not detected.
[a] Two values represent S and L splice variants, respectively.

mRNA was not affected by long-term treatment with diazepam (Heninger et al., 1990) or flurazepam (Tietz et al., 1999a). Long-term treatment with flurazepam did not affect β1 polypeptide expression in the hippocampus (Chen et al., 1999). Triazolam up-regulated β1 mRNA in the cingulate cortex and hippocampal CA1 region and down-regulated it in the interpeduncular nucleus (Ramsey-Williams and Carter, 1996). Flurazepam withdrawal for 2 or 7 days had no effect on β1 mRNA expression (Tietz et al., 1999a). Treatment of rat cultured CGCs with diazepam increased β1 expression by 47% (Follesa et al., 2002). Similar treatment with zaleplon or zolpidem had no effect on β1 expression (Follesa et al., 2002). Withdrawal from diazepam, zaleplon, and zolpidem increased β1 mRNA expression (31–57%) (Follesa et al., 2002). Most in vivo studies suggest that β1 subunit is not affected by long-term BZ treatment.

8. β2 Subunit. A reduction in β2 mRNA expression in vivo was found after long-term treatment with abecarnil in the cerebral cortex (Holt et al., 1996), with alprazolam in the brainstem (Tanay et al., 2001), with flurazepam in the cerebral cortex, cerebellum, and hippocampus (Zhao et al., 1994a), and with triazolam in the interpeduncular nucleus (Ramsey-Williams and Carter, 1996) (Table 3). No effects on β2 mRNA expression in any brain region studied were found after long-term treatment with diazepam, flurazepam (Tietz et al., 1999a), imidazenil, or zolpidem (Wu et al., 1994; Holt et

TABLE 6
Effect of long-term benzodiazepine treatment in vivo on $GABA_A$ receptor subunit polypeptide expression

Compound, Species, Dose, Duration, Brain Region	Subunit mRNA						Reference
	$\alpha1$	$\alpha2$	$\alpha5$	$\beta2$	$\beta3$	$\gamma2$	
	% change						
Diazepam							
Rat, 3×5 to 3×20 mg/kg/d, 14 d							Impagnatiello et al., 1996
Frontoparietal motor cortex	−37		+158	$+47^{\beta2/3}$		+50	
Frontoparietal somatosensory cortex	N.S.		+209	$N.S.^{\beta2/3}$		N.S.	
Rat, 3×5 to 3×20 mg/kg/d, 14 d							Pesold et al., 1997
Frontoparietal motor cortex	$-37^{LIII-IV}$	N.S.	+150	$+48^{\beta2/3}$		+48	
Frontoparietal somatosensory cortex	N.S.	N.S.	+221	$N.S.^{\beta2/3}$		N.S.	
Flurazepam							
Rat, 100 mg/kg/day, 7 d							Chen et al., 1999
Hippocampus							
CA1	−20	N.S.		N.S.	−19	N.S.	
CA2	N.S.	N.S.		N.S.	-25^{SR}	N.S.	
CA3	−38	N.S.		N.S.	-14^{SL}	N.S.	
Dentate gyrus	−34	N.S.		N.S.	−18	-8^{ML}	
Cerebral cortex							
Layer I	N.S.				N.S.	N.S.	
Layer II/III	-24^{Fr}				$-23^{Fr,Par}$	N.S.	
Layer IV	-26^{Par}				-20^{Par}	N.S.	
Layer V	$-19^{Fr,Occ}$				-42^{Fr}	N.S.	
Layer VI	−23				-38^{Fr}	N.S.	
Caudate-putamen	—	N.S.		—	N.S.	N.S.	
Thalamus	N.S.	—		N.S.	—	N.S.	
Substantia nigra reticulata	N.S.	—		N.S.	—	N.S.	
Inferior colliculus	N.S.	—		N.S.	—	N.S.	
Superior colliculus	—	N.S.		—	N.S.	N.S.	
Cerebellum	N.S.	—		N.S.	N.S.	N.S.	
Imidazenil							
Rat, 3×1 to 3×4 mg/kg/d, 14 d							Impagnatiello et al., 1996
Frontoparietal motor cortex	N.S.		N.S.	$N.S.^{\beta2/3}$		N.S.	
Frontoparietal somatosensory cortex	N.S.		N.S.	$N.S.^{\beta2/3}$		N.S.	
Rat, 3×1 to 3×4 mg/kg/d, 14 d							Pesold et al., 1997
Frontoparietal motor cortex	N.S.	N.S.	N.S.	$N.S.^{\beta2/3}$		N.S.	
Frontoparietal somatosensory cortex	N.S.	$-22^{LIII-IV}$	N.S.	$N.S.^{\beta2/3}$		N.S.	

d, day(s); N.S., not significantly different from the control value; —, not detected; $\beta2/3$, detected using an antibody specific to $\beta2$ and $\beta3$ subunits; LIII-IV, layers III and IV; SR, stratum radiatum; SL, stratum lacunosum; ML, molecular layer; Fr, frontal; Par, parietal; Occ, occipital.

al., 1996, 1997a, 1999; Impagnatiello et al., 1996) (Table 3). Flurazepam withdrawal for 2 days up-regulated $\beta2$ mRNA expression in the hippocampal CA1 and CA3 regions and dentate gyrus (Tietz et al., 1999a). On day 7 after discontinuation of flurazepam treatment, these changes returned to control level (Tietz et al., 1999a) (Table 5). Flurazepam-induced $\beta2$ mRNA down-regulation observed by Zhao et al. (1994a) was reversed after a 2-day withdrawal. Long-term treatment with diazepam up-regulated $\beta2/3$ subunit immunoreactivity in the frontoparietal motor cortex but not in somatosensory cortex, whereas imidazenil had no effects (Impagnatiello et al., 1996; Pesold et al., 1997). Long-term flurazepam treatment had no effect on $\beta2$ polypeptide (Chen et al., 1999). Treatment of cultured rat CGCs with diazepam decreased $\beta2$ mRNA (Follesa et al., 2002), whereas zaleplon or zolpidem had no effect (Follesa et al., 2002). Long-term treatment with flunitrazepam down-regulated $\beta2/3$ immunoreactivity in CGCs (Brown et al., 1998). Withdrawal from diazepam, zaleplon, and zolpidem treatments reduced $\beta2$ mRNA by 27 to 48% in CGCs (Follesa et al., 2002). In summary, studies on in vivo effects of long-term BZ treatment on $\beta2$ subunit expression suggest brain region-specific down-regulation of the subunit with some BZs.

TABLE 7
Effect of withdrawal from long-term benzodiazepine treatment in vivo on $GABA_A$ receptor subunit polypeptide expression

Compound, Species, Dose Duration, Withdrawal Time Brain Region	Subunit mRNA		Reference
	$\alpha1$	$\beta3$	
	% change		
Flurazepam			
Rat, 100 mg/kg/d, 7 d, 2 d			Tietz et al., 1999b
Hippocampus			
CA1	-14^{SO}	N.S.	
CA3	N.S.	$+10^{SO}, 9^{SR}$	
Dentate	N.S.	N.S.	
Cerebral cortex	N.S.		
Inferior colliculus	N.S.		
Cerebellum	N.S.		

d, day(s); N.S., not significantly different from the control value; SO, stratum oriens; SR, stratum radiatum.

9. $\beta3$ Subunit. Expression of $\beta3$ mRNA in vivo was reduced by long-term flurazepam treatment in the cerebellum and hippocampus (Zhao et al., 1994a) and in the frontal cortex, hippocampus, and caudate-putamen (Tietz et al., 1999a). Long-term treatment with triazolam strongly down-regulated $\beta3$ mRNA in the olfactory and sensory cortices, central amygdala, and lateral geniculate nucleus (Ramsey-Williams and Carter, 1996). Long-term treatment of rats with abecarnil, diazepam, or zolpidem had no effect on $\beta3$ mRNA expression in the

TABLE 8
Effect of long-term benzodiazepine treatment on $GABA_A$ receptor subunit mRNA expression in cultured cells in vitro

Cells, Compound, Concentration, Duration	Subunit mRNA								Reference
	α1	α3	α4	α5	β1	β2	β3	γ2	
	% change								
Rat hippocampal cells									
Etizolam, 10 μM, 5 d	N.S.	N.S.	N.S.	−17				−26	Sanna et al., 2005
Lorazepam, 10 μM, 5 d	−19	+59	N.S.	N.S.				−21	Sanna et al., 2005
Rat cerebellar granule cells									
Diazepam, 10 μM, 5 d	−20		N.S.					−24, −26[a]	Follesa et al., 2001a
Diazepam, 10 μM, 5 d	−23		N.S.		+47	−27	N.S.	−25, −27[a]	Follesa et al., 2002
Imidazenil, 10 μM, 5 d	N.S.		N.S.					−21, N.S.[a]	Follesa et al., 2001a
Zaleplon, 10 μM, 5 d	N.S.		N.S.		N.S.	N.S.	N.S.	N.S., N.S.[a]	Follesa et al., 2002
Zolpidem, 10 μM, 5 d	N.S.		N.S.		N.S.	N.S.	N.S.	N.S., N.S.[a]	Follesa et al., 2002

d, day(s); N.S., not significantly different from the control value.
[a] Two values represent S and L splice variants, respectively.

cerebral cortex (Wu et al., 1994; Holt et al., 1996, 1997a), with the exception of diazepam given as daily injections (Arnot et al., 2001). Long-term alprazolam had no effect on β3 mRNA expression in the brainstem (Tanay et al., 2001). Withdrawal from flurazepam for 2 days down-regulated β3 mRNA expression in all hippocampal subregions (Tietz et al., 1993, 1999a) (Table 5). In addition, β3 was down-regulated in the cerebellar granule cell layer (Tietz et al., 1999a). On day 7 after discontinuation of flurazepam treatment these changes returned to control level (Tietz et al., 1999a). Long-term flurazepam treatment down-regulated β3 polypeptide in the cerebral cortex and hippocampus (Chen et al., 1999; Tietz et al., 1999b). However, after 2-day withdrawal β3 polypeptide was returned to control levels and even slightly up-regulated in hippocampal CA3 region (Tietz et al., 1999b). Treatment of CGCs with diazepam, zaleplon, and zolpidem or withdrawal had no effect on β3 mRNA expression (Follesa et al., 2002). In conclusion, in vivo studies suggest brain region-specific down-regulation of β3 mRNA and polypeptide in several hippocampal and cortical subregions after long-term flurazepam treatment.

10. γ2 Subunit. Long-term treatment of rats with abecarnil, diazepam, and lorazepam down-regulated γ2 mRNA expression in the cerebral cortex (Kang and Miller, 1991; Primus and Gallager, 1992; Wu et al., 1994; Holt et al., 1996). A reduction in γ2 mRNA was seen in the frontoparietal motor cortex by diazepam but not by imidazenil treatment (Impagnatiello et al., 1996; Longone et al., 1996). Long-term treatment with flurazepam for 7 days (Tietz et al., 1999a) or 14 days (Zhao et al., 1994b) did not affect cerebral cortical γ2 mRNA expression, but a 4-week treatment down-regulated it for 31% (Zhao et al., 1994b). The same applies to the hippocampus, where flurazepam-induced down-regulation was seen only after the long, 4-week treatment (Zhao et al., 1994b). Long-term treatment with zolpidem had no effect on cerebral cortical γ2 mRNA expression (Holt et al., 1997a). After discontinuation of long-term treatment with diazepam, cortical γ2 mRNA level of the treated rats returned to control rat level (Wu et al., 1994; Longone et al., 1996). The expression of γ2 mRNA was up-regulated on withdrawal day 7 from long-term treatment with flurazepam (34%) while being at control level on day 2 (Tietz et al., 1993, 1999a). Diazepam up-regulated γ2 polypeptide measured with immunogold labeling in the frontoparietal motor cortex (50%), but not in the somatosensory cortex, whereas imidazenil had no effects (Impagnatiello et al., 1996; Pesold et al., 1997). No effect of long-term treatment with flurazepam treatment was found on cortical γ2 polypeptide (Chen et al., 1999). Long-term diazepam treatment increased γ2 mRNA expression in the cerebellum in one (Holt et al., 1999) of three studies (Wu et al., 1994; Impagnatiello et al., 1996), whereas no effect of long-term treatment with

TABLE 9
Effect of withdrawal from long-term benzodiazepine treatment on $GABA_A$ receptor subunit mRNA expression in cultured cells in vitro

Cells, Compound, Concentration, Duration, Withdrawal Time	Subunit mRNA								Reference
	α1	α2	α3	α4	β1	β2	β3	γ2	
	% change								
Rat hippocampal neurons									
Etizolam, 10 μM, 5 d, 6 h	N.S.	+24	+30	N.S.				−21(S)	Sanna et al., 2005
Lorazepam, 10 μM, 5 d, 6 h	N.S.	+17	+38	+23				−18(S)	Sanna et al., 2005
Rat cerebellar granule cells									
Diazepam, 10 μM, 5 d, 6 h	−37			+40				−18, −23[a]	Follesa et al., 2001a
Diazepam, 10 μM, 5 d, 6 h	−32			+38	+57	−48	N.S.	−19, −25[a]	Follesa et al., 2002
Imidazenil, 10 μM, 5 d, 6 h	−20			+42				−37, −21[a]	Follesa et al., 2001a
Zaleplon, 10 μM, 5 d, 6 h	−23			+59	+42	−27	N.S.	−20, −32[a]	Follesa et al., 2002
Zolpidem, 10 μM, 5 d, 6 h	−29			+32	+31	−31	N.S.	−19, −27[a]	Follesa et al., 2002

d, day(s); N.S., not significantly different from the control value.
[a] Two values represent S and L splice variants, respectively.

TABLE 10
Effect of long-term benzodiazepine treatment on $GABA_A$ receptor subunit polypeptide expression in cultured cells in vitro

Cells, Compound, Concentration, Duration	Subunit mRNA					Reference
	α1	α4	α6	β2/3	γ2	
	% change					
Rat hippocampal neurons						
Etizolam, 10 μM, 5 d	N.S.	N.S.				Sanna et al., 2005
Lorazepam, 10 μM, 5 d	−36	N.S.				Sanna et al., 2005
Rat cerebellar granule cells						
Bretazenil, 1 μM, 2 d	−11					Johnston and Bristow, 1998
Diazepam, 1 μM, 2 d	−19					Johnston and Bristow, 1998
Diazepam, 10 μM, 5 d	−37	N.S.			−50	Follesa et al., 2001a
Flunitrazepam, 1 μM, 2 d	−20					Johnston and Bristow, 1998
Flunitrazepam, 1 μM, 2 d	−41		N.S.			Brown and Bristow, 1996
Flunitrazepam, 1 μM, 2 d	−40			−67		Brown et al., 1998
Imidazenil, 1 μM, 2 d	N.S.					Johnston and Bristow, 1998

d, day(s); N.S., not significantly different from the control value.

diazepam, lorazepam, or imidazenil was found on hippocampal γ2 mRNA expression (Kang and Miller 1991; Wu et al., 1994; Impagnatiello et al., 1996; Tietz et al., 1999). In accordance, long-term treatment with flurazepam treatment had no effect on γ2 polypeptide in the cerebellum or hippocampus (Chen et al., 1999). Long-term treatment of cultured CGCs with diazepam reduced the γ2 mRNA expression by 24 to 27% and γ2 polypeptide by 50%, whereas zaleplon and zolpidem had no effect (Follesa et al., 2001a, 2002). Withdrawal from long-term treatment with diazepam, zaleplon, zolpidem, and imidazenil reduced CGC γ2 mRNA expression by 18 to 37%, and withdrawal from diazepam treatment reduced the γ2 polypeptide by 63% (Follesa et al., 2001a, 2002). Long-term treatments of cultured hippocampal cells with etizolam and lorazepam reduced γ2S mRNA expression by 21 and 26%, respectively (Sanna et al., 2005). The reduced expression of γ2S mRNA was sustained during 6-h withdrawal from lorazepam and etizolam (Sanna et al., 2005). Studies on the effects of in vivo long-term BZ agonist treatment and withdrawal suggest down-regulation of γ2 expression in the cerebral cortex by some, but not all agonists, but had little effect on γ2 expression in the cerebellum or hippocampus.

11. γ1 and γ3 Subunits. Long-term treatment of rats with continuous diazepam infusion using minipumps up-regulated γ1 mRNA expression (Arnot et al., 2001). No other significant effects of long-term BZ treatment on γ1 mRNA or polypeptide expression have been found thus far (Table 3). Long-term in vivo treatment with diazepam, but not abecarnil, increased γ3 mRNA expression in the cerebral cortex (Holt et al., 1996).

12. δ Subunit. Long-term treatment with diazepam, imidazenil, or zolpidem did not affect δ mRNA expression in several brain regions studied (Impagnatiello et al., 1996; Holt et al., 1997a). In contrast, triazolam treatment strongly down-regulated δ expression in several brain regions with a strong basal expression, such as the thalamus (Ramsey-Williams and Carter, 1996) (Table 3). These data do not allow any clear conclusion on the effects of long-term BZ administration on δ subunit expression in the brain. This would be important to clarify because the δ subunit-containing receptors are regarded to form BZ-insensitive receptor populations (but see Hanchar et al., 2006) that function especially at extrasynaptic and perisynaptic areas.

13. Conclusions on Effects of Long-Term Benzodiazepine Administration on Receptor Subunit Expression. Studies on regulation of $GABA_A$ receptor subunit expression in vivo by long-term BZ treatment have shown that the regulation is subunit-specific, is brain region-specific, and occurs at subunit-specific time scales. In addition, the inverse agonist studied (FG 7142) often had opposite effects on the expression as compared with BZ agonists. The partial agonist imidazenil was mostly without an effect on expressions of receptor subunits (Tables 2 and 3, Impagnatiello et al., 1996; Longone et al., 1996; Pesold et al., 1997; Johnston and Bristow, 1998). In vitro studies with cultured cells treated with long-term BZs produced surprisingly controversial results compared with studies in

TABLE 11
Effect of withdrawal from long-term benzodiazepine treatment on $GABA_A$ receptor subunit polypeptide expression in cultured cells in vitro

Cells, Compound, Concentration, Duration, Withdrawal Time	Subunit Polypeptide			Reference
	α1	α4	γ2	
	% change			
Rat hippocampal neurons				
Etizolam, 10 μM, 5 d/6 h	N.S.	N.S.		Sanna et al., 2005
Lorazepam, 10 μM, 5 d/6 h	−26	+81		Sanna et al., 2005
Rat cerebellar granule cells				
Diazepam, 10 μM, 5 d/6 h	−75	+45	−63	Follesa et al., 2001a

d, day(s); N.S., not significantly different from the control value.

vivo. The in vitro results from rat CGCs and hippocampal neurons are often opposite those received from hippocampus or cerebellum of rats treated in vivo. Thus, the in vitro results should be interpreted very cautiously.

Quantitative RT-PCR studies usually monitor the steady-state mRNA levels, not the rates of mRNA synthesis or degradation. Therefore, it has not been possible to deduce whether down-regulation of a subunit mRNA results from reduced transcription rate or increased degradation rate. However, using a nuclear run-off assay, Holt et al. (1997b) showed a 65% decrease in the $\gamma 2$ mRNA synthesis rate in the cerebral cortex of rats continuously treated with diazepam, whereas in the cerebellum, the rate was increased by 42% (Holt et al., 1999). The changes in $\gamma 2$ mRNA transcription rate paralleled the changes in $\gamma 2$ mRNA steady-state levels indicating that diazepam predominantly regulates $\gamma 2$ mRNA at the level of transcription (Holt et al., 1997a, 1999). The results suggest a brain region-specific regulation of $\gamma 2$ mRNA transcription by long-term treatment with diazepam. Lorazepam-induced reduction in transcriptional activity is suggested in $\alpha 1$ subunit down-regulation (Kang et al., 1994). The group isolated human $\alpha 1$ gene promoter and showed that long-term lorazepam treatment down-regulates transcriptional activity of $\alpha 1$ promoter in neurons transiently transfected with a $\alpha 1$ promoter-firefly luciferase construct (Kang et al., 1994). Lorazepam treatment dose-dependently attenuated expression of luciferase activity in the cells (Kang et al., 1994). The signaling mechanism by which lorazepam represses $\alpha 1$ gene promoter activity is unknown. However, it has been shown that GABA agonist-induced reduction of cell surface $GABA_A$ receptors occurs before down-regulation of receptor subunit mRNA expression (Baumgartner et al., 1994; Miranda and Barnes, 1997). It was subsequently found that long-term BZ treatment also induces $GABA_A$ receptor internalization (Tehrani and Barnes, 1997). How this receptor internalization is signaled into cell nucleus to suppress receptor subunit gene transcription is currently not known.

Studies on long-term BZ treatment in vivo indicate alterations in $GABA_A$ receptor subunit expression. The clearest effects are the down-regulation of $\alpha 1$ and $\beta 3$ in several cortical and hippocampal subregions and the down-regulation of $\gamma 2$ in the cerebral cortex. In addition, $\alpha 4$ subunit is strongly up-regulated in BZ withdrawal. There is also an indication of up-regulation of $\gamma 3$ subunit. BZ-induced alterations in $GABA_A$ receptor subunits may produce receptors with lower sensitivity or insensitivity to BZs (e.g., formation of $\alpha 4\beta\gamma 2$ and $\alpha\beta\gamma 3$-containing receptors). The changes, however, are quantitatively rather small and short-lasting. Therefore, the changes cannot solely explain the development of BZ tolerance, dependence or withdrawal syndrome after the discontinuation, but they may partially contribute to mechanisms underlying these phenomena.

14. Benzodiazepine-Induced Changes in the Expression of Other Genes. BZ administration induces a wide variety of other changes in neuronal gene expression that may participate to the development of tolerance and dependence. In a microarray study using wild-type and $\alpha 1$(H101R) knockin mouse lines, it was shown that even a single dose of diazepam significantly changed the expression of 54 transcripts (0.43% of the transcripts on the array), 34 transcripts being down-regulated and 20 transcripts being up-regulated (Huopaniemi et al., 2004). Changes in the expression of six transcripts, CaMKIIα, BDNF, MKP-1, GIF, c-*fos*, and NGFI-A, were mediated via action of diazepam on $\alpha 1$ subunit-containing $GABA_A$ receptors (Huopaniemi el al., 2004).

According to glutamate hypothesis of BZ tolerance and dependence, excitatory mechanisms become up-regulated to compensate for BZ-induced enhancement of inhibition (Stephens, 1995). Expression of *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptors has been shown to be regulated after long-term BZ treatment. Cerebral cortical GluN1 and GluN2B, but not GluN2A subunits of NMDA receptors (Tsuda et al., 1998), glutamic acid decarboxylase (GAD_{67}), and GluA1 subunit of AMPA receptor were increased in diazepam-withdrawn rats (Izzo et al., 2001). In rats withdrawn from flurazepam, amplitudes of AMPA receptor-mediated miniature excitatory postsynaptic currents were increased in hippocampal CA1 neurons (Van Sickle et al., 2004; Xiang and Tietz, 2007). The 50% enhancement in AMPA receptor function was attributed to an increase in GluA1 polypeptide trafficking from the endoplasmic reticulum and its subsequent incorporation into membranes (Song et al., 2007; Das et al., 2008), whereas NMDA receptor-mediated currents were reduced in this brain region (Van Sickle et al., 2004; Xiang and Tietz, 2007). Mice deficient in GluA1 subunits have reduced short-term tolerance (i.e., prolonged short-term impairment to high doses of flurazepam) and develop less tolerance but show increased withdrawal signs after a 7-day flurazepam treatment and challenge with flumazenil (Aitta-Aho et al., 2009). Even a single injection of diazepam to young mice increases the AMPA receptor function over that of NMDA receptors in dopamine neurons of the ventral tegmental area, measured in vitro 24 h after the drug injection in the ventral tegmental area dopamine neurons in vitro (Heikkinen et al., 2009).

Long-term BZ administration up-regulates L-type high voltage-gated calcium channels in the cerebral cortex (Katsura et al., 2007) and potentiates high voltage-gated calcium channel currents in the hippocampal CA1 neurons (Xiang et al., 2008).

B. Neurosteroids

Endogenous neurosteroids 3α-OH-5α-pregnan-20-one (3α,5α-THP) and its active 5β isomer 3α-OH-5-pregnan-20-one are metabolites of the ovarian/adrenal steroid

progesterone (Barbaccia, 2004). Allopregnanolone can also be synthesized de novo in the brain from cholesterol (Compagnone and Mellon, 2000). Other endogenous neurosteroids include 5α-pregnane-3α,21-diol-20-one, which is a metabolite of the adrenal steroid corticosterone (Barbaccia, 2004). These neurosteroids are potent positive allosteric modulators of $GABA_A$ receptors (Lambert et al., 2009), the δ-containing receptors ($\alpha4\beta\delta$, $\alpha6\beta\delta$) being especially sensitive to them (Wohlfarth et al., 2002; Spigelman et al., 2003). In addition to modulation of $GABA_A$ receptor function, fluctuations in brain neurosteroid concentrations (e.g., during estrous cycle, pregnancy, and stress) affect $GABA_A$ receptor subunit expression via an action that does not need the activation of steroid hormone receptors (Maguire and Mody, 2007). During estrous cycle, the *hippocampal* δ polypeptide level decreases 30% from the late diestrus (high-progesterone phase) to estrus (low-progesterone phase), whereas the pattern of $\gamma2$ subunit during the cycle is complementary to that of δ (Maguire et al., 2005). The expression of $\alpha4$ does not change, suggesting an up-regulation of $\alpha4\beta\delta$ receptors, down-regulation of $\alpha4\beta\gamma2$, and increase in tonic inhibition in late diestrus and the reverse effects at estrus (Maguire et al., 2005).

In vivo treatment of rats with progesterone or allopregnanolone up-regulated $\alpha4$ and δ polypeptide expression in the hippocampus (Gulinello et al., 2001; Hsu et al., 2003; Shen et al., 2005; Maguire et al., 2005; Maguire and Mody, 2007), whereas the $\gamma2$ polypeptide was down-regulated by 40 to 50% (Shen et al., 2005). Therefore, exogenous neurosteroids also up-regulate $\alpha4\beta\delta$ receptors and increase tonic inhibition. Discontinuation of administration of a neurosteroid agonist or the parent compound progesterone (i.e., withdrawal) leads to a 3-fold increase in hippocampal $\alpha4$ and δ (but no change in $\gamma2$ expression) (Smith et al., 1998; Sundstrom-Poromaa et al., 2002; Gangisetty and Reddy, 2009) and in premenstrual syndrome-like symptoms such as anxiety and increased seizure susceptibility (Dennerstein et al., 1985; Herzog, 2009).

Although studies on the effects of neurosteroids on $GABA_A$ receptor subunit expression in vivo have been focused on the hippocampus, studies in cultured cells in vitro have been performed using mouse cortical neurons and rat cerebellar granule cells. Progesterone treatment (1 μM, 5 days) had no effect on $\alpha4$ mRNA in cortical neurons or CGCs, whereas 6-h progesterone withdrawal up-regulated the expression in both cultures (Follesa et al., 2000, 2001b). In contrast, in differentiated P19 cells, allopregnanolone (1 μM, 4 days) down-regulated $\alpha4$ mRNA expression, but 1-day withdrawal from the treatment did not affect the expression (Grobin and Morrow, 2000). Long-term treatment with allopregnanolone (0.1 μM, 2 days) up-regulated $\alpha4$ polypeptide by 146% in IMR-32 neuroblastoma cells (Zhou and Smith, 2007, 2009).

Long-term treatment with progesterone and allopregnanolone had no effect on $\gamma2$ mRNA in cultured cortical neurons (Yu et al., 1996; Follesa et al., 2001b), whereas both compounds down-regulated it in CGCs (Follesa et al., 2000). Progesterone withdrawal down-regulated $\gamma2$ mRNA in both types of cell cultures (Follesa et al., 2000, 2001b). The δ polypeptide tends to be down-regulated in CGCs by long-term treatment with progesterone and allopregnanolone (Biggio et al., 2006), which effect is increased at withdrawal (Biggio et al., 2006).

C. Barbiturates

Barbiturates were introduced in early 20th century as relatively nonselective general depressants (Estes, 1995). Although also affecting several other ionotropic receptors, $GABA_A$ receptors are the primary target of barbiturates (Smith and Riskin, 1991). Barbiturates have been used in clinical practice as sedatives, anxiolytics, hypnotics, anesthetics, and anticonvulsants (Ito et al., 1996b).

There are a number of studies on the effects of long-term treatment with pentobarbital on $GABA_A$ receptor subunit mRNA expression (Tables 12 and 13). Treatment durations have been 6 to 14 days and daily pentobarbital doses 30 to 120 mg/kg/day (intraperitoneal injection) or 24 to 225 mg/day when using osmotic minipumps. Long-term treatment with pentobarbital had no effect on $\alpha1$ mRNA in the cerebral cortex, cerebellum, and several other brain regions, although it slightly down-regulated $\alpha1$ mRNA in the hippocampus and inferior and superior colliculi (Morrow et al., 1990, 1991; Tseng et al., 1994) (Table 12). During pentobarbital withdrawal (1 day), $\alpha1$ mRNA was up-regulated in the neocortex and cerebellar granule and Purkinje cells (Tseng et al., 1994) (Table 13). The expression of cerebellar $\alpha6$ mRNA was up-regulated by long-term treatment with pentobarbital (Ito et al., 1996a) (Table 12), and during withdrawal (1 day), the expression returned to control levels (Ito et al., 1996). Long-term treatment with pentobarbital drastically up-regulated $\beta1$ mRNA 600% over basal expression in the CA1 and CA2 regions of the hippocampus, although the expression was not affected in other hippocampal areas (Yin and Lee, 1998). On 1-day pentobarbital withdrawal, the $\beta1$ expression was still elevated by 130 to 180% but returned to control levels in 7-day withdrawal (Yin and Lee, 1998). Long-term treatment with pentobarbital had no effect on $\beta3$ mRNA expression (Tseng et al., 1994) (Table 12). During withdrawal $\beta3$ expression was up-regulated in the neocortex, whereas no changes in $\beta3$ expression were found in other brain regions studied (Tseng et al., 1994) (Table 13). The expression of $\gamma2$ mRNA was slightly reduced by long-term treatment with pentobarbital in the superior and inferior colliculi (Tseng et al., 1993a). Long-term treatment with pentobarbital up-regulated δ mRNA expression in the cerebellum but not in the frontal cortex (Lin and Wang, 1996) (Table 12). Withdrawal from pentobarbital down-regulated δ expression in the cerebellum, whereas no effect was found in the frontal cortex (Lin and Wang., 1996) (Table 13).

TABLE 12
Effect of long-term pentobarbital treatment in vivo on $GABA_A$ receptor subunit mRNA expression

Species, Dose, Duration, Regimen, Brain Region	Subunit mRNA						Reference
	α1	α6	β1	β3	γ2	δ	
	% change						
Rat, 30 mg/kg, 14 d							Morrow et al., 1990
Intraperitoneal injection							
Cerebral cortex	N.S.						
Rat, increasing dose							Morrow et al., 1991
30 mg->120 mg/kg, 14 d							
Intraperitoneal injection							
Cerebral cortex	N.S.						
Rat, 7.2 mg/d,[a] 6 d							Tseng et al., 1993a
Infusion, osmotic minipump							
Neocortex					N.S.		
Piriform cortex					N.S.		
Hippocampus					N.S.		
Caudate putamen					N.S.		
Medial habenular nucleus					N.S.		
Thalamus					N.S.		
Superior colliculus					−12		
Inferior colliculus					−8		
Central gray					N.S.		
Cerebellum					N.S.		
Rat, 7.2 mg/d,[a] 6 d							Tseng et al., 1994
Infusion, osmotic minipump							
Neocortex	N.S.			N.S.			
Piriform cortex	N.S.			N.S.			
Hippocampus							
CA1	−19			N.S.			
CA3	−10			N.S.			
Dentate gyrus	−10			N.S.			
Caudate putamen	N.S.			N.S.			
Medial habenular nucleus	N.S.			N.S.			
Thalamus	N.S.			—			
Superior colliculus	−25			—			
Inferior colliculus	−16			—			
Central gray	N.S.			—			
Cerebellum							
Molecular cells	N.S.			—			
Granule cells	N.S.			N.S.			
Purkinje cells	N.S.			—			
Rat, 7.2 mg/d,[a] 6 d							Ito et al., 1996a
Infusion, osmotic minipump							
Cerebellum							
Granule cells		+47					
Mouse, 3×75 mg/d, 7 d							Lin and Wang, 1996
Infusion, osmotic minipump							
Frontal cortex						N.S.	
Cerebellum						+44	
Rat, increasing dose							Yin and Lee, 1998
30 mg->120 mg/kg, 9 d							
Intraperitoneal injection							
Hippocampus							
CA1			+636				
CA2			+623				
CA3			N.S.				
CA4			N.S.				
Dentate gyrus			N.S.				

d, day(s); N.S., not significantly different from the control value; —, not detected.
[a] Continuous infusion.

The effect of long-term pentobarbital treatment on $GABA_A$ receptors has also been studied at polypeptide level using ligand binding assays. Consistent with slight or no effects of long-term treatment with pentobarbital on subunit mRNA expressions, the maximal number of binding sites (B_{max}) for [^{3}H]flunitrazepam, [^{3}H]muscimol, and [^{35}S]*t*-butylbicyclophosphorothionate binding to brain sections of rats chronically treated with pentobarbital did not differ from the control levels (Tseng et al., 1993b; Miyaoka et al., 1994). One-day pentobarbital withdrawal increased the [^{3}H]flunitrazepam B_{max} values in the frontal cortex, cerebellum and striatum (Tseng et al., 1993b; Miyaoka et al., 1994). The B_{max} value of [^{3}H]muscimol was increased in the frontal cortex and that of [^{35}S]*t*-butylbicyclophosphorothionate in the frontal cortex and striatum (Tseng et al., 1993b). Consistent with increased α6 mRNA expression in the study of Ito et al. (1996a), cerebellar [^{3}H]Ro 15-4513 binding was

TABLE 13
Effect of withdrawal from long-term pentobarbital treatment in vivo on $GABA_A$ receptor subunit mRNA expression

Species, Dose, Duration, Withdrawal Time, Regimen, Brain Region	Subunit mRNA					Reference
	α1	α6	β1	β3	δ	
			% change			
Rat, 7.2 mg/d,[a] 6 d, 1 d						Tseng et al., 1994
Infusion, osmotic minipump						
Neocortex						
Layer II/III	+52			+21		
Layer IV	+32			+14		
Layer V/VI	+39			+16		
Piriform cortex	+14			+14		
Hippocampus						
CA1	N.S.			N.S.		
CA3	N.S.			N.S.		
Dentate gyrus	N.S.			N.S.		
Caudate putamen	N.S.			N.S.		
Medial habenular nucleus	N.S.			N.S.		
Thalamus	N.S.			—		
Superior colliculus	N.S.			—		
Inferior colliculus	N.S.			—		
Central gray	N.S.			—		
Cerebellum						
Molecular layer	N.S.			—		
Granule cells	+18			N.S.		
Purkinje cells	+45			—		
Rat, 7.2 mg/d,[a] 6 d, 1 d						Ito et al., 1996a
Infusion, osmotic minipump						
Cerebellum						
Granule cells		N.S.				
Mouse, 3×75 mg/d, 7 d, 1 d						Lin and Wang, 1996
Infusion, osmotic minipump						
Frontal cortex					N.S.	
Cerebellum					−41	
Rat, increasing dose						Yin and Lee, 1998
60→90 mg/kg/d, 9 d, 1 d						
Intraperitoneal injection						
Hippocampus						
CA1			+128			
CA2			+176			
CA3			N.S.			
CA4			N.S.			
Dentate gyrus			N.S.			
Rat, increasing dose						Yin and Lee, 1998
60→90 mg/kg/d, 9 d, 7 d						
Intraperitoneal injection						
Hippocampus						
CA1			N.S.			
CA2			N.S.			
CA3			N.S.			
CA4			N.S.			
Dentate gyrus			N.S.			

d, day(s); N.S., not significantly different from the control value; —, not detected.
[a] Continuous infusion.

increased by long-term pentobarbital treatment and on pentobarbital withdrawal (Ito et al., 1996a).

In conclusion, there are rather few studies on the effects of barbiturates on the expression of $GABA_A$ receptors/subunits. Long-term treatment studies with pentobarbital suggest that receptor subunit expressions are usually slightly down-regulated, whereas pentobarbital withdrawal leads in receptor up-regulation.

D. Ethanol

The pharmacological actions of ethanol include anxiolysis, sedation, motor incoordination, impairment of judgment, and, at high concentrations, anesthesia. According to current view, the primary targets mediating ethanol intoxication are $GABA_A$ (Wallner et al., 2003; Lovinger and Homanics, 2007), NMDA (Ron, 2004), glycine (Crawford et al., 2007), 5-hydroxytryptamine 3 (serotonin) (Lovinger, 1999), and nicotinic acetylcholine receptors (Cardoso et al., 1999) as well as L-type Ca^{2+} channels (Wang et al., 1994), G-protein-activated inwardly rectifying K^+ channels (Kobayashi et al., 1999), and Ca^{2+}-activated K^+ channels (Brodie et al., 2007) (for review, see Vengeliene et al., 2008). Long-term administration of ethanol produces tolerance, dependence, and withdrawal signs upon cessation. Desensitization of ethanol-sensitive mechanisms obviously plays a role in the development of tolerance (Dopico and Lovinger, 2009), and the activity of the glutamatergic system is enhanced during withdrawal (De Witte et al., 2003; Nagy, 2008).

The effects of ethanol administration on $GABA_A$ receptor subunit expression have been studied using various ethanol doses (5–15 g/kg/day), durations of treatment (6–84 days), and routes of ethanol administration (for details, see Tables 14–24). The administration has been continuous [continuous ethanol (CE)] or intermittent [continuous intermittent ethanol (CIE)]. Furthermore, effects of withdrawal from ethanol on subunit expression have been determined at varying times after cessation (3 h–7 days). The variability in the results between the studies is obviously due, at least partially, to these differences in experimental schedules and conditions.

1. α1 Subunit. Long-term treatment of adult male Sprague-Dawley rats in vivo with ethanol down-regulated α1 mRNA in the cerebral cortex (Morrow et al., 1990, 1991; Montpied et al., 1991b; Mhatre and Ticku, 1992; Devaud et al., 1995) and, correspondingly, the α1 polypeptide was down-regulated by long-term ethanol treatment (Mhatre et al., 1993; Charlton et al., 1997; Devaud et al., 1997; Matthews et al., 1998; Marutha Ravindran et al., 2007a,b) (Tables 14 and 18). Variability was seen in the effects of ethanol on α1 polypeptide expression in different parts of cerebral cortex: down-regulation in the prefrontal, parietal, and piriform cortices, but no effect in the cingulate and motor cortices (Grobin et al., 2000). Down-regulation of rat cerebral cortical α1 mRNA was also observed after 1-day withdrawal from continuos ethanol treatment (wdr-CE) (Mhatre and Ticku, 1992). Down-regulation of cortical α1 polypeptide was also detected after 6 to 8 h wdr-CE (Devaud et al., 1997), but reverted back at 48 h of withdrawal (Marutha Ravindran et al., 2007a). In cultured mouse cortical neurons α1 mRNA and polypeptide were down-regulated with CE and CIE treatments (Marutha Ravindran and Ticku, 2006; Ravindran and Ticku, 2006; Sheela Rani and Ticku, 2006) (Tables 21 and 23). Down-regulation of α1 polypeptide was still present after a 7-day withdrawal period in CIE-treated but not CE-treated cells (Marutha Ravindran and Ticku, 2006; Ravindran and Ticku, 2006). In contrast, α1 mRNA or polypeptide was not significantly affected on 5-day wdr-CE or wdr-CIE in cultured cortical neurons (Sheela Rani and Ticku, 2006) (Tables 22 and 24).

In the cerebellum, CE treatment down-regulated α1 mRNA expression in male Sprague-Dawley rats (Mhatre and Ticku, 1992; Morrow et al., 1992) and in female C57BL/6 mice (Wu et al., 1995). After 1-day wdr-CE, α1 mRNA down-regulation was still present (Mhatre and Ticku, 1992). Expression of α1 mRNA was not down-regulated by CE in cultured rat CGCs (Follesa et al., 2003), but the down-regulation occurred after with-

TABLE 14
Effect of long-term ethanol treatment in vivo on $GABA_A$ receptor α subunit mRNA expression

Species, Dose, Duration, Regimen, Brain Region	Subunit mRNA						Reference
	α1	α2	α3	α4	α5	α6	
	% change						
Rat, 14 d							Morrow et al., 1990
Inhalation, BEC > 150 mg/100 ml							
Cerebral cortex	−45						
Rat, 14 d							Montpied et al., 1991
Inhalation, BEC > 150 mg/100 ml							
Cerebral cortex	−49	−29	N.S.				
Rat, 14 d, BEC > 150 mg/100 ml							Morrow et al., 1991
Inhalation							
Cerebral cortex	−45	−29	N.S.				
Rat, 8–10 g/kg/d, 14 d							Morrow et al., 1992
Forced ethanol drinking							
Cerebellum	−20					+50	
Rat, 5 g/kg/d, 6 d							Mhatre and Ticku, 1992
Intragastric intubation							
Cerebral cortex	−61	−61, −45	N.S.		−51		
Cerebellum	−28					+76	
Rat, 10–12 g/kg/d, 14 d							Devaud et al., 1995
Forced ethanol drinking							
Cerebral cortex	−43			+89	N.S.		
Mouse, 5 g/kg/d, 14 d							Wu et al., 1995
Intragastric intubation							
Cerebellum							
Purkinje cell layer	−40						
Granule cell layer	−23					+36	
Deep cerebellar cells	−40						
Rat, 10 g/kg/d, 84 d							Charlton et al., 1997
EtOH cont. liquid diet							
Hippocampus	−47				+64		
Rat, 5 g/kg/d, 18 months							Sarviharju et al., 2006
Forced ethanol drinking							
Frontoparietal cortex	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
Olfactory bulb	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
Olfactory tubercle	N.S.	N.S.	N.S.	−30	N.S.	N.S.	

d, day(s); BEC, blood ethanol concentration; N.S., not significantly different from the control value.

TABLE 15
Effect of long-term ethanol treatment in vivo on $GABA_A$ receptor β, γ and δ subunit mRNA expression

Species, Dose, Duration, Regimen, Brain Region	Subunit mRNA							Reference
	β1	β2	β3	γ1	γ2	γ3	δ	
	% change							
Rat, 5 g/kg/d, 6 d								Mhatre and Ticku, 1994
Intragastric intubation								
Cerebral cortex	+29	+55	+72					
Rat, 10–12 g/kg/d, 14 d								Devaud et al., 1995
Forced ethanol drinking								
Cerebral cortex	N.S.	N.S.	N.S.	70	+32, N.S.[a]	N.S.	N.S.	
Mouse, 5 g/kg/d, 14 d								Wu et al., 1995
Intragastric intubation								
Cerebellum								
Purkinje cell layer		N.S.			+82			
Granule cell layer		N.S.	N.S.		+46			
Deep cerebellar cells		N.S.			+62			
Rat, 5 g/kg, 18 months								Sarviharju et al., 2006
Forced ethanol drinking								
Frontoparietal cortex	N.S.	N.S.	−20	N.S.	N.S.	N.S.	N.S.	
Olfactory bulb	N.S.	N.S.	−18	N.S.	N.S.	N.S.	N.S.	
Olfactory tubercle	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	

d, day(s); N.S., not significantly different from the control value.
[a] Two values represent S and L splice variants, respectively.

drawal from the treatment (Follesa et al., 2003; Biggio et al., 2007). CE down-regulated α1 polypeptide expression in the cerebellum (Mhatre et al., 1993; Charlton et al., 1997; Marutha Ravindran et al., 2007a). After a 2-day withdrawal, the α1 polypeptide returned to control level (Marutha Ravindran et al., 2007a).

In the hippocampus, CE down-regulated α1 mRNA expression (Charlton et al., 1997), whereas the expression of α1 polypeptide was not affected (Charlton et al., 1997; Matthews et al., 1998; Marutha Ravindran et al., 2007a). A 2-day withdrawal from 6-day CE treatment had no effect on α1 polypeptide expression in the hippocampus (Marutha Ravindran et al., 2007a). In contrast, a 2-day withdrawal from 60-day continuous intermittent ethanol (wdr-CIE) down-regulated it by 48% (Cagetti et al., 2003). In cultured hippocampal neurons, α1 mRNA was down-regulated by CE and on withdrawal from CE (Sanna et al., 2003). Expression of α1 mRNA was down-regulated by CE in the amygdala (Papadeas et al., 2001) but not in the nucleus accumbens or ventral tegmental area (Charlton et al., 1997; Papadeas et al., 2001). CE decreased the α1 mRNA content in whole-brain samples of withdrawal seizure-prone mice but not in withdrawal seizure-resistant mice (Buck et al., 1991).

The effects of CE have also been studied in $GABA_AR$ α1 knockin mice harboring S270H and L277A mutations in

TABLE 16
Effect of withdrawal from long-term ethanol treatment in vivo on $GABA_A$ receptor α subunit mRNA expression

Species, Dose, Duration, Withdrawal Time Regimen, Brain Region	Subunit mRNA						Reference
	α1	α2	α3	α4	α5	α6	
	% change						
Rat, 5 g/kg/d, 6 d, 1d							Mhatre and Ticku, 1992
Intragastric intubation							
Cerebral cortex	−71	−61, −43	N.S.		−45		
Cerebellum	−34					+150	
Rat, 6 g/kg/d, 60 d, 2 d							Mahmoudi et al., 1997
Intragastric intubation, CIE							
Hippocampus							
CA1				+26	N.S.		
CA3				+46	N.S.		
Dentate gyrus				+30	N.S.		
Cerebral cortex							
Layer II				+12			
Layer III				+18			
Thalamus				+15			
Rat, 6 g/kg/d, 30 d, 2 d							Petrie et al., 2001
Intragastric intubation, CIE							
Cerebellum	N.S.					N.S.	
Rat, 6 g/kg/d, 60 d, 2 d							Petrie et al., 2001
Intragastric intubation, CIE							
Hippocampus				N.S.	N.S.		
Rat, 6 g/kg/d, 60 d, 2 d							Cagetti et al., 2003
Intragastric intubation, CIE							
Hippocampus		N.S.					

d, day(s); N.S., not significantly different from the control value.

TABLE 17
Effect of withdrawal from long-term ethanol treatment in vivo on $GABA_A$ receptor β, γ, and δ subunit mRNA expression

Species, Dose, Duration, Withdrawal Time Regimen, Brain Region	Subunit mRNA						Reference
	β1	β2	β3	γ1	γ2	δ	
	% change						
Rat, 5 g/kg/d, 6 d, 1 d							Mhatre and Ticku, 1994
Intragastric intubation							
Cerebral cortex	N.S.	+47	+28				
Rat, 6 g/kg/d, 30 d, 2 d							Petrie et al., 2001
Intragastric intubation, CIE							
Cerebellum		N.S.				N.S.	
Rat, 6 g/kg/d, 60 d, 2 d							Petrie et al., 2001
Intragastric intubation, CIE							
Hippocampus				N.S.	N.S., N.S.[a]	N.S.	
Rat, 6 g/kg/d, 60 d, 2 d							Cagetti et al., 2003
Intragastric intubation, CIE							
Hippocampus				+80	+48, N.S.[a]		

d, day(s); N.S., not significantly different from the control value.
[a] Two values represent S and L splice variants, respectively.

α1 subunit (Borghese et al., 2006). Recombinant receptors containing these mutations are insensitive to ethanol (Ueno et al., 2000; Borghese et al., 2006). CE treatment did not affect α1 polypeptide level in α1 knockin mice, although it reduced the level in wild-type mice (Werner et al., 2009). The results strongly suggest that CE down-regulates wild-type α1 mRNA and polypeptide. Furthermore, the study suggests that potentiation of α1-containing receptors by ethanol would be needed for CE-induced α1-receptor down-regulation.

The only study where an increase of α1 mRNA was observed is that of Hirouchi et al. (1993). They found a

TABLE 18
Effect of long-term ethanol treatment in vivo on $GABA_A$ receptor α subunit polypeptide expression

Species, Dose, Duration, Regimen, Brain Region	Subunit Polypeptide						Reference
	α1	α2	α3	α4	α5	α6	
	% change						
Rat, 5 g/kg/d, 6 d							Mhatre et al., 1993
Intragastric intubation							
Cerebral cortex	−61	−47	−30				
Cerebellum	−56						
Rat, 10 g/kg/d, 28 d							Charlton et al., 1997
Ethanol cont. liquid diet							
Frontoparietal cortex	−49				N.S.		
Cerebellum	−30				−36		
Hippocampus	N.S.				N.S.		
Ventral tegmental area	N.S.						
Rat, 10–12 g/kg/d, 14 d							Devaud et al., 1997
Forced ethanol administration							
Cerebral cortex	−33			+26			
Rat, 10 g/kg/d, 14 d							Matthews et al., 1998
Forced ethanol administration							
Hippocampus	N.S.	N.S.	N.S.	N.S.			
Rat, 10 g/kg/d, 40 d							Matthews et al., 1998
Forced ethanol administration							
Cerebral cortex	−18	N.S.	N.S.	+28			
Hippocampus	N.S.	N.S.	N.S.	+43			
Rat, 15 g/kg/d, 14 d							Grobin et al., 2000
Forced ethanol administration							
Prefrontal cortex	−18			+24			
Cingulate cortex	N.S.			+110			
Motor cortex	N.S.			+40			
Parietal cortex	−45			N.S.			
Piriform cortex	−21			+60			
Rat, 15 g/kg/d, 14 d							Papadeas et al., 2001
Forced ethanol administration							
Amygdala	−21			−22			
Nucleus accumbens	N.S.			−28			
Ventral tegmental area	N.S.			N.S.			
Rat, 9 g/kg/d, 6 d							Marutha Ravindran et al., 2007a
Intragastric intubation							
Cerebral cortex	−41	−41		+29			
Cerebellum	−28	−27				+66	
Hippocampus	N.S.	N.S.		+34			

d, day(s); N.S., not significantly different from the control value.

TABLE 19
Effect of long-term ethanol treatment in vivo on $GABA_A$ receptor β, γ, and δ subunit polypeptide expression

Species, Dose, Duration, Regimen, Brain Region	Subunit Polypeptide						Reference
	β1	β2	β3	γ1	γ2	δ	
	% change						
Rat, 5g/kg/d, 6 d							Mhatre and Ticku, 1994
Intragastric intubation							
Cerebral cortex		+23	+23				
Rat, 10–12 g/kg/d, 14 d							Devaud et al., 1997
Forced ethanol administration							
Cerebral cortex		+36	+36	+30	N.S.		
Rat, 10 g/kg/d, 14 d							Matthews et al., 1998
Forced ethanol administration							
Hippocampus		N.S.	N.S.	N.S.			
Rat, 10 g/kg/d, 40 d							Matthews et al., 1998
Forced ethanol administration							
Cerebral cortex	N.S.	N.S.	N.S.				
Hippocampus	N.S.	N.S.	N.S.				
Rat, 9 g/kg/d, 6 d							Marutha Ravindran et al., 2007a
Intragastric intubation							
Cerebral cortex		+34			N.S.		
Cerebellum		+31			N.S.		
Hippocampus		N.S.			+32		
Rat, 9 g/kg/d, 6 d							Marutha Ravindran et al., 2007b
Intragastric intubation							
Cerebral cortex						N.S.	
Cerebellum						−35	
Hippocampus						−25	

d, day(s); N.S., not significantly different from the control value.

37% increase in α1 mRNA in samples purified from total brain of mice receiving a daily 1 mmol/kg injection of the alcohol dehydrogenase inhibitor pyrazole and kept 7 days at continuous inhalation of ethanol vapor. The presence of pyrazole and the absence of acetaldehyde on α1 mRNA expression were not studied, but these factors may be responsible for the opposite result received.

2. α2 Subunit. Long-term ethanol treatment down-regulated α2 mRNA (Montpied et al., 1991b; Mhatre and Ticku, 1992; Morrow et al., 1992) and α2 polypeptide (Mhatre et al., 1993; Marutha Ravindran et al., 2007a) in the cerebral cortex (Tables 14 and 18). This down-regulation was not seen by Matthews et al. (1998). Down-regulation of cortical α2 mRNA was also detected after a 1-day wdr-CE (Mhatre and Ticku, 1992). However, during 2-day wdr-CE, cortical α2 polypeptide expression was reversed to control levels (Marutha Ravindran et al., 2007a). CE and CIE down-regulated α2 mRNA in cultured mouse cortical neurons (Sheela Rani and Ticku, 2006). The expression of α2 polypeptide was down-regulated in cortical neurons with CE, whereas CIE had no effect on expression (Sheela Rani and Ticku, 2006). Withdrawal from CE or CIE had no effect on α2 mRNA or polypeptide expression in cultured mouse cortical neurons (Sheela Rani and Ticku, 2006).

Long-term ethanol treatment down-regulated α2 polypeptide in the cerebellum (Marutha Ravindran et al., 2007a), and the expression returned to control levels

TABLE 20
Effect of withdrawal from long-term ethanol treatment in vivo on $GABA_A$ receptor subunit polypeptide expression

Species, Dose, Duration, Withdrawal Time Regimen, Brain Region	Subunit Polypeptide								Reference
	α1	α2	α4	β2	β3	γ1	γ2	δ	
	% change								
Rat, 10–12 g/kg/d, 14 d, 6–8 h									Devaud et al., 1997
Forced ethanol administration									
Cerebral cortex	−34		+30	+32	+32	+54	N.S.		
Rat, 6 g/kg/d, 60 d, 2 d									Cagetti et al., 2003
Intragastric intubation, CIE									
Hippocampus	−48		+50				+38	−52	
Rat, 9 g/kg/d, 6 d, 2 d									Marutha Ravindran et al., 2007a
Intragastric intubation									
Cerebral cortex	N.S.	N.S.	+29	N.S.			N.S.		
Cerebellum	N.S.	N.S.		N.S.			N.S.		
Hippocampus	N.S.	N.S.	+36	N.S.			N.S.		
Rat, 9 g/kg/d, 6 d, 2 d									Marutha Ravindran et al., 2007b
Intragastric intubation									
Cerebral cortex								N.S.	
Cerebellum								N.S.	
Hippocampus								N.S.	

d, day(s); N.S., not significantly different from the control value.

TABLE 21
Effect of long-term ethanol treatment on $GABA_A$ receptor subunit mRNA expression in cultured cells in vitro

Cells, [EtOH], Treatment Time	Subunit mRNA										Reference
	α1	α2	α3	α4	α5	α6	β2	β3	γ2	δ	
	% change										
Rat hippocampal neurons											
100 mM, 5 d	−24	N.S.	−23	N.S.	N.S.				−22, −34[a]		Sanna et al., 2003
100 mM, 5 d										+120	Follesa et al., 2005
Rat cerebellar granule cells											
100 mM, 5 d	N.S.			N.S.		N.S.			−23, −32[a]		Follesa et al., 2003
100 mM, 5 d	N.S.										Follesa et al., 2004
100 mM, 5 d										N.S.	Follesa et al., 2005
Mouse cortical neurons											
75 mM, 5 d	−24	−24		N.S.			N.S.	N.S.	−32		Sheela Rani and Ticku, 2006
75 mM, 5 d, CIE	−55	−52		48			N.S.	N.S.	−29		Sheela Rani and Ticku, 2006

d, day(s); N.S., not significantly different from the control value.
[a] Two values represent S and L splice variants, respectively.

after a 2-day withdrawal from the treatment (Marutha Ravindran et al., 2007a). In cultured rat CGCs, CE had no effect on α2 mRNA (Follesa et al., 2004), whereas withdrawal from CE drastically up-regulated α2 mRNA and polypeptide expression (Follesa et al., 2004; Biggio et al., 2007).

In the hippocampus, wdr-CIE had no effect on α2 mRNA expression (Cagetti et al., 2003). Hippocampal α2 polypeptide level was not affected by CE treatment or withdrawal from the treatment (Matthews et al., 1998; Marutha Ravindran et al., 2007a). In cultured hippocampal neurons α2 mRNA expression was not affected by CE, whereas it was up-regulated by withdrawal from the treatment (Sanna et al., 2003). Studies on α2 subunit in vivo suggest that α2 is down-regulated brain region-specifically in the cerebral cortex and cerebellum by CE.

3. α3 Subunit. The expression of α3 mRNA in the cerebral cortex is generally not affected by CE (Montpied et al., 1991b; Morrow et al., 1991; Mhatre and Ticku, 1992) or wdr-CE (Mhatre and Ticku, 1992) (Tables 14 and 18). Down-regulation of cortical α3 polypeptide expression has been observed (Mhatre et al., 1993). CE had no effect on hippocampal α3 polypeptide expression (Matthews et al., 1998). In cultured hippocampal neurons, CE down-regulated α3 mRNA expression, whereas withdrawal from the treatment up-regulated its expression (Sanna et al., 2003). The results on ethanol regulation of α3 expression are controversial but suggest mostly that ethanol does not regulate the α3 expression.

4. α4 Subunit. Cerebral cortical α4 mRNA expression was up-regulated after CE (Devaud et al., 1995) or withdrawal from the treatment (Mahmoudi et al., 1997) (Tables 14 and 18). Likewise, CE up-regulated cortical α4 polypeptide expression (Devaud et al., 1997; Matthews et al., 1998; Grobin et al., 2000; Marutha Ravindran et al., 2007a). There was heterogeneity in the up-regulation, the increase in expression being greatest (110%) in the cingulate cortex, although no effect was found in the parietal cortex (Grobin et al., 2000). The expression of α4 mRNA was up-regulated by CIE and α4 polypeptide by CE in cultured mouse cortical neurons (Sheela Rani and Ticku, 2006). Withdrawal from CE also up-regulated cortical α4 in vivo in rats (Devaud et al., 1997; Marutha Ravindran et al., 2007a). In contrast, wdr-CE and wdr-CIE had no effect on α4 mRNA in cultured mouse cortical neurons (Sheela Rani and Ticku, 2006). CE did not affect α4 mRNA expression in cultured rat cerebellar granule cells, whereas up-regulation of α4 mRNA and polypeptide was detected after withdrawal

TABLE 22
Effect of withdrawal from long-term ethanol treatment on $GABA_A$ receptor subunit mRNA expression in cultured cells in vitro

Cells, [EtOH], Duration, Withdrawal Time	Subunit mRNA										Reference
	α1	α2	α3	α4	α5	α6	β2	β3	γ2	δ	
	% change										
Rat hippocampal neurons											
100 mM, 5 d, 3 h	−15	+30	+40	+30	N.S.				−21, −40[a]		Sanna et al., 2003
100 mM, 5 d, 9 h										N.S.	Follesa et al., 2005
Rat cerebellar granule cells											
100 mM, 5 d, 3–12 h	−29			+46		−27			−76, −64[a]		Follesa et al., 2003
100 mM, 5 d, 3 h		+159									Follesa et al., 2004
100 mM, 5 d, 6 h										−27	Follesa et al., 2005
100 mM, 5 d, 6 h	−22	+160		+43		−28			−33, −22[a]	−23	Biggio et al., 2007
Mouse cortical neurons											
75 mM, 5 d, 5d	N.S.	N.S.		N.S.			N.S.	N.S.	N.S.		Sheela Rani and Ticku, 2006
75 mM, 5 d, 5d, CIE	N.S.	N.S.		N.S.			N.S.	N.S.	N.S.		Sheela Rani and Ticku, 2006

d, day(s); N.S., not significantly different from the control value.
[a] Two values represent S and L splice variants, respectively.

TABLE 23
Effect of long-term ethanol treatment on $GABA_A$ receptor subunit polypeptide expression in cultured cells in vitro

Cells, [EtOH], Treatment Time	Subunit Polypeptide						Reference
	α1	α2	α4	β2	γ2	δ	
	% change						
Rat hippocampal neurons							
100 mM, 5 d						+85	Follesa et al., 2005
Rat cerebellar granule cells							
100 mM, 5 d						N.S.	Follesa et al., 2005
Mouse cortical neurons							
75 mM, 5 d	−30			+35	N.S.		Marutha Ravindran and Ticku, 2006
75 mM, 5 d, CIE	−25			+29	N.S.		Marutha Ravindran and Ticku, 2006
75 mM, 5 d	−26	−30	+74	+24	N.S.		Sheela Rani and Ticku, 2006
75 mM, 5 d, CIE	−15	N.S.	N.S.	+158	N.S.		Sheela Rani and Ticku, 2006

d, day(s); N.S., not significantly different from the control value.

from the treatment (Follesa et al., 2003; Biggio et al., 2007). CE up-regulated hippocampal α4 mRNA and polypeptide expression (Devaud et al., 1995, 1997; Matthews et al., 1998; Marutha Ravindran et al., 2007a). Withdrawal from CE up-regulated hippocampal α4 mRNA in the study by Mahmoudi et al. (1997) [but not in that of Petrie et al. (2001)] and hippocampal α4 polypeptide in studies of Cagetti et al. (2003) and Marutha Ravindran et al. (2007a) (Table 16). CE did not affect α4 mRNA expression in cultured rat hippocampal neurons (Sanna et al., 2003), whereas withdrawal from the treatment up-regulated α4 mRNA and especially polypeptide expression (Sanna et al., 2003). CE down-regulated α4 polypeptide in the amygdala and nucleus accumbens (Papadeas et al., 2001). Life-long ethanol drinking down-regulated α4 mRNA in the olfactory tubercle (Sarviharju et al., 2006). Withdrawal from long-term ethanol treatment slightly up-regulated α4 mRNA in the thalamus (Mahmoudi et al., 1997).

Studies on α4 expression suggest that α4 subunit is strongly up-regulated by long-term ethanol treatment and especially on withdrawal. The effect is brain region-specific. More recently, Pignataro et al. (2007) have described a number of genes that are up-regulated by a short-term alcohol treatment of mouse cultured cortical neurons. It is noteworthy that one the genes was *GABRA4*, the expression of which can be up-regulated by relevant concentrations of ethanol (10–60 mM) via activation of heat shock factor 1 that binds to "alcohol response element" of the α4 subunit gene promoter. The detailed mechanisms of other $GABA_A$ receptor subunit gene regulation by alcohol are not known.

5. α5 Subunit. The expression of α5 mRNA was down-regulated by CE in the cerebral cortex (Mhatre and Ticku, 1992), whereas no effect was found by Devaud et al. (1995) (Table 14). CE did not affect cerebral cortical α5 polypeptide expression (Charlton et al., 1997). Withdrawal from CE down-regulated cortical α5 mRNA expression (Mhatre and Ticku, 1992). CE down-regulated α5 polypeptide expression in the cerebellum (Charlton et al., 1997). In the hippocampus, CE up-regulated α5 mRNA expression, although it had no effect on α5 polypeptide expression (Charlton et al., 1997). Withdrawal from CE did not affect hippocampal α5 mRNA expression (Mahmoudi et al., 1997; Petrie et al., 2001). Long-term ethanol treatment or withdrawal from it did not affect α5 mRNA expression in cultured rat hippocampal neurons (Sanna et al., 2003). The results of studies on the CE effect on α5 subunit suggest brain region-specific modulation of the expression.

TABLE 24
Effect of withdrawal from long-term ethanol treatment on $GABA_A$ receptor subunit polypeptide expression in cultured cells in vitro

Cells, [EtOH], Duration, Withdrawal Time	Subunit Polypeptide						Reference
	α1	α2	α4	β2	γ2	δ	
	% change						
Rat hippocampal neurons							
100 mM, 5 d, 6 h			+120				Sanna et al., 2003
100 mM, 5 d, 6 h						−27	Follesa et al., 2005
100 mM, 5 d, 12 h						N.S.	Follesa et al., 2005
Rat cerebellar granule cells							
100 mM, 5 d, 3 h		+159					Follesa et al., 2004
100 mM, 5 d, 6 h						+82	Follesa et al., 2005
100 mM, 5 d, 12 h						N.S.	Follesa et al., 2005
100 mM, 5 d, 6 h			+140				Biggio et al., 2007
Mouse cortical neurons							
75 mM, 5 d, 2d,	N.S.			N.S.	N.S.		Marutha Ravindran and Ticku, 2006
75 mM, 5 d, 7d, CIE	−20			+26	N.S.		Marutha Ravindran and Ticku, 2006
75 mM, 5 d, 5d	N.S.	N.S.	N.S.	N.S.	−61		Sheela Rani and Ticku, 2006
75 mM 5 d, 5d, CIE	N.S.	N.S.	N.S.	N.S.	−17		Sheela Rani and Ticku, 2006

N.S., not significantly different from the control value.

6. α6 Subunit. Cerebellar α6 mRNA expression was up-regulated by CE in rat (Mhatre and Ticku, 1992; Morrow et al., 1992) and mouse (Wu et al., 1995) cerebellum (Table 14). Withdrawal from CE resulted in a drastic increase in α6 mRNA expression in the study by Mhatre and Ticku (1992), but not in that of Petrie et al. (2001). Cerebellar α6 polypeptide was up-regulated by CE (Marutha Ravindran et al., 2007a), and after a 2-day wdr-CE, it was reversed to expression level of the control animals (Marutha Ravindran et al., 2007a). CE had no effect on α6 mRNA expression in cultured rat CGCs (Follesa et al., 2003), whereas wdr-CE resulted in α6 mRNA down-regulation (Follesa et al., 2003; Biggio et al., 2007). Long-term ethanol treatment of the α6 knockout mice expressing *Escherichia coli* β-galactosidase under the control of α6 gene promoter (Jones et al., 1997) did not alter β-galactosidase activity, suggesting that α6 up-regulation requires functional α6 subunits (Vekovischeva et al., 2000). The results suggest that CE up-regulates α6 expression in the cerebellum.

7. β1 Subunit. Long-term ethanol up-regulated β1 mRNA in the cerebral cortex (Mhatre and Ticku (1994), whereas no effect was found by Devaud et al. (1995) (Table 15). Expression of β1 mRNA returned to control level after a 1-day wdr-CE (Mhatre and Ticku., 1994). CE had no effect on cortical or hippocampal β1 polypeptide expression (Matthews et al., 1998). CE does not seem to consistently regulate β1 expression.

8. β2 Subunit. Cerebral cortical β2 mRNA was up-regulated by CE (Mhatre and Ticku (1994), whereas no effect was found by Devaud et al. (1995) (Table 15). In cultured mouse cortical neurons, CE, CIE, wdr-CE, and wdr-CIE had no effect on β2 mRNA expression (Sheela Rani and Ticku, 2006). Cerebral cortical β2 polypeptide was up-regulated by CE in studies by Mhatre and Ticku (1994), Devaud et al. (1997), and Marutha Ravindran et al. (2007a), whereas no effect was found by Matthews et al. (1998). Expression of cortical β2 mRNA was slightly up-regulated after a 1-day wdr-CE (Mhatre and Ticku, 1994), but it was not affected after a 2-day wdr-CIE (Petrie et al., 2001). Cortical β2 polypeptide was found to be up-regulated by wdr-CE Devaud et al. (1997) but not by Marutha Ravindran et al. (2007a). In cultured mouse cortical neurons, β2 polypeptide was up-regulated by CE and CIE (Marutha Ravindran and Ticku, 2006; Ravindran and Ticku, 2006; Sheela Rani and Ticku, 2006). The up-regulation persisted for at least 7 days after wdr-CIE (Marutha Ravindran and Ticku, 2006; Ravindran and Ticku, 2006) but not wdr-CE (Marutha Ravindran and Ticku, 2006; Ravindran and Ticku, 2006; Sheela Rani and Ticku, 2006). Cerebellar β2 polypeptide was up-regulated by CE in rats in vivo (Marutha Ravindran et al., 2007), whereas it had no effect on cerebellar β2 mRNA in mice (Wu et al., 1995). After 2-day wdr-CE (Marutha Ravindran et al., 2007) and 2-day wdr-CIE (Petrie et al., 2001), the expressions of cerebellar β2 mRNA and polypeptide, respectively, were not different from control values. CE or wdr-CE did not affect β2 polypeptide expression in the hippocampus (Matthews et al., 1998; Marutha Ravindran et al., 2007a). Studies on β2 subunit in vivo suggest that β2 is up-regulated brain region-specifically in the cerebral cortex and cerebellum.

9. β3 Subunit. Treatment of rats with CE up-regulated cerebral cortical β3 mRNA in the study of Mhatre and Ticku (1994), but not in that of Devaud et al. (1995) (Table 15). After 1-day wdr-CE, β3 mRNA value was still greater than in the corresponding control animals (Mhatre and Ticku, 1994). In cultured mouse cortical neurons CE, CIE, wdr-CE, and wdr-CIE had no effect on β3 mRNA (Sheela Rani and Ticku, 2006). Cortical β3 polypeptide was up-regulated by CE in the studies of Mhatre and Ticku (1994) and Devaud et al. (1997), but not in that of Matthews et al. (1998). The up-regulation in β3 polypeptide persisted 6 to 8 h after wdr-CE (Devaud et al., 1997). In contrast to short CE exposure, a life-long ethanol consumption in alcohol-preferring rats down-regulated β3 mRNA expression in the frontoparietal cortex and olfactory bulb (Sarviharju et al., 2006). The results suggest up-regulation of β3 by short-term treatment, but down-regulation in some brain regions after life-long consumption.

10. γ2 Subunit. Long-term ethanol treatment up-regulated the short (S) but not the long (L) splice variant of γ2 mRNA in the cerebral cortex (Devaud et al., 1995) (Table 15). CE or wdr-CE had no effect on cortical γ2 polypeptide expression (Devaud et al., 1997; Marutha Ravindran et al., 2007a). In cultured mouse cortical neurons, CE, CIE, wdr-CE, or wdr-CIE had no effect on γ2 polypeptide expression in the studies of Marutha Ravindran and Ticku (2006) and Ravindran and Ticku (2006). In contrast, CE down-regulated γ2 mRNA but not polypeptide expression (Sheela Rani and Ticku, 2006). In that study, wdr-CE and wdr-CIE had no effect on γ2 mRNA, but γ2 polypeptide expression was down-regulated after withdrawal from both types of long-term ethanol administration (Sheela Rani and Ticku, 2006). CE or wdr-CE had no effect on cerebellar γ2 polypeptide expression in rats in vivo (Marutha Ravindran et al., 2007a). The S and L variants of γ2 mRNA were down-regulated by CE and wdr-CE in cultured rat CGCs (Follesa et al., 2003; Biggio et al., 2007). Expressions of γ2 mRNA splice variants in the hippocampus were not affected by wdr-CIE according to Petrie et al. (2001), whereas an increase in γ2S but not in γ2L mRNA after wdr-CIE was found by Cagetti et al. (2003). The γ2 polypeptide was up-regulated by a 6-day CE in the hippocampus (Marutha Ravindran et al., 2007a). Expression of hippocampal γ2 polypeptide was up-regulated after wdr-CIE (Cagetti et al., 2003), but not wdr-CE (Marutha Ravindran et al., 2007a). In cultured rat hippocampal neurons, CE and wdr-CE down-regulated γ2S and γ2L mRNA expression (Sanna et al., 2003). The

results suggest brain region-specific up-regulation of γ2 expression by CE in the hippocampus.

11. γ1 and γ3 Subunits. Long-term ethanol up-regulated γ1 mRNA and polypeptide expression in the cerebral cortex (Devaud et al., 1995, 1997) (Tables 15 and 19). Increased expression of cerebral cortical γ1 polypeptide persisted on wdr-CE (Devaud et al., 1997). Hippocampal γ1 mRNA expression was found to be up-regulated on wdr-CIE by Cagetti et al. (2003) but not by Petrie et al. (2001). CE did not up-regulate hippocampal γ1 polypeptide (Matthews et al., 1998). Long-term ethanol had no effect on γ3 mRNA expression in the cerebral cortex (Devaud et al., 1995). The results suggest up-regulation of γ1 expression in ethanol withdrawal and unaltered expression of γ3 by ethanol.

12. δ Subunit. The expression of δ mRNA and polypeptide was not affected by CE or wdr-CE in the cerebral cortex (Devaud et al., 1995; Marutha Ravindran et al., 2007b) (Tables 15, 17, 19, and 20). Expression of δ polypeptide in the cerebellum and hippocampus was down-regulated by CE (Marutha Ravindran et al., 2007b). After wdr-CE (Marutha Ravindran et al., 2007b) or wdr-CIE (Petrie et al., 2001), the expression of cerebellar and hippocampal δ mRNA and polypeptide did not differ from the expression in control animals, whereas a down-regulation of hippocampal δ polypeptide after wdr-CIE was detected (Cagetti et al., 2003). In cultured rat CGCs, CE had no effect on δ mRNA and polypeptide expression, whereas wdr-CE slightly down-regulated both δ mRNA and polypeptide (Follesa et al., 2005; Biggio et al., 2007). In cultured rat hippocampal neurons, CE strongly up-regulated δ mRNA and polypeptide expression (Follesa et al., 2005). The δ mRNA and polypeptide levels elevated by CE in hippocampal neurons returned to control levels by 12 h (Follesa et al., 2005). The results suggest brain region-specific down-regulation of δ subunit.

13. Studies of Continuous Intermittent Ethanol Administration on Subunit Cell Surface Expression. The group of Olsen and Spigelman have further investigated their CIE model in the rat. Cagetti et al. (2003) found that CIE strongly down-regulates hippocampal α1 and δ subunits but up-regulates synaptic α4 and γ2 subunits. They dissected the $GABA_A$ receptor-related molecular events in hippocampal CA1 slices after 1-h ethanol intoxication (Liang et al., 2007). The cell surface fractions of α4 and δ subunits (extrasynaptic), but not those of α1, α5, or γ2 subunits, were decreased. This was accompanied by decreased magnitude of tonic $GABA_A$ current, the enhancement of which was reduced by ethanol. At 48 h, the cell surface subunit content of α4 (80%) and γ2 (82%) increased, whereas that of α1 (−50%) and δ (−79%) decreased (Liang et al., 2007). These changes were fully reversible, but persisted long after withdrawal from CIE treatment. The authors hypothesized that early ethanol tolerance might result from activation and subsequent internalization of extrasynaptic α4βδ receptors, subsequently leading to transcriptionally regulated increases in α4 and γ2 subunits, resulting in insertion of the newly formed α4βγ2 receptors at synapses.

14. Conclusions on Effects of Continuous Ethanol Administration on $GABA_A$ Receptor Subunit Expression. CE and CIE treatments of rats in vivo down-regulate α1 expression in most brain regions studied and α2 expression specifically in the cerebral cortex and cerebellum. These treatments and especially withdrawal from them up-regulate α4 expression in the cerebral cortex and hippocampus. CE up-regulates α6 expression in the cerebellum. α1 and α6 subunits are located in β2-α6-α1-γ2 $GABA_A$ receptor subunit gene cluster in the same positions as α2 and α4 in the homologous β1-α4-α2-γ1 gene cluster (Russek, 1999). These two clusters have obviously evolved from a common ancestral cluster. The α1/α2 genes, homologous genes from β2-α6-α1-γ2 and β1-α4-α2-γ1 $GABA_A$ receptor subunit gene clusters may contain similar regulatory elements responsible for their down-regulation by CE; likewise, the α6/α4 genes may contain similar regulatory elements responsible for the up-regulation by CE. Up-regulation of β2 and β3 is suggested by most studies. CE and CIE brain region-specifically up-regulate γ2 in the hippocampus. The δ subunit is down-regulated brain region-specifically in the cerebellum and in hippocampus. These changes suggest down-regulation of α1βγ2, α2βγ2, α4βδ, and α6βδ receptor subtypes and up-regulation of α4βγ2 and α6βγ2 receptor subtypes. The CE-induced changes in $GABA_A$ receptor expression are short-lived (usually 1–2 days), whereas CIE-induced changes take much longer to revert.

In vitro studies with cultured cells treated with CE or CIE have produced quite controversial results compared with studies in vivo. The in vitro results from rat hippocampal neurons or cerebellar granule cells are often opposite the results received from hippocampus or cerebellum of rats treated in vivo. Therefore, the in vitro results should be interpreted very cautiously.

IV. Novel Opportunities to Target the $GABA_A$ Receptor System with Therapeutics

The drugs now used clinically that target the $GABA_A$ receptor system are often very efficacious in the short term, but they lose their pharmacological effects to a great extent during repeated administration. The most important disease group to find more efficient pharmacological treatment is the anxiety disorders, but the treatment of many other indications also would benefit from compounds with more selective action either at BZ-site of synaptic $GABA_A$ receptors or at other sites of, for example, extrasynaptic receptors in the hippocampus, cortex, and thalamus or at selective cell types, such as cortical interneurons. We give here some examples.

Medical genetics has progressed to the level that we know several $GABA_A$ receptor subunit genes that are

somehow associated with neuropsychiatric illnesses (Korpi and Sinkkonen, 2006). Unfortunately, in most cases, it is premature to make detailed hypotheses regarding the mechanisms by which these genetic associations might affect the development or progress of a particular set of symptoms or a disease. However, there is one very good example for rational drug development work, namely schizophrenia, in which deficient function in cortical interneurons has been established (Benes et al., 1991; Lewis et al., 2005; Benes, 2009) and specific $GABA_A$ receptor $\alpha 2$ subunit changes have been documented in principal neurons (Cruz et al., 2009), provoking a simple idea of targeting the $\alpha 2$ subunits with subtype-selective compounds, apparently with agonists to counteract the reduced interneuron activity. Similar compounds should be tested also for generalized anxiety and other anxiety disorders, the idea being based on results from a study on mouse model with targeted inactivation of $\alpha 2$ BZ-sites [so called $\alpha 2$(H101R) mice]. In that model, diazepam is inactive in inducing short-term anxiolysis (Löw et al., 2000). This idea was corroborated in experiments with $\alpha 2$ knockout mice (Dixon et al., 2008). It is noteworthy that work with $\alpha 3$ knockout mice suggests a role for reduced function of $\alpha 3$ subunit-containing $GABA_A$ receptors in schizophrenia-like sensorimotor deficits and excessive dopamine function (Yee at al., 2005); also, in this mouse model, diazepam had little anxiolytic effect. Experimental compounds having $\alpha 2$ and/or $\alpha 3$ agonist selectivity already exist (e.g., Atack et al., 2006; Jennings et al., 2006; Van Laere et al., 2008; Taliani et al., 2009), but they have not been well tested in patients with anxiety (Atack, 2008). Furthermore, neurobiological findings suggest that these same receptor subtypes at the spinal level should be tested as targets for alleviating neuropathic pain (Knabl et al., 2008).

As discussed previously (Korpi and Sinkkonen, 2006), the arsenal for hypnotic drugs acting on the BZ-site is already satisfactory; triazolam, midazolam, zolpidem, zopiclone, and zaleplon demonstrate acceptable short-term efficacies with a selection of half-lives and pharmacokinetic variations to fit needs of different patients. It is also possible that these compounds retain their hypnotic activity in long-term treatment (up to 12 months) as has been recently shown for the *S* isomer of zopiclone (eszopiclone) (Krystal et al., 2003; Roth et al., 2005). The GABA-site agonist gaboxadol acts differently from BZ-site ligands to alleviate insomnia (Wafford and Ebert, 2006), but its phase III trial as a hypnotic was stopped as a result of adverse effects in some patient populations. The role of direct GABA-site agonists should still be examined, because gaboxadol seems to target preferentially a clearly different receptor population than BZ compounds. Gaboxadol targets the extrasynaptic $GABA_A$ receptors (Chandra et al., 2006), its high-affinity binding occurs in different brain regions from BZ binding (Friemel et al., 2007), and it does not show cross-tolerance with BZs in rodent motor function tests (Voss et al., 2003). It is interesting that neuroprotective efficacy (e.g., ischemia models) has been stronger with direct GABA agonists such as muscimol than with BZs (Green et al., 2000). In a number of knockout and transgenic mouse models, the sedative actions of gaboxadol and muscimol correlate with the mouse forebrain density of high-affinity muscimol binding, presumably reflecting an extrasynaptic population of $GABA_A$ receptors containing $\alpha 4$ and δ subunits (Chandra et al., 2010).

Neurosteroids and neuroactive steroids have actions on $GABA_A$ receptors (Schumacher et al., 2003; Lambert et al., 2003), especially on δ subunit-containing extrasynaptic receptors (Chandra et al., 2006), and mediate the fast nongenomic effects of these compounds either by enhancing or inhibiting the receptor activity (Majewska, 1992). There have been several attempts to develop neurosteroid compounds as anesthetics; since the pioneering studies of Hans Selye, they have been long known to possess powerful, rapidly acting and quickly residing (quick metabolism) effects in rats. A synthetic steroid ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) is a potent $GABA_A$ receptor modulator without efficacy on nuclear hormone receptors (Carter et al., 1997). It holds promise in some forms of epilepsy, such as infantile spasms and catamenial epilepsy (Rogawski and Reddy, 2002), when there is a probable deficiency in brain concentrations of neurosteroids.

Inverse agonists of the $GABA_A$ receptors might be used to treat alcoholism (and eating disorders/obesity?) and cognitive impairment (Korpi and Sinkkonen, 2006). In this context, it seems to be clear that the new compounds should have subtype selectivity rather than being general nonselective inverse agonists. Nonselective BZ-site inverse agonists are strongly anxiogenic and proconvulsant or convulsant, and in long-term treatment, these actions might get sensitized and abolish any initial treatment effect. Subtype-selective inverse agonists might be safer in this regard, but it remains to be fully established. Inverse agonists for the $\alpha 5$ subunit-containing receptors should be tested for cognitive improvement, because both genetically and pharmacologically rendered impairment of $\alpha 5$ subunits seems to improve learning and memory in mouse and monkey models (Crestani et al., 2002; Collinson et al., 2006; Ballard et al., 2009).

Regarding alcoholism, the situation is still unclear. Ethanol actions at extrasynaptic $GABA_A$ receptors containing δ and $\alpha 4$ or $\alpha 6$ subunits have been shown to be antagonized by the BZ ligand Ro 15-4513 in some experiments (Wallner et al., 2006; Hanchar et al., 2006) but not in others (Borghese et al., 2007; Korpi et al., 2007). The excitement on alcohol-antagonistic effects of Ro 15-4513 and several other inverse agonists started already in the 1980s (Suzdak et al., 1986; Bonetti et al., 1988) without any therapeutic breakthroughs up to now. Still needed are more selective molecules and/or better ani-

mal models to study the mechanisms of the putative alcohol antagonistic action.

In addition to the drugs targeting the main $GABA_A$ receptor subtypes, one might also attempt to target the minor ones (e.g., ε and θ subunit-containing receptors) that are highly enriched (e.g., in monoaminergic nuclei) (Sinkkonen et al., 2000; Moragues et al., 2002) and might serve as selective targets for non-BZ site compounds to regulate neuronal activity in ascending monoamine pathways. The ε and θ subunits are most likely assembled with the α3 subunits, but hardly anything has been published in terms of selective pharmacology and functional activity of these receptor subtypes (Ranna et al., 2006). Neuronal cell population-specific modulation may eventually be needed (e.g., for the pharmacological regulation of behaviors such as feeding), because brain regionally discrete effects are often different from systemic drug effects.

Finally, it should be remembered that the $GABA_A$ receptor is a large molecular complex having surprisingly many different binding sites and interactions with other proteins. Therefore, it is possible that the future will bring more compounds having novel target sites at the receptor complex or at the receptor-associated proteins, which might then provide different pharmacological profiles and effects in human.

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