Full-length review

An update on GABA$_A$ receptors

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Abstract

Recent advances in molecular biology and complementary information derived from neuropharmacology, biochemistry and behavior have dramatically increased our understanding of various aspects of GABA$_A$ receptors. These studies have revealed that the GABA$_A$ receptor is derived from various subunits such as $\alpha_1$-$\alpha_6$, $\beta_1$-$\beta_3$, $\gamma_1$-$\gamma_6$, $\delta$, $e$, $\pi$, and $\rho_1$-$2$. Furthermore, two additional subunits ($\beta_3$, $\gamma_5$) of GABA$_A$ receptors in chick brain, and five isoforms of the $\rho$-subunit in the retina of white perch (Roccus americana) have been identified. Various techniques such as mutation, gene knockout and inhibition of GABA$_A$ receptor subunits by antisense oligodeoxynucleotides have been used to establish the physiological/pharmacological significance of the GABA$_A$ receptor subunits and their native receptor assemblies in vivo. Radioligand binding to the immunoprecipitated receptors, co-localization studies using immunoaffinity chromatography and immunocytochemistry techniques have been utilized to establish the composition and pharmacology of native GABA$_A$ receptor assemblies. Partial agonists of GABA$_A$ receptors are being developed as anxiolytics which have fewer and less severe side effects as compared to conventional benzodiazepines because of their lower efficacy and better selectivity for the GABA$_A$ receptor subtypes. The subunit requirement of various drugs such as anxiolytics, anticonvulsants, general anesthetics, barbiturates, ethanol and neurosteroids, which are known to elicit at least some of their pharmacological effects via the GABA$_A$ receptors, have been investigated during the last few years so as to understand their exact mechanism of action. Furthermore, the molecular determinants of clinically important drug-targets have been investigated. These aspects of GABA$_A$ receptors have been discussed in detail in this review article.

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Keywords: GABA$_A$ receptors; Anxiolytics; Anticonvulsants; General anesthetics; Ethanol; Neurosteroids

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1. Introduction

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the vertebrate central nervous system. GABA activates three different receptor classes such as GABA_A, GABA_B, and GABA_C receptors. GABA_A receptors are ligand-gated chloride ion channels [21,286]. These receptors are activated by GABA, muscimol and isoguvacine, and are inhibited by bicuculline, gabazine (SR 95531) and (+)-β-hydrazine [345]. GABA_B receptors are activated by GABA, (−)-baclofen, (±)-4-amino-3-(5-chloro-2-thienyl)butanoic acid and 3-aminopropyl-trans-4-aminocrotonic acid (TPMPA) [260] but are insensitive to bicuculline, phaclofen, saclofen and 2-hydroxysaclofen [275].

These receptors are known to be coupled to Ca^2+ or K^+ channels via G proteins so as to activate the second messenger systems within the cell [21,24]. GABA_C receptors are derived from various isoforms of the ρ-subunit, and are directly associated with chloride ion channels. These receptors are activated by GABA and certain conformationally restricted analogues of GABA such as cis-4-amino-2-thiename acid (CACA) and trans-4-amino-2-thiename acid (TACA), and are inhibited by imidazole-4-acetic acid [22] and [(1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid] (TPMPA) [260] but are insensitive to bicuculline, barbiturates, benzodiazepines and baclofen [83,253,353].

Recently, it has been proposed that GABA_A receptors should be classified as a specialized set of the GABA_A receptors [10]. The GABA_A receptors are of great importance as they play a pivotal role in the regulation of brain excitability, and many important drugs such as benzodiazepines, barbiturates, neurosteroids, ethanol, and some of the anticonvulsants and general anesthetics interact with these receptors so as to elicit their pharmacological effects.

2. GABA_A receptor subunits

GABA_A receptor is a transmembrane hetero-oligomeric protein which is expressed in the peripheral and central nervous system. The deduced amino acid sequences of GABA_A receptor subunits show significant sequence identity (20% to 30%) with other ligand-gated ion channels such as the nicotinic acetylcholine receptor, glycine receptor and 5-HT_3 receptor. Several subunit classes and isoforms within each class of the GABA_A receptor have been cloned in the mammalian brain. Amino acid identity among isoforms of the same class is ~70% whereas the identity among classes is ~30%. Various subunits of the GABA_A receptors are α_1−α_6, β_1−β_3, γ_1−γ_3, δ, ε, π, and ρ_1−ρ_3 [34,55−57,61,121,177,181,230,232,280,365]. Receptor assemblies derived from various isoforms of the ρ-subunit are suggested to be classified as GABA_C receptors [230,280] as these receptors are insensitive to both bicuculline and baclofen. However, it has been proposed recently that the ρ-subunit containing receptors are best classified as a specialized set of the GABA_A receptors [10] since ρ-subunits are structurally part of the family of GABA_A receptor subunits despite of their distinctive regulatory binding sites [230,280]. Furthermore, two additional subunits (β_2, γ_2) of GABA_A receptors in chick brain [12,119], and five isoforms of the ρ-subunit in the retina of white perch (Roccus americana) [254] have been identified. There are also reports that some of the subunits exist as splice variants [12,118,156,158,346]. The γ_2-subunit is expressed as γ_2S (short form) and γ_2L (long form) variants. The γ_2L variant contains an eight amino acid insert between TM3 and TM4 which provides a potential phosphorylation site [156,346]. Two splice variants of the α_c-subunit have also been reported [158]. However, the short form of α_c-subunit does not form functional receptors [158]. Other examples of subunit splice variants are chicken β_2 and β_4 genes [12,118], and human β_3 gene [154]. Distribution of major subunits in various regions of brain has been investigated [354]. Briefly, the α_1-subunit is present predominantly in the cerebellum, whereas the hippocampus has low levels. In contrast, the α_3-subunit are present predominantly in the hippocampus, whereas cerebellum is practically devoid of these subunits. Cerebral cortex has intermediate levels of α_2−α_4-subunits and low levels of α_c-subunit. The α_c-subunit is present in the cerebellar granule cells, whereas hippocampus and cerebral cortex are devoid of this subunit. The relative distribution of β_1- and β_3-subunits is as follow: hippocampus > cortex > cerebellum, whereas the rank for the β_2-subunit levels is cerebellum > cortex > hippocampus [171,354]. Interestingly, the γ_1-subunit mRNA is expressed predominantly in amygdala and septum, in contrast to most of the GABA_A receptor subunits which are mainly expressed in the cerebral cortex and cerebellum, and shows little change with postnatal development [343,354,359]. The γ_2-subunit is present in abundant amount in almost every region of the brain, whereas the γ_3-subunit is present mainly in the cortex and basal nuclei [354]. There is also report that the levels of γ_2S-subunit are more abundant than the γ_2L-subunit levels in the hippocampus, cerebral cortex and olfactory bulb [103]. In contrast, the inferior colliculus, medulla...
and cerebellum have more $\gamma_{2L}$- vs. $\gamma_{2S}$-subunits [103]. The $\delta$-subunit is present predominantly in cerebellum, whereas hippocampus and cortex have low levels of this subunit [354]. Thalamus contains intermediate levels of $\delta$-subunit, and high levels of $\alpha_3$-subunit [354]. The $\epsilon$-subunit mRNA is present in amygdala, thalamus and subthalamic nucleus [57]. On the other hand, the $\pi$-subunit has been reported to be present in lung, thymus, prostate and uterus [121]. Although, various isoforms of the $\rho$-subunit are known to be localized in the retina [55,56,73,195,230,254], it has been shown recently that the $\rho_{1-3}$-subunits are also expressed in the rat brain regions such as superior colliculus, cerebellar Purkinje cells and hippocampus [23]. Immunoblot studies using antibodies to various subunits of GABA$_A$ receptors have revealed the molecular mass of these subunits as follows: $\alpha_1 = 50–51$ kDa, $\alpha_2 = 52–53$ kDa, $\alpha_3 = 58–61$ kDa, $\alpha_4 = 66–67$ kDa, $\alpha_5 = 53–55$ kDa, $\alpha_6 = 57–58$ kDa, $\beta_1 = 57$ kDa, $\beta_2 = 54–57$ kDa, $\beta_3 = 57$ kDa, $\gamma_1 = 45–51$ kDa, $\gamma_2 = 43–49$ kDa, $\gamma_3 = 45$ kDa, $\gamma_4 = 47$ kDa, $\gamma_5 = 43–46$ kDa, and $\delta = 54$ kDa [16–19, 61, 69, 72, 149, 150, 152, 153, 171, 178, 194, 195, 207, 257, 259, 298, 305]. The polyclonal anti-$\alpha_1$ antibody, raised by us in a rabbit against the synthetic peptide corresponding to the amino acids 1–15 of the rat $\alpha_1$-subunit of GABA$_A$ receptors, recognized a protein band of 51 kDa in immunoblots, whereas the polyclonal anti-$\beta_2$ antibody, raised by us in a rabbit against the synthetic peptide AGPLPRHSGRNA [171] corresponding to the amino acid residues 382–393 of the rat $\beta_2$-subunit of GABA$_A$ receptors, recognized a protein band of 55 kDa in immunoblots.

3. Subunit composition of native GABA$_A$ receptors and their binding characteristics

GABA$_A$ receptor is a pentameric assembly derived from a combination of various subunits. Elucidation of these assemblies of native GABA$_A$ receptors is a very challenging task since several different types of receptor assemblies can be derived theoretically from a combination of various subunits. There are reports indicating that the $\alpha_1$, $\beta_2$, and $\gamma_2$-subunits co-exist in many native GABA$_A$ receptors [16,149,298]. Furthermore, it is known that two different isoforms of the $\alpha_1$- [5,8,19,69,72, 151,152,160,176,207,242,243,330], $\beta_1$ [171], or $\gamma$-subunit [17,150,151,257] can co-localize in the same receptor assembly. However, the preferred pentameric combination includes two $\alpha$, two $\gamma$, and one $\beta$-subunits [8], or two $\alpha$, two $\beta$, and one $\gamma$-subunits [39]. Recently, it has been suggested that a total of four alternating $\alpha$ and $\beta$-subunits are connected by a $\gamma$-subunit in a pentameric GABA$_A$ receptor assembly [319]. The preferred stoichiometry of GABA$_A$ receptor subtype probably varies among different brain regions. In an attempt to elucidate the complete pentameric composition of a brain receptor, the cerebellar receptor assemblies have been proposed as $\alpha_1\alpha_6\gamma_{2S}$-$\gamma_{2L}$/$\beta_{2/3}$/$\alpha_1\alpha_6\beta_2/3\gamma_2$/ $\alpha_1\beta_2/3\gamma_2$/ $\alpha_1\alpha_6\beta_2/3\gamma_2$.$\delta$, $\alpha_1\beta_2/3\gamma_2$ and $\alpha_1\beta_2/3\gamma_2$.$\delta$ [36,37,151,152,159,160,207,243,258]. However, recent studies have demonstrated that $\delta$-subunit does not co-localize with $\gamma$-subunit in native GABA$_A$ receptor assemblies [4,259]. The diazepam-insensitive benzodiazepine-site in the human cortex has been reported to be composed of the $\alpha_1$, $\beta_2$, and $\gamma_{2L}$-subunits [357].

Although co-expression of the $\alpha$, $\beta$- and $\gamma$-subunits is required for the formation of fully functional GABA$_A$ receptors and most of the native GABA$_A$ receptor assemblies consist of these subunits yet the co-localization of these three types of subunits is not an absolute requirement for the formation of the GABA$_A$ receptor. It is known that the mice devoid of the $\gamma_2$-subunit were able to express functional GABA$_A$ receptors, though devoid of the benzodiazepine binding site, in unaltered numbers from the remaining subunits [101]. In contrast, when the $\alpha_5$ [88], $\alpha_2$ [127,140], or $\beta_2$-subunit [126] gene was inactivated, some of the brain GABA$_A$ receptors were lost. These observations suggest that the $\alpha$- and $\beta$-subunits, unlike the $\gamma_2$-subunit, are essential components for the receptor assembly and membrane targeting of the GABA$_A$ receptors. Furthermore, it has been suggested that each receptor subtype has its own target identity depending on the subunits, and highly selective recognition processes operate to ensure differential assembly and subcellular distribution of the receptor subtypes within a cell [88–90,216,228].

During the last few years, localization of various binding sites on the GABA$_A$ receptors has been investigated. These studies have indicated that the high affinity GABA/muscimol binding site is localized at the interface of the $\alpha$- and $\beta$-subunits of GABA$_A$ receptors [246,364]. The $\beta_2$-subunit of GABA$_A$ receptors has been suggested as a necessary requirement for the TBPS binding site (picrotoxin site) [288,364]. Although the homo-oligomeric GABA$_A$ receptors derived from $\beta_2$-subunit exhibit a specific high affinity binding for $[^{35}]$SBPS, the GABA$_A$ receptor assemblies consisting of $\alpha_1\beta_2\gamma_2$- or $\alpha_1\beta_3$-subunits have higher affinity for $[^{35}]$TBPS over those consisting of $\beta_3\gamma_2$- or $\beta_3$-subunits [288,364]. Benzodiazepine (BZ) site is localized at the interface of the $\alpha$- and $\gamma$-subunits of GABA$_A$ receptors [355,364]. Therefore, the GABA$_A$ receptor assemblies containing two isoforms of the $\alpha$- as well as $\gamma$-subunit would have two benzodiazepine sites in the same receptor assembly [152]. Receptors containing the $\alpha_1$-subunit mimic BZ I pharmacology, whereas those containing the $\alpha_2$, $\alpha_3$, or $\alpha_4$-subunit variant display BZ II pharmacology [16,189,194,207, 247,363]. BZ II receptors have lower affinity for CL 218872, $\beta$-CCM and zolpidem as compared to BZ I receptors [16,189,194,207,247,363]. However, receptors containing the $\alpha_5$-subunit variant lack affinity for zolpidem and its analogues [109,194,197,207,247] but have high affinity for Ro 15-4513 (sarmazenil) [109,197], a partial inverse agonist of the benzodiazepine site. BZ I receptors,
The GABA_A receptor assemblies containing α_1- and γ-subunits, are present predominantly in the cerebellum but are scarce in the hippocampus, whereas BZ II receptors are expressed in abundance in the hippocampus and almost absent in the cerebellum [80,81,197,231]. However, both receptor types, i.e., BZ I and BZ II receptors are equally expressed in the cerebral cortex [81,231]. It is virtually lacked sensitivity to zolpidem [17]. The BZ-I and BZ-II receptor types, i.e., BZ-I and BZ-II receptors are equally absent in the cerebellum [80,81,197,231]. However, both the α_5- and α_6-subunits are expressed in the cerebral cortex [81,231]. A specific ligand, [3H]flumazenil, binds with high affinity to the [3H]benzodiazepine receptor partial agonists have high affinity for these diazepam-insensitive receptors [152,153,178,258,313]. Moreover, the α_4β_2γ_2L vs. α_6β_2γ_2L GABA_A receptors [357], [3H]Muscimol binds with high affinity to the α_6 (153,352) as well as the α_5-subunit [152] containing GABA_A receptors. The α_6-subunit containing receptor assemblies are localized in cerebellar granule cells [178,258,305], whereas those containing the α_5-subunit variant are localized in various brain regions such as cerebral cortex, hippocampus, thalamus/hypothalamus, striatum and olfactory bulb [19,153,352], and retina [195]. Radioligand binding to the immunoprecipitated receptors using polyclonal antibodies to various γ-subunits has revealed that the γ_1- or γ_2-subunit containing receptor assemblies are of low abundance [17]. Furthermore, the γ_2-receptor population displayed a reduced affinity for flumazenil and flunitrazepam, and virtually lacked sensitivity to zolpidem [17]. The γ_1-receptor assembly displayed low affinity for most of the benzodiazepine site ligands except flunitrazepam, and can be differentiated from those containing the γ_2- or γ_3-subunit by its low affinity for the inverse agonist β-CCM and its lack of affinity for the partial inverse agonist Ro 15-4513 (sarmazenil) as well as for the Ro 15-1788 (flumazenil), a competitive antagonist of the benzodiazepine site [17]. It is thus evident that the γ_1-subunit receptor assemblies are not involved in mediating all major effects of benzodiazepines in vivo since these effects are susceptible to blockade by flumazenil. Replacement of the γ-subunit by a δ-subunit in the expression studies produces a GABA_A receptor which is not potentiated by benzodiazepines [269,270,281]. Polyclonal antisera raised against the δ-subunit has been reported to immunoprecipitate the [3H]Ro 15-1788 binding sites [18], thereby suggesting that the GABA_A receptors containing δ-subunit have a benzodiazepine binding site which has a novel pharmacology [207]. Although the δ-subunit is reported to co-localize with α_1-, α_2-, α_3-, α_5- and β_3/δ-subunits in various receptor assemblies [140,207], it does not co-localize with the γ-subunit in native GABA_A receptors according to recent studies [4,259]. These results make it difficult to explain the discrepancy between the expression study data and the immunoprecipitation results. However, recent studies have indicated that the polyclonal antisera raised against δ-subunit immunoprecipitates only the [3H]muscimol binding sites, whereas the [3H]Ro 15-1788 or [3H]flunitrazepam binding sites are not immunoprecipitated by this antibody in the rat brain [4,259] in contradiction to the findings of Benke et al. [18]. Transfection of cells with a combination of α-, β- and γ-subunits results in the expression of GABA-activated currents and [35S]TBPS binding sites [57]. However, these transfected cell membranes did not bind either [3H]flunitrazepam [57]. Transfection of cells with a combination of α, β, and γ-subunits results in the expression of both [3H]muscimol and [35S]TBPS binding sites but no [3H]Ro 15-1788 or [3H]flunitrazepam binding sites were detected [121]. When the δ-subunit was expressed with α/β/γ-subunit combination in transfection system, there was a reduction in the density of [3H]Ro 15-1788 binding sites [121]. In contrast to earlier view regarding the homomeric nature of the p-subunit receptor assemblies, it has been shown recently that the rat retinal receptor assemblies derived from these subunits are heteromeric and composed of at least p_1- and p_2-subunits [365], and both the p_1- and p_2-subunits are necessary to form a functional receptor assembly [23].

Radioligand binding to the immunoprecipitated receptors using subunit-specific antibodies has been used as a tool to establish and understand the composition and pharmacology of native GABA_A receptor assemblies. These studies have been conducted primarily in cerebral cortex, cerebellum and whole brain using ligands of the GABA binding site ([3H]muscimol and the benzodiazepine site ([3H]flunitrazepam, [3H]Ro 15-1788 and [3H]Ro 15-4513]. The reported percentage of the binding sites immunoprecipitated by antisera to various subunits of GABA_A receptors in the adult rat brain regions are as follows: α_1 = 70–97%, α_2 = 4–28%, α_3 = 12–24%, α_4 = 0–15%, α_5 = 4–14%, α_6 = 30–39% (cerebellum), β_1 = 2–32%, β_2 = 55–96%, β_3 = 19–52%, γ_1 = 0–19%, γ_2 = 50–94%, γ_3 = 31–32%, γ_4 = 37–65%, γ_5 = 0–18%, and δ = 0–23% [16,19,61,151–153,171,178,189,194,207,257,258,283,347]. We have recently observed that the polyclonal anti-α_1 antibody, raised by us in a rabbit against the synthetic peptide corresponding to the amino acids 1–15 of the rat α_1-subunit of GABA_A receptors, elicits higher percentage immunoprecipitation values of the [3H]flunitrazepam (97% vs. 79%) and [3H]muscimol (76% vs. 68%) binding activity in the adult rat cerebellum vs. cerebral cortex, whereas the immunoprecipitation values for [3H]Ro 15-4513 were comparable (≈ 80%) in both the brain regions [Mehta and Ticku, unpublished observations]. Furthermore, the polyclonal anti-β antibody, raised by us in a rabbit against the synthetic peptide AGPRHSGR (171) corresponding to the amino acid residues 382–393 of the rat β_2-subunit.
of GABA
receptors, elicited higher percentage immunoprecipitation values of the \(^{1}H\)flunitrazepam as well as \(^{1}H\)muscimol binding activity in the adult rat cerebellum vs. cerebral cortex [Mehta and Ticku, unpublished observations]. Aging process is reported to affect the expression of GABA
receptor subunits (mRNA and protein) in various regions of the rat brain [102,104—106,209,211] as well as radioligand binding to the GABA
receptors [75,211,264,265]. However, age-related changes in the mRNA expression of a particular subunit does not necessarily lead to similar changes in protein or assembly into mature GABA
receptors, and it has been proposed that complex regulatory mechanisms of GABA
receptor expression exist at the transcriptional, translational and post-translational and/or assembly levels, which vary with the subunit and brain area [105]. Recently, insulin has been reported to increase the number of functional postsynaptic GABA
receptor assemblies [340], whereas chronic GABA exposure results in the down-regulation of GABA
receptors [108,186,204,263].

4. Molecular determinants of GABA
receptors

In an attempt to characterize the molecular determinants of clinically important drug-targets, several amino acids have been identified that are important for binding of ligands to the GABA and benzodiazepine site. It has been reported that Tyr
, Thr
, and Tyr
 amino acid residues of the \(\beta\)-subunit [3] and Phe
 of the \(\alpha\)-subunit [284,291] are important for the binding of GABA. His
 (or His
 in human) [70,349] and Gly
 (or Gly
 in human) [248] amino acid residues of the \(\alpha\)-subunit, and Phe
, Met
 and Thr
 amino acid residues of the \(\gamma\)-subunit [31,33,214,350] are reported to be key determinants of the benzodiazepine site of the rat GABA
receptors. Moreover, His
 amino acid residue of the \(\alpha\)-subunit interacts directly with the pendant phenyl group of diazepam, flunitrazepam, chlordiazepoxide and other 5-phenyl benzodiazepines [193,366]. This His residue is absent in the \(\alpha\)-subunit, and it is replaced by Arg (Arg
) in the \(\alpha\)-subunit of the human/rat GABA
receptors. These differences in the \(\alpha\)- and \(\alpha\)-subunits make the GABA
receptor assemblies, derived from the \(\alpha\)- or \(\alpha\)-subunit, insensitive to conventional benzodiazepines such as diazepam, flunitrazepam and clonazepam [152, 153,178,198,258,313]. However, the replacement of Arg
 residue by Gln
 in the \(\alpha\)-subunit of alcohol-sensitive (alcohol-nontolerant) ANT rat cerebellum alters the normal diazepam-insensitive GABA
receptors into diazepam-sensitive ones [157]. A single amino acid change, from a glutamate to a glycine at position 225 in the \(\alpha\)-subunit sequence, substantially increases the affinity of the \(\alpha\)-subunit containing GABA
receptors for CL218872 and zolpidem [248]. Amino acid residues at positions 159 (Tyr
), 161 (Tyr
), 162 (Thr
), 206 (Thr
), 209 (Tyr
) and 211 (Val
) on the \(\alpha\)-subunit of GABA
receptors influence affinities of the ligands for the benzodiazepine site, thereby suggesting that these amino acids also form part of the benzodiazepine binding site [2,30,32,272,348]. However, mutations of \(\alpha\)-Gln
, the amino acid residue located at equal distance to \(\alpha\)-Gly
 and \(\alpha\)-Thr
, did not affect the ligand binding, thereby indicating that this amino acid does not interact with benzodiazepines and related compounds [272]. It is thus evident from these studies that three separate domains of the \(\alpha\)-subunit such as near His
, Tyr
 and Gly
—Val
 are involved in the benzodiazepine binding. Similarly, two domains of the \(\gamma\)-subunit, Lys
—Trp
 and Arg
—Asp
, have been identified which are required for the high affinity benzodiazepine binding [20]. Although it has been speculated that the co-localization of two isoforms of \(\alpha\)-subunit and one \(\gamma\)-subunit can form two benzodiazepine sites in the same receptor-assembly provided both the \(\alpha\)-subunits and one \(\gamma\)-subunit contribute key amino acids to the benzodiazepine site as described above.

Ser
 as well as Ala
 amino acid residues of the \(\alpha\)-subunit, and Ser
 as well as Met
 of the \(\beta\)-subunit of GABA
receptor are reported to be important amino acids for the action of clinically relevant concentrations of the inhalational anesthetic isoflurane [162]. Met
 amino acid residue of the \(\beta\)-subunit is important for the potentiation of the GABA response by the intravenous anesthetic agent propofol but this amino acid is not involved in the activation of the receptor by the high concentrations of propofol [162]. Furthermore, the extracellular amino-terminal half of the \(\alpha\)-subunit plays an important role in the propofol potentiation of pentobarbital-response [328]. The extracellular N-terminal domain of the \(\alpha\)-subunit [86], His
 amino acid residue of the \(\alpha\)-subunit [86], His
 residue of the \(\beta\)-subunit [130], and His
 amino acid of the \(\beta\)-subunit [356] are important molecular determinants of the Zn
 binding site on the GABA
 receptors. Furthermore, it has been suggested that the Cys—Cys loop of the large extracellular NH
-terminal domain of the GABA
 receptor \(\rho\)-subunit plays an important role in the receptor/channel functions [175].

Several amino acids such as \(\beta\)-Ser
, \(\gamma\)-Ser
 and \(\gamma\)-Ser
 have been identified as potential target sites on the GABA
receptors for the phosphorylation process [164,182,223] in an on-going attempt to investigate the role of the phosphorylation process, mediated through PKA, PKC and other kinases such as tyrosine kinase, on the modulation of GABA
 receptor functions [107,116, 118,148,164,169,180,182,223,224,296,312,339,344]. These studies have also revealed that all the major subunits of GABA
 receptors contain consensus sites for the phosphorylation process, and this process may be important in a wide range of physiological and pathological processes.
in the central nervous system. Recently, it has been demonstrated that the $\beta_{2/3}$ subunit(s) of native GABA$_A$ receptors are phosphorylated in situ, thereby providing a direct evidence that the native GABA$_A$ receptors are indeed phosphorylated and modulated in situ by endogenous kinases [339]. However, the detailed mechanism of phosphorylation and its physiological relevance are not precisely known currently.

5. Physiological/pharmacological role of various GABA$_A$ receptor subunits and their receptor assemblies

GABA$_A$ receptors play a very important role in anxiety. Currently, drugs which activate the benzodiazepine site of GABA$_A$ receptors are widely used to relieve anxiety. However, these classical anxiolytics are full agonists at most of the GABA$_A$ receptor subtypes, and have several side effects such as sedation, ataxia, amnesia, tolerance, and physical dependence. During the last decade, attempts have been made to dissociate their useful therapeutic effects from side effects by developing partial agonists of the GABA$_A$ receptor subtypes. Unfortunately, the physiological/pharmacological role of various native GABA$_A$ receptor assemblies is not yet known. Once this information becomes available, it will be much easier to synthesize compounds selective for a particular receptor assembly so as to get a desired therapeutic effect without any major side effect. Several approaches such as mutation, gene knockout and the inhibition of expression of GABA$_A$ receptor subunits by antisense oligodeoxynucleotides have been tried to establish the role of various subunits and their receptor assemblies with a limited success so far as described below and summarized in Table 1.

It has been suggested that motor control is a distinct behavioral correlate of the $\alpha_4$-subunit containing GABA$_A$ receptors because the point mutation in the cerebellar $\alpha_4$-subunit containing GABA$_A$ receptors (replacement of $\text{Arg}^{100}$ by $\text{Gln}^{100}$) results in ethanol intolerance (enhanced sensitivity to ethanol) in ANT rats (alcohol-nontolerant rats), thereby making the animals highly susceptible to the impairment of postural reflexes by ethanol and benzodiazepines [157]. However, there were no differences in the metabolism of ethanol [129], its hypnotic effect [127], withdrawal-hyperexcitability [129], or reversal of ethanol’s effects by Ro 15-4513 [127] in the $\alpha_4$-deficient mice vs. wild-type mice. Furthermore, loss of righting reflex (inability to recover normal posture) as well as the immobilizing response to tail-clamp-stimulus in mice anesthetized with volatile anesthetics, and sleep-time-response to pentobarbital did not differ between the $\alpha_4$-deficient and wild-type mice [127]. Interestingly, in the $\alpha_4$-deficient cerebellar granule cells, the $\delta$-subunit is selectively degraded as seen by immunoprecipitation, immunocytochemistry and immunoblot analysis with $\delta$-subunit specific antibody, thereby providing an evidence for a specific association between the $\alpha_4$ and $\delta$-subunits in cerebellar granule cells [140]. Cerebellar granule cells of the $\alpha_4$-subunit deficient mutant mice also have severe deficits in the high affinity $[^3]H$-muscimol/$[^3]H$Ro 15-4513 binding to the GABA sites, and furosemide-induced increases in the $[^3]S$-butylbicyclic phosphorothionate (TBPS) binding to the picrotoxin-sensitive convulsant sites [185]. DMCM, allopregnanolone and zinc are less efficacious in these mice in altering the $[^3]S$TBPS binding in the presence of those concentrations of GABA that fail to activate the $\alpha_4$-subunit-containing GABA$_A$ receptors [185]. Furthermore, the inhibition of $\alpha_4$-subunit by antisense oligodeoxynucleotide (ODN) in cerebellar granule cells shifts the GABA dose–response curve to the right and significantly increases the EC$_{50}$ value of GABA (a decreased GABA-response), whereas the flunitrazepam-induced potentiation of GABA-activated currents are enhanced [369]. Some of the above mentioned changes may be adaptive consequences of altered GABA$_A$ receptor subunit expression pattern in response to the gene knockout. On the other hand, the $\gamma_2$-subunit antisense ODN reduces the EC$_{50}$ value and shifts the dose–response curve for GABA to the left (an enhanced GABA-response), and decreases the flunitrazepam-induced potentiation of GABA-activated currents [369]. Furthermore, the bolus infusion of antisense ODN to the GABA$_A$ receptor $\gamma_2$-subunit is reported to inhibit the in vivo formation of the benzodiazepine binding sites in the rat cerebral cortex and striatum [146], and in vivo formation of the GABA, picrotoxin and benzodiazepine binding sites are also inhibited in the hippocampus [147], thereby resulting in an increase in the convulsive threshold for $\beta$-CCM [367], an inverse agonist for the benzodiazepine site. Targeted disruption of the $\gamma_2$-subunit gene in mice [101] resulted in the depletion of 94% of the benzodiazepine sites in the brain of neonatal mice, while the number of GABA sites was only slightly reduced. Diazepam was inactive behaviorally in these mice. Furthermore, these mice died within a few days after birth, although their birth weight was normal and the major
peripheral organs, including pituitary, pancreas and adrenal gland which are known to express GABA$_{\alpha}$ receptors, did not reveal any pathological changes. The surviving mutants exhibited excessive hyperactivity, impaired grasping, impaired righting reflexes and abnormal gait. None of these mutant mice survived beyond P18, although feeding was apparently not impaired [101]. Recently, mice devoid of the $\gamma_{2L}$-subunit have been produced [128]. These mice are viable, healthy, and overtly indistinguishable from the wild-type. However, there was up-regulation of the $\gamma_{2S}$-subunit as a result of compensatory mechanism in these mutant mice devoid of the $\gamma_{2L}$-subunit [128]. Potentiation of the GABA current by ethanol as well as several behavioral effects of ethanol such as sleep time, anxiolysis, acute function tolerance and chronic withdrawal hyperexcitability were not altered in these mutant mice, thereby suggesting that the $\gamma_{2L}$-subunit is not required for the modulatory effects of ethanol at the GABA$_{\alpha}$ receptors [128]. These results contradict the previous view that the $\gamma_{2S}$-subunit of GABA$_{\alpha}$ receptor is a critical molecular site of action for ethanol [335] but support the reports indicating that the presence of $\gamma_{2L}$-subunit is not an absolute requirement for the potentiation of GABA-induced responses by ethanol [190,285]. Mice deficient in the $\beta_{3}$-subunit of GABA$_{\alpha}$ receptor usually die as neonates [126]. The mice that survive are runted until weaning, and have a reduced life span. These mutant mice are fertile but mothers fail to nurse offspring [126]. Brain morphology is grossly normal but these mice are hyperactive, hyperresponsive to human contact and often run continuously in tight circles [126]. When held by tail, these mutant mice hold all the paws in like a ball. They also exhibit tremor, jerky gait, cleft palate [53,54,126] and Angelman syndrome [179,225,262,267], an inherited neurobehavioral disorder that is characterized in humans by seizures, ataxia, stiff jerky movements, absence of speech and severe mental retardation. Angelman syndrome in the $\beta_{3}$-deficient mice [179,225,262,267] is consistent with the report that the gene encoding the GABA$_{\alpha}$ receptor $\beta_{3}$-subunit maps to the Angelman syndrome region on chromosome 15, and deletion of this gene (GABRB3) is found in the Angelman syndrome patients [337]. Furthermore, based on gene knockout study, it has been reported that the $\beta_{3}$-subunit is important in the mediation of the tonic suppression response due to the volatile anesthetic agents such as enflurane and halothane [255], whereas the loss of righting reflex due to the volatile anesthetic agents and pentobarbital is not affected by the absence of $\beta_{3}$-subunit [255]. However, the loss of righting reflex due to midazolam and etomidate is attenuated by the absence of the $\beta_{3}$-subunit [255]. Administration of the antisense oligodeoxynucleotide to the GABA$_{\alpha}$ receptor $\alpha_{5}$- or $\beta_{3}$-subunit into striatum results in the increased behavioral sensitivity to cocaine in rats, thereby suggesting the role of these subunits in determining cocaine-sensitivity [236]. Although it was thought that the deletion of $\alpha_{5}$- and $\gamma_{3}$-subunits may result in neurological phenotype [225], this possibility was ruled out based on the observation that mutation of these subunits does not result in any overt neurological phenotype in mice and thus does not provide useful animal models for the Angelman syndrome in humans [54,225]. The above-mentioned studies suggest that the antisense oligodeoxynucleotide treatment and gene-knockout techniques are useful tools for studying the regulation of receptor structure/function and revealing the physiological and pharmacological significance of various GABA$_{\alpha}$ receptor subunits and their receptor assemblies.

In an attempt to investigate the role of $\alpha_{5}$-subunit in seizures, it has been shown that $\alpha_{5}$-subunit mRNA is increased in the dentate gyrus at 4 h after the fifth amygdaloid kindled seizure [44]. However, another group of investigators did not find any change in the $\alpha_{5}$-subunit mRNA levels at 24 h after the sixth fully kindled seizure elicited by Schaffer collateral stimulation [144]. Role of the GABA$_{\alpha}$ receptor $\alpha_{3}$-subunit in kainic acid-induced seizures in rats has also been investigated by several researchers because these seizures in rats represent an animal model for human temporal lobe epilepsy. The neuropathological events underlyng these seizures include acute limbic seizures (status epilepticus) followed by neurodegeneration in the hippocampus, thereby ultimately manifesting the spontaneous recurrent seizures after about 3 weeks. Interestingly, recurrent spontaneous seizures following the administration of kainic acid result in the up-regulation of $\alpha_{3}$-subunit mRNA [322]. Furthermore, the $\alpha_{4}$-subunit immunoreactivity is also reported to be increased in the rat hippocampus at 30 days after the administration of kainic acid [274]. Electroconvulsive shock (ECS) treatment has been shown recently to increase the $\alpha_{4}$-subunit mRNA and the diazepam-insensitive $[^{3}H]$Ro 15-4513 binding in the dentate gyrus, thereby suggesting that the $\alpha_{4}$-subunit of GABA$_{\alpha}$ receptor could be implicated in the clinical effects of electroconvulsive therapy [43]. Furthermore, the inhibition of $\alpha_{4}$-subunit by the antisense oligodeoxynucleotide is reported to prevent the increased seizure susceptibility observed following progesterone withdrawal [292]. Therefore, it has been speculated that the $\alpha_{4}$-subunit of GABA$_{\alpha}$ receptor plays an important role during periods of fluctuations in the levels of endogenous neuroactive steroids associated with the menstrual and pregnancy cycles in females and during stress in males as well as females [292,293]. A long-lasting kindling phenomenon associated with chronic intermittent ethanol (CIE) treatment is also reported to result in an increase in the $\alpha_{4}$-subunit mRNA levels in the thalamus, dentate gyrus, CA1 and CA3 regions of hippocampus, and layers II and III of the cortex, thereby further supporting the role of $\alpha_{4}$-subunit of GABA$_{\alpha}$ receptors in seizures [183]. Although the genetic studies have revealed that the differences between the highly prone DBA/2J mice to juvenile audiogenic seizures and the seizure-resistant C57BL/6J mice are due to several genetic factors [226] yet these two
strains of mice do not differ in the $\alpha_{1-3}$ [342], $\beta_{1-3}$ [143], $\delta$ [341], $\gamma_{1}$ [343] and $\gamma_{2}$-subunits [156]. However, the comparison of $\alpha_{1}$-subunit of GABA$_A$ receptors has not been investigated in these mice.

6. Interaction of drugs with GABA$_A$ receptors

Several drugs such as benzodiazepines, barbiturates, neurosteroids, ethanol, some of the anticonvulsants, and general anesthetics interact with GABA$_A$ receptors so as to elicit their pharmacological effects. The role of GABA$_A$ receptors in the action of these categories of drugs has been reviewed from time to time by us and other researchers [282,308–310,313–316,347]. In this section, we would summarize the recent developments in this area.

6.1. Anxiolytics

In an attempt to dissociate the desired therapeutic effect of anxiolytics acting at the benzodiazepine site of GABA$_A$ receptors from their side effects, several new compounds such as imidazenido [7,60,98,100,277,306], abecarnil [155, 196,198,233,234,276,278,297,325], bretazenil [27,84,111,155,191,198,219,245,249], and divaplon [82,93,137,155] have been synthesized and evaluated for their pharmacological effects. Some of these compounds are partial agonists at some GABA$_A$ receptor subtypes and full agonists at others as is the case with abecarnil which is a partial agonist at the $\alpha_{1}\beta_{2}\gamma_{2}$ GABA$_A$ receptor assembly and a full agonist at $\alpha_{1}\beta_{2}\gamma_{1}$ GABA$_A$ receptor assembly [155]. On the other hand, divaplon is a partial agonist at both $\alpha_{1}\beta_{2}\gamma_{3}$ and $\alpha_{1}\beta_{2}\gamma_{2}$ GABA$_A$ receptor assemblies [155], and imidazenido has low efficacy at a broad spectrum of GABA$_A$ receptor subtypes [100]. As a result of these features, these compounds are devoid of many, if not all, side effects associated with conventional anxiolytic benzo- diazepines because the lower intrinsic efficacy of the GABA$_A$ receptor partial agonists is probably sufficient to maintain the low efficacy responses such as anxiolytic and anticonvulsant responses, but insufficient to induce sedation, myorelaxation, dependence and ethanol-potentiation, which require high fractional receptor occupancy [78,94,138,238]. Accordingly, abecarnil is a potent anxiolytic and elicits no or only weak effects in tests of motor incoordination and muscle relaxation, and has a relatively weak ability to potentiate the effects of ethanol and barbiturates in contrast to diazepam [297]. The therapeutic safety, defined as the ratio between the anticonvulsant efficacy and the muscle relaxant action, is more favorable for abecarnil as compared to diazepam in rodents and baboon (Papio papio) [325]. Abecarnil also elicits potent anticonflict and taming effects with little sedative and ataxic effects in primates [233]. Chronic administration of abecarnil was found to elicit persistent anxiety-like and anticonvulsant effects without any amnesia in rats [234], and it did not induce tolerance or withdrawal syndromes in mice [278]. Interestingly, imidazenido, a potent anxiolytic, is devoid of sedation, ataxia and ethanol-potentiation, and blocks the sedative and ataxic effects of diazepam in rats [100]. It also completely attenuates the benzodiazepine-induced cognition deficit in monkeys [306]. Furthermore, it causes only low tolerance and dependence liabilities in rats [7]. Chronic treatment with a pharmacologically effective dose of imidazenido failed to induce tolerance to the effects of this drug on GABA$_A$ receptor function in mouse brain [98]. Bretazenil elicits potent anticonflict and anticonvulsant activity but it results in a lower degree of sedation, tolerance [111], dependence liability [27,219], and ethanol-potentiation [191] as compared to conventional benzodiazepines. Likewise, divaplon elicits potent anticonflict activity, and it results in lower sedation [93] and tolerance [82,137] as compared to diazepam. According to a recent report, chronic exposure of primary cultured cerebellar granule cells to the agonists of the benzodiazepine site results in a reduction of the $\alpha_{1}$-subunit protein expression, and the magnitude of response depends upon the efficacy of these agents [139]. Therefore, it has been suggested that this model may serve as an indicator of benzodiazepine agonist efficacy with the ability to differentiate between partial agonists of the benzodiazepine site [139]. Chronic treatment with conventional benzodiazepines, having full agonistic effect, decreases the mRNA levels of the $\alpha_{1}$- and $\gamma_{2}$-subunits [123,145], and increases the levels of the $\alpha_{3}$- and $\alpha_{6}$-subunit mRNA in the rat brain [229]. Furthermore, chronic treatment with the conventional benzodiazepines decreases the efficacy of barbiturates as well as agonists/inverse agonists of the benzodiazepine site [133], uncouples the GABA and barbiturate sites from the benzodiazepine site [132], and decreases coupling between the benzodiazepine site and GABA$_A$ receptor-gated chloride channels [132]. These changes may be responsible for tolerance and dependence following chronic treatment with conventional benzodiazepines.

6.2. Anticonvulsants

Loreclezole, an anticonvulsant, is reported to interact with GABA$_A$ receptors [334]. It does not require the presence of either an $\alpha$- or a $\gamma$-subunit of GABA$_A$ receptor, but is highly selective for GABA$_A$ receptor assemblies containing the $\beta_{2/3}$-subunit versus $\beta_{1}$-subunit [334]. It has a negligible affinity for the benzodiazepine site, and the anticonvulsant effect could be reversed by some inverse agonists of the benzodiazepine site but not by Ro 15-1788 [59]. The interaction of loreclezole with GABA$_A$ receptors has also been demonstrated in recombinant receptor system [85,351]. Tiagabine, another anticonvulsant agent [240,300], elicits its effects as a result of inhibition of the GABA uptake [25], and it has a potential utility in the treatment of chronic seizure disorders such as generalized clonic–tonic epilepsy, photomyoclonic seizures, myoclonic
petit mal epilepsy and complex partial epilepsy [300]. Vigabatrin, i.e., γ-vinyl GABA (GVG), is an inhibitor of GABA aminotransferase, and it crosses the blood–brain barrier (unlike GABA), thereby resulting in a dose-dependent long-lasting enhancement of the GABA concentration in the brain [114,174]. It is an effective treatment for partial complex seizures in pediatric and adult patients with pharmacoresistance to the conventional antiepileptic drugs [38,273]. Gabapentin, a structural analogue of GABA, was synthesized with the idea that it would elicit the physiological actions of GABA in the brain. Although gabapentin, unlike GABA, crosses the blood–brain barrier and elicits anticonvulsant effects [11,62], it does not interact with GABA_A or GABA_B receptors. Moreover, it is not converted metabolically into GABA or agonist of GABA receptor and it is not an inhibitor of GABA uptake or of GABA degradation [333]. However, it has been reported to increase the GABA turnover by activation of glutamate decarboxylase [304]. It has also been reported recently that gabapentin increases the levels of GABA in the brains of epileptic patients [239] and relieves partial epilepsy [38]. Gabapentin increases the levels of GABA in the brains of epileptic patients [239] and relieves partial epilepsy [38]. Gabapentin, unlike GABA, crosses the blood–brain barrier and elicits anticonvulsant effects [11,62], it does not interact with GABA_A or GABA_B receptors. Moreover, it is not converted metabolically into GABA or agonist of GABA receptor and it is not an inhibitor of GABA uptake or of GABA degradation [333]. However, it has been reported to increase the GABA turnover by activation of glutamate decarboxylase [304]. It has also been reported recently that gabapentin increases the levels of GABA in the brains of epileptic patients [239] and relieves partial epilepsy [38].

6.3. General anesthetics

Several general anesthetics of diverse structure such as isoflurane, enflurane, halothane, barbiturates, etomidate, steroid anesthetics and propofol have been reported to interact with GABA_A receptors at clinically relevant concentrations [9,46,112,115,141,172,237,327]. Their exact binding site on GABA_A receptor and subunit requirement are not yet fully established. Mutation of the residues within either α2 (S270 or A291) or β3 (S265 or M286) subunits of GABA_A receptor is reported to cause insensitivity to clinically relevant concentrations of the inhalational anesthetic isoflurane [162]. The β-subunit of GABA_A receptor appears to contribute to the direct actions of propofol (2,6-diisopropylphenol), an intravenous general anesthetic which is used clinically these days [58,268]. A point mutation in the β3-subunit (M286W) abolished potentiation of the GABA response by propofol but it did not alter direct activation of the receptor by high concentrations of propofol [162]. Furthermore, the α- and γ-subunits influence both the direct and modulatory actions of propofol on GABA_A receptor function [167]. Propofol is also reported to potentiate the pentobarbital-induced current, and the α-subunit of GABA_A receptor is necessary for this modulatory action [328]. It seems that the extracellular amino-terminal half of the α-subunit is sufficient to support the propofol potentiation of pentobarbital-response [328]. However, the direct activation of GABA_A receptor by propofol has been suggested to be a distinct process mediated through a distinct site [328]. Interestingly, propofol, unlike conventional benzodiazepines, potentiated the α3β2GABA_A receptor-mediated responses comparable to those of the α3β2GABA_A receptor assembly [336]. However, it failed to elicit a direct activation of the α3β2GABA_A receptors [336].

6.4. Barbiturates

Barbiturates have been known to elicit their pharmacological effects via the GABA_A receptors. These drugs potentiate the effect of GABA at lower concentrations and activate the receptor directly at higher concentrations through two distinct sites [3]. The α-subunit is reported to influence the degree of efficacy (but not affinity) of pentobarbital-induced potentiation of the GABA responses [307]. However, the β-subunit does not have any marked effect on the affinity or efficacy of the potentiating response [110]. Recently, pentobarbital has been shown to activate the homomeric GABA_A receptors containing β1-subunit in Xenopus oocytes [328]. The α-subunit is reported to influence the affinity as well as efficacy of direct activation of GABA_A receptors by pentobarbital at higher concentrations [307]. Interestingly, pentobarbital, unlike conventional benzodiazepines, potentiates the GABA responses mediated through α6β1γ2 as well as α6β2γ2 GABA_A receptors [336]. Moreover, pentobarbital was able to activate the α6β1γ2 GABA_A receptors directly, but it failed to elicit a similar effect at the α6β2γ2 GABA_A receptors [336]. Chronic pentobarbital administration leads to tolerance and physical dependence. The δ-subunit mRNA in the cerebellum is upregulated in the pentobarbital-tolerant mice, and is down-regulated in the pentobarbital-withdrawn mice [173]. These observations implicate the role of the δ-subunit of GABA_A receptor in the action of pentobarbital. Furthermore, the α5-subunit mRNA in the rat cerebellum is upregulated in the pentobarbital-tolerant animals, thereby resulting in an enhancement of the diazepam-insensitive [3H]Ro 15-4513 binding [136]. The pentobarbital-dependent rats are also reported to express the increased levels of the α1- and γ2-subunit mRNA of GABA_A receptor, whereas the decreased levels of mRNA
Similarly, the mRNA levels are increased in the rat hippocampus [320]. The expression of $\beta_1$ mRNA is increased in the hippocampal pyramidal cells of CA1 and CA2, but not in hippocampal CA3–4 or cerebral cortex, following chronic treatment of rats with pentobarbital [358]. Chronic treatment of primary cultured cerebellar granule cells with pentobarbital decreased the GABA$_A$ receptor subunit $\gamma_{2L}/\gamma_{2S}$ mRNA ratio but this effect is not mediated via direct activation of the receptor itself [326]. Neither the mechanism nor the impact of decreasing the $\gamma_{2L}/\gamma_{2S}$ mRNA ratio by pentobarbital is known, but changes in the ratio could produce a decreased phosphorylation of the GABA$_A$ receptor with subsequent alterations to GABA$_A$ receptor function [326].

6.5. Ethanol

Ethanol shares several pharmacological actions with the benzodiazepines and barbiturates, and several of its effects are mediated through GABA$_A$ receptors [1,166,200, 205,301,302,311,313]. On the other hand, GABA$_B$ receptors do not seem to be involved in the pharmacological effects of ethanol [203]. It has been suggested that ethanol sensitivity is correlated with the presence of BZ1 binding sites ($[^{3}H]$zolpidem binding sites) [26,51,52] as well as with the $\alpha_3$, $\beta_2$, and $\gamma_2$-subunits of GABA$_A$ receptors [51,71]. Over the last decade, effects of chronic ethanol administration and its abrupt withdrawal on the mRNA and polypeptide levels of GABA$_A$ receptor subunits have been investigated in several brain regions in an attempt to explain the mechanisms underlying its dependence and tolerance. Chronic ethanol administration is reported to reduce the levels of $\alpha_1$, $\alpha_2$, $\alpha_3$-subunit mRNA and polypeptide in cerebellum and cerebral cortex [29,41, 63,210,212,217,220]. However, chronic administration of ethanol or its abrupt withdrawal following chronic administration did not result in the down-regulation of the GABA$_A$ receptor assemblies containing $\alpha_1$-subunit in both the brain regions as determined by the $[^{3}H]$muscimol, $[^{3}H]$flunitrazepam, $[^{3}H]$Ro 15-4513 and $[^{3}H]$zolpidem binding to the immunoprecipitated GABA$_A$ receptors using $\alpha_1$-subunit-specific polyclonal antibody [Mehta and Ticku, unpublished observations]. In contrast to the decrease in the mRNA and polypeptide levels of the $\alpha_1$-subunit by chronic ethanol administration, the $\alpha_3$-subunit mRNA levels are increased in the rat hippocampus [41]. Similarly, the $\alpha_4$, $\gamma_1$, $\gamma_{2S}$ [65], $\alpha_6$ [212] and $\beta_{2,3}$-subunits [213] mRNA levels increase in the ethanol-dependent animals. Furthermore, the polypeptide levels of the $\alpha_4$, $\beta_{2,3}$, and $\gamma_1$-subunits of GABA$_A$ receptors are also increased in the cerebral cortex of ethanol-dependent and ethanol-withdrawn rats [63]. However, chronic administration of ethanol did not alter the mRNA levels of $\gamma_{2L}$, $\gamma_1$ and $\delta$-subunits [65] as well as the polypeptide levels of $\gamma_2$-subunit in cerebral cortex. During the behavioral peak of ethanol withdrawal, mRNA levels of the $\alpha_1$, $\alpha_4$, and $\gamma_{2S}$-subunits revert nearly to control levels, whereas significant increase in the $\beta_2$, $\beta_3$, and $\gamma_1$-subunit mRNA levels are observed in the rat cerebral cortex [64]. Chronic intermittent ethanol (CIE) treatment in rats leads to an increase in the $\alpha_3$-subunit mRNA levels in hippocampus, cerebral cortex and thalamus [183]. A new in vitro model of CIE using cultured cortical neurons has been developed for studying the biochemical and molecular mechanisms underlying the CIE-induced kindling-like phenomenon observed in humans [134]. In the postmortem frontal cortex of human alcoholics, the GABA$_A$ receptor $\alpha_1$, $\alpha_2$, $\alpha_3$, and $\beta_{2,3}$-subunit peptide expression did not differ as compared to control group [215]. Furthermore, no differences in GABA$_A$ receptor $\alpha_1$ or $\alpha_3$-subunit mRNA were found, while higher levels of $\beta_2$-subunit mRNA were found in human alcoholics [215]. These results suggest that the effects of chronic ethanol exposure in human alcoholics differ from the rat model of ethanol-dependence.

Chronic ethanol treatment increases the $[^{3}H]$Ro 15-4513 binding in the rat brain [208] and mouse cerebellum [14], and alters the behavioral effects of Ro 15-4513 (sarmazenil) [13,202], a partial inverse agonist of the benzodiazepine site which is reported to antagonize the pharmacological effects of low doses of ethanol mediated through GABA$_A$ receptors [200,309,313]. The $[^{3}H]$Muscimol binding sites are increased in the alcoholic human cerebral cortex [318] and superior frontal gyrus of noncirrhotic alcoholics [67]. There is a possibility that phosphorylation plays a role in the biochemical events following chronic administration of ethanol and its abrupt withdrawal [344]. Selectively bred mouse lines (SS: short sleep; LS: long sleep) and rat lines [AT: alcohol-tolerant or alcohol-insensitive; ANT: alcohol-nontolerant or alcohol-sensitive; alcohol-prefering rats and alcohol-nonpreferring rats] have also implicated the GABA$_A$ receptors in the action of ethanol [1,77,299,329]. Ethanol potentiates the GABA responses of certain brain regions in LS mice but not in SS mice, which is consistent with the much longer ethanol-induced sleep time in the LS mice [1]. On the other hand, the alcohol-sensitive ANT rats differ from the alcohol-insensitive AT rat lines by a critical point mutation of the $\alpha_3$-subunit of GABA$_A$ receptors (Arg$^{100}$ residue is replaced by Gln$^{100}$ in the ANT rat line), thereby altering the normal diazepam-insensitive receptors into diazepam-sensitive ones [157]. Although the ANT rats are more sensitive to the motor impairment induced by ethanol [77] or benzodiazepines [122] as compared to the AT rats, there is no substantial difference in the sensitivity to the hypnotic doses of ethanol [76,161]. The ANT rats exhibit a diminished stress-responses to a variety of stressors, even in the non-intoxicated state [323]. Furthermore, Ro 15-4513 has almost two times more affinity for the ANT rat cerebellum versus the AT rat cerebellum [329]. Alcohol-prefering rats are reported to be more active in the open field [142,338], and are innately anxious as assessed by the less time spent on the open arms as compared to alcohol-nonpreferring rats.
6.6. Neurosteroids

Several neurosteroids have been shown to interact with the GABA<sub>Α</sub> receptors [184,206,251], as was first demonstrated for a synthetic neurosteroid anesthetical alphasaline [117]. These drugs interact with a distinct neurosteroid-binding site, different from the barbiturate-site, on the GABA<sub>Α</sub> receptors [97,324]. Furthermore, the presence of multiple distinct steroid recognition sites or conformational states on the GABA<sub>Α</sub> receptors has also been suggested [120,221]. However, it has been suggested recently that dehydroepiandrosterone sulfate (DHEAS), a neurosteroid, binds to the picrotoxin site of the GABA<sub>Α</sub> receptors, whereas dehydroepiandrosterone (DHEA) does not interact with the GABA<sub>Α</sub> receptor complex [294]. Neurosteroids, in general, interact with the GABA<sub>Α</sub> receptor assemblies composed of α<sub>1</sub>-, β<sub>1</sub>-, and γ<sub>2</sub>-subunits or β<sub>2</sub>-subunits alone [251]. Modulation of the GABA response by steroids requires the presence of β-subunit, and does not depend on the presence of a γ-subunit unlike the benzodiazepine site [251]. However, the substitution of a γ<sub>1</sub>-subunit for a γ<sub>2</sub>-subunit greatly enhances the sensitivity to neuroactive steroids [250,252]. On the other hand, δ- and ε-subunits inhibit the neurosteroid modulation of the GABA<sub>Α</sub> receptors [57,368], whereas the type of α-subunit isomorph does not seem to play a major role in determining the neurosteroid efficacy or potency as a positive modulator [250]. The α<sub>2</sub>-subunit suppression prevents withdrawal effects of progesterone which include symptoms of premenstrual syndrome (PMS) such as anxiety and seizure susceptibility [292]. The pseudopregnancy paradigm has been suggested as a useful model for periods of endogenous neurosteroid withdrawal such as PMS and postpartum or postmenopausal dysphoria [293] which are associated with increased emotional liability and benzodiazepine-insensitivity [47,292,293]. Chronic neurosteroid treatment decreases the mRNA levels of α<sub>2</sub>-, α<sub>4</sub>-, β<sub>2</sub>- and β<sub>3</sub>-subunits [360], and results in a heterologous uncoupling between the GABA, barbiturate, neurosteroid and benzodiazepine site [87,362], and a reduced efficacy of GABA, the benzodiazepine site ligands and neurosteroids at the GABA<sub>Α</sub> receptors in a heterologous manner [361]. Recently, neurosteroids are reported to be more effective in inhibiting bicuculline-induced seizures in the ethanol-withdrawn rats and in potentiating the GABA<sub>Α</sub> receptor-mediated chloride uptake in synaptosomes prepared from the cerebral cortex of ethanol-withdrawn rats as compared to control group [64]. Interestingly, the α<sub>ε</sub>-containing recombinant receptors are more sensitive to the inhibition of [1<sup>35</sup>S]TBPS binding by 5α-pregnan-3α-ol-20-one than those containing the α<sub>α</sub>-subunits [160]. 5α-Pregnan-3α-ol-20-one is a neurosteroid and a natural active metabolite of progesterone which is also known as allo-pregnanolone or allopregnan-3α-ol-20-one or 3α-hydroxy-5α-pregnan-20-one, and is almost equi-potent to benzodiazepines in potentiating the GABA<sub>Α</sub> receptor-mediated chloride ion flux [222]. It has hypnotic and anxiolytic effects [50,125] which correlate with its efficacy on GABAergic transmission in the CNS, and potentiates the binding of [1<sup>3</sup>H]flunitrazepam (a ligand for the benzodiazepine-site), and [1<sup>3</sup>H]muscimol (a ligand for the GABA-binding site) in the rat cerebral cortex, cerebellum and hippocampus [206]. 5α-Pregnan-3α-ol-20-one also elicited a biphasic response, i.e., it potentiated the binding of [1<sup>35</sup>S]TBPS (a ligand for the picrotoxin-site) at lower concentrations and inhibited the binding at higher concentrations in the rat cerebral cortex, cerebellum and hippocampus [206]. Furthermore, it is more efficacious in enhancing the binding of [1<sup>3</sup>H]flunitrazepam and [1<sup>3</sup>H]muscimol in the cerebellum of ethanol-dependent rats as compared to control group [206], and is more efficacious in inhibiting the binding of [1<sup>35</sup>S]TBPS in the hippocampus of ethanol-dependent and ethanol-withdrawn rats [206], thereby suggesting that the neurosteroid binding site associated with the GABA<sub>Α</sub> receptors plays an important role during ethanol-dependence and ethanol-withdrawal [206]. However, 5α-pregnan-3α-ol-20-one has poor oral availability. To improve bioavailability, a new synthetic neuroactive steroid ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) has been synthesized and is currently under clinical trials. It is a potent and efficacious anticonvulsant agent for the management of generalized absence and partial seizures [35] as well as for convulsions due to cocaine poisoning [96]. It is more potent and efficacious as compared to valproate and diazepam in blocking the development of pentylentetrazole (PTZ)-kindled seizures [95]. Recently, it was reported that ganaxolone is superior to valproate, ethosuximide, clonazepam, diazepam, and phenobarbital in preventing the PTZ-induced convulsions and the behavioral effects of PTZ including its depressant effects on locomotor activity and rearing in mice, thereby suggesting that ganaxolone may provide additional benefits in the treatment of epilepsy by controlling anxiety, mood changes and other behavioral alterations associated with pre-seizure activity [15].

6.7. Miscellaneous

The divalent cation zinc is reported to block the GABA<sub>Α</sub> receptor-mediated responses in a non-competitive and volt-
Receptor assemblies derived from a combination of α- and β-subunits are highly sensitive to zinc-blockade but those containing a γ-subunit are almost resistant to blockade by zinc [68,290]. In other words, GABA\textsubscript{A} receptors with a benzodiazepine-site are resistant to blockade by zinc while those devoid of the benzodiazepine-site are sensitive to blockade by zinc in most instances. The extracellular N-terminal domain of the α\textsubscript{1}-subunit [86], His\textsuperscript{273} amino acid residue of the α\textsubscript{6}-subunit [86], His\textsuperscript{367} residue of the β\textsubscript{1}-subunit [130], and His\textsuperscript{292} aminoacid of the β\textsubscript{3}-subunit [356] are reported to be important determinants of the Zn\textsuperscript{2+} binding site on the GABA\textsubscript{A} receptors. Thyroid hormones such as l-triiodothyronine (T3) and l-thyroxine are also reported to interact with the GABA\textsubscript{A} receptors [40,192], and it has been suggested that the α\textsubscript{1}-subunit imparts T3 sensitivity to the GABA\textsubscript{A} receptors [40]. Although somatostatin-14, a biologically active tetradecapeptide, is known to mediate its biological actions through the G protein-coupled membrane receptors [332], its interaction with GABA\textsubscript{A} receptor complex has also been reported [74,91,331]. Chronic administration of antipsychotic drugs such as clozapine and olanzapine is reported to decrease the rat cortical and limbic GABA\textsubscript{A} receptors probably in response to the increased GABA release following the blockade of multiple neurotransmitter receptors on GABAergic interneurons, and it has been suggested that these antipsychotic drugs would assist in substantially reversing an under-active GABAergic system in schizophrenia [79]. The pineal gland hormone melatonin [45,170,187,227], lanthanum [135,271], γ-butyrolactones as well as γ-thiobutyrolactones [124], penicillin [131], antihelmintic compound ivermectin [165,244], pesticide compounds lindane, endrin and dieldrin [317], anxiolytic/anticonvulsant compounds chloromethiazole and trichloroethanol [113,218,235], polyamines such as spermine and spermidine [99], and antidepressants such as amoxapine and mianserin [295] have been reported to interact with GABA\textsubscript{A} receptors but the exact site of action of these drugs on the GABA\textsubscript{A} receptors and their subunits requirement are not known currently.

7. Concluding remarks

The heterogeneity of GABA\textsubscript{A} receptors in the brain is much larger than originally thought. There is a large number of different GABA\textsubscript{A} receptor subunits with distinct regional distribution. The pentameric GABA\textsubscript{A} receptor assembly can be derived from a permutation and combination of two, three, four, or even five different subunits. Composition of various GABA\textsubscript{A} receptor assemblies can differ not only in different parts of the brain or in different cells but also in the same cell. Although the α\textsubscript{1}, β\textsubscript{1/3}, and γ\textsubscript{2/3}-subunits co-exist in many native GABA\textsubscript{A} receptors, the composition of major receptor assemblies in various regions of brain has not been characterized fully. Radioligand binding to the immunoprecipitated receptors using subunit-specific antibodies, co-localization studies using immunooaffinity chromatography, immunocytochemistry, and the recombinant receptors system are useful tools to provide this information in future. Furthermore, characterization of molecular determinants of clinically important drug-targets is under way. Based on new information available so far with these techniques, better anxiolytics are being developed. These drugs are partial agonists for GABA\textsubscript{A} receptor subtypes, and have fewer and less severe side effects as compared to conventional benzodiazepine anxiolytics. However, the progress in this area is hindered by the fact that we still do not know the composition and physiological/pharmacological significance of various native GABA\textsubscript{A} receptor assemblies. Various experimental approaches such as mutation, gene knockout and inhibition of expression of GABA\textsubscript{A} receptor subunits by antisense oligodeoxynucleotides are likely to provide answers to some of these questions, and help us in developing newer drugs devoid of major side effects associated with currently available anxiolytics as a result of their better selectivity for various GABA\textsubscript{A} receptor assemblies.

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