The complexity of the $GABA_A$ receptor shapes unique pharmacological profiles

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Gamma-amino butyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system (CNS) and many physiological actions are modulated by $GABA_A$ receptors. These chloride channels can be opened by GABA and are a target for a variety of important drugs such as benzodiazepines, barbiturates, neuroactive steroids, convulsants and anaesthetics. $GABA_A$ receptors are involved in anxiety, feeding and drinking behaviour, circadian rhythm, cognition, vigilance, and learning and memory. Moreover, deficits in the functional expression of $GABA_A$ receptors have been implicated in multiple neurological and psychiatric diseases. This review aims to discuss the unique physiological and pharmacological properties of the multitude of $GABA_A$ receptor subtypes present in the CNS, making this receptor an important target for novel rational drug therapy.

Classification of neurotransmitter receptors

There are two major classes of neurotransmitter receptors: ionotropic and metabotropic. Binding of the appropriate ligand to ionotropic receptors (ligand-gated ion channels) results in a conformational change that opens a pore in the receptor and permits the transfer of ions across the cell membrane. Metabotropic receptors are associated with G proteins (i.e. they are G-protein-coupled receptors; GPCRs) that transduce the extracellular signal into an intracellular response. Typically, ligand-gated ion channels give rise to fast postsynaptic responses that last only a few milliseconds. This type of receptor contains two functional domains: an extracellular domain to which the neurotransmitter binds and a membrane-spanning domain that forms the ion channel. The superfamily of the ligand-gated ion channels contains cys-loop receptors such as $GABA_A$ receptors ($GABA_AR$), glycine (GlyR), serotonin ($5HT_3$) and nicotinic acetylcholine (nAChR) receptors, ionotropic glutamate receptors (AMPA, Kainate and NMDA) and ATP-gated channels (P2X). By contrast, metabotropic receptors produce slower, long-lasting postsynaptic effects. G proteins may alter the properties of these ion channels directly, or may activate intracellular second-messenger pathways that indirectly modulate the channel activity [3]. Metabotropic receptors include metabotropic glutamate receptors, muscarinic acetylcholine receptors, $GABA_B$ receptors and most serotonin receptors, as well as receptors for norepinephrine, epinephrine, histamine, dopamine, neuropeptides and endocannabinoids.

$GABA_A$ receptors

GABA receptors are the most important inhibitory receptors in the brain. Inhibitory GABAergic synapses may either contain ionotropic $GABA_A$ receptors, which are blocked by bicuculline, or metabotropic $GABA_B$ receptors that are baclofen-stimulated and bicuculline-insensitive. This review will primarily focus on $GABA_A$ receptors.

Binding of GABA to the $GABA_A$ receptor opens channels that are permeable to Cl^- (Figure 1). When these channels open, negatively charged chloride ions can flow across the membrane. $GABA_A$ receptors can mediate inhibitory neurotransmission by hyperpolarizing the membrane of the postsynaptic neuron, resulting in an inhibitory postsynaptic potential (IPSP) that decreases the probability of firing

GABA metabolism

GABA is synthesized from glutamate by the enzyme glutamic acid decarboxylase (GAD), which is found almost exclusively in GABAergic neurons. GAD requires a cofactor, pyridoxal phosphate, derived

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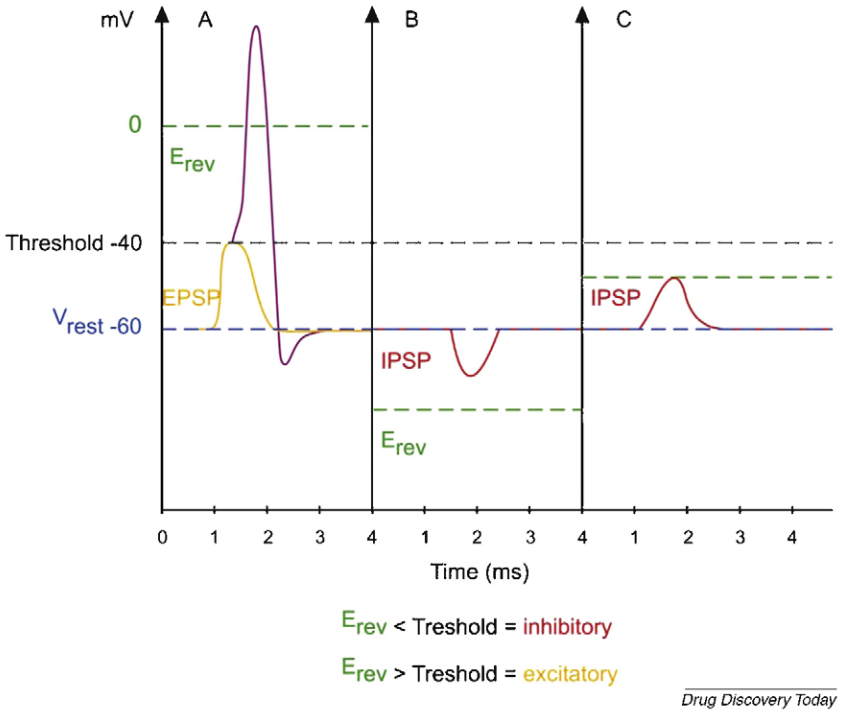

FIGURE 1

In general, postsynaptic potentials (PSPs) alter the probability that an action potential will be produced in the postsynaptic cell. PSPs are called excitatory (EPSP) if they increase the likelihood of a postsynaptic potential occurring, and inhibitory (IPSP) if they decrease the likelihood. The principles of postsynaptic inhibition are much the same as for excitation. In both cases, neurotransmitters binding to receptors open or close ion channels in the postsynaptic cell. Whether a postsynaptic response is an EPSP or an IPSP depends on the type of ion channel that is coupled to the receptor. In fact, the only factor that distinguishes postsynaptic excitation from inhibition is the reversal potential of the PSP in relation to the threshold voltage for generation of action potentials in the postsynaptic cell [3]. (A) If the reversal potential (Erev, green) is more positive than the action potential threshold (−40 mV), the neurotransmitter is excitatory and it generates EPSPs. (B) In the case of activation of a GABA synapse, hyperpolarization of the postsynaptic membrane results in a reversal potential that is more negative than the threshold for firing, preventing the occurrence of an action potential. (C) IPSPs can nonetheless depolarize the postsynaptic cell if their reversal potential is between the resting potential and the threshold for firing. (adapted from Purves *et al.* [3]).

from vitamin B_6. Consequently, a dietary deficiency of vitamin B_6 can lead to diminished GABA synthesis, resulting in seizures and mental retardation. Both neurons and glia contain high-affinity transporters that remove GABA from the synapse (GAT1-3 [1]). Most GABA is eventually converted into succinate, which is metabolized further in the tricarboxylic acid cycle that mediates cellular ATP synthesis by the mitochondrial enzymes GABA transaminase and succinic semi-aldehyde dehydrogenase. Inhibition of GABA breakdown causes a rise in tissue GABA content and an increase in the activity of inhibitory neurons. GABA metabolism is discussed in more detail elsewhere [2].

$GABA_A$ receptor subtypes

Structure and heterogeneity

$GABA_A$ receptors are heteropentameric assemblies that consist of a large N-terminal extracellular domain, four transmembrane-spanning regions (TM 1–4), and a large intracellular loop between TM3 and TM4 [4]. Nineteen different $GABA_A$ receptor subunits have been identified so far in mammals. On the basis of the sequence similarity (Figure 2), these are divided into eight subunit classes, some of which have multiple members: α(1–6), β (1–3), γ (1–3), δ, ε, ρ (1–3), θ and π [reviewed by [5]]. The diversity of the subunits is further increased by alternative splicing, which generates multiple forms of the α_5 and α_6, the β_2 and β_3 and the γ_2 subunits (into γ_{2L} and γ_{2S}). This set is the largest of any among the mammalian ion channel receptors but, at least for the human brain, it seems to be final [6].

In addition there are three ρ subunits that occur exclusively in the retina: ρ_1, ρ_2 and ρ_3, which assemble in a specialized form of $GABA_A$ receptors, sometimes referred to as $GABA_C$ receptors (homo- or hetero-oligomers) [7]. The $GABA_C$ responses are also of the fast type associated with the opening of an ion channel; they are, however, unaffected by typical modulators of the $GABA_A$ receptors, such as benzodiazepines and barbiturates and are not blocked by bicuculline.

$GABA_A$ receptor assembly and location in the CNS

$GABA_A$ receptors are widely distributed within the CNS and show a considerable heterogeneity [8]. Owing to restrictions during the assembly of $GABA_A$ receptors, approximately 500 of the 150 000 theoretically possible $GABA_A$ receptor subtypes can be found in the CNS. [9,8]. The majority of the native receptors are composed of α, β and γ subunits (Figure 3) and receptors of this type can have at least one of three general compositions: 2α.2β.γ; 2α.β.2γ; α.2β2γ [10]. Concatenated receptors were used to confirm that the majority of the receptors are organised in a

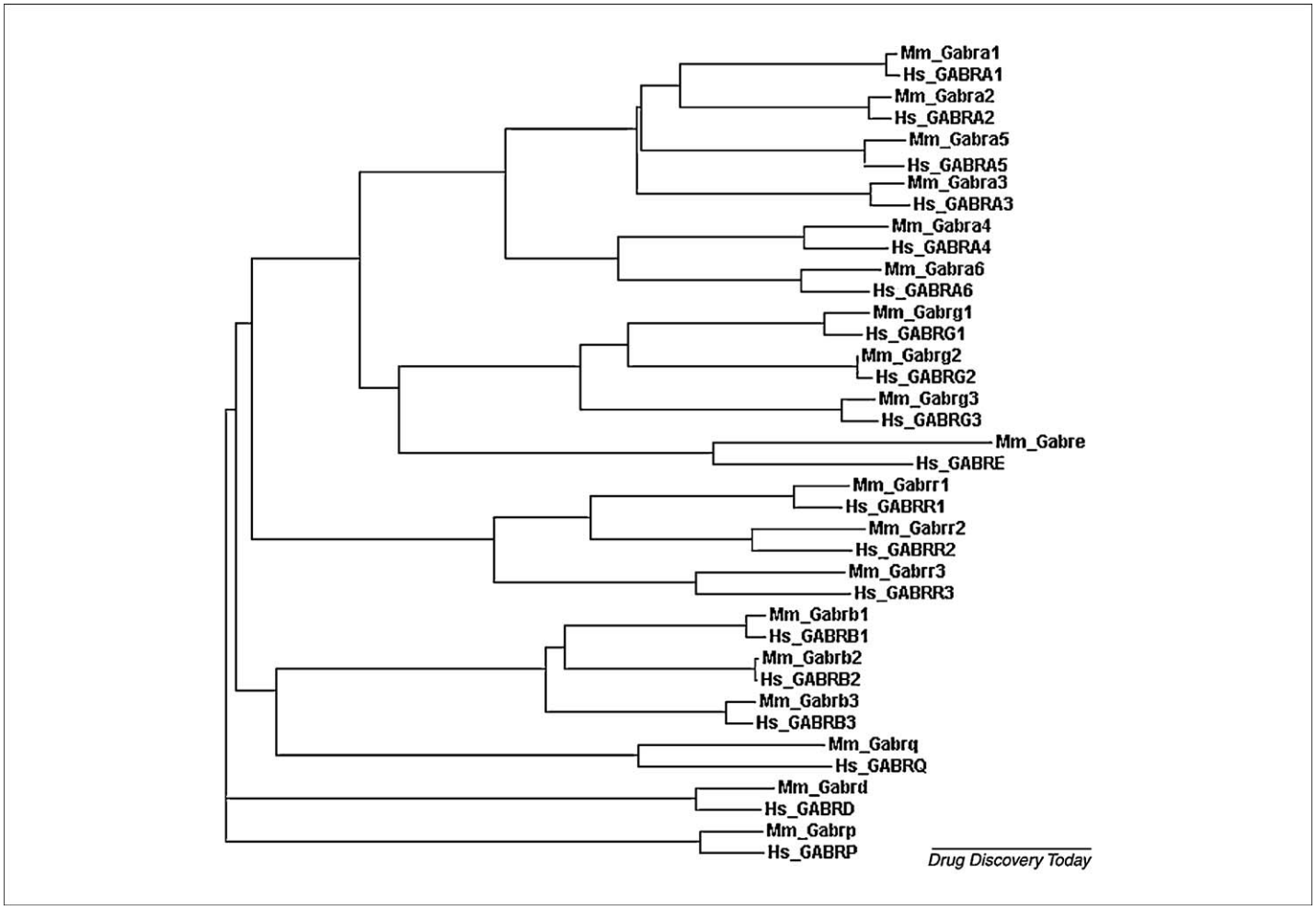

FIGURE 2

Phylogram showing the relative similarity between the different $GABA_A$ receptor subunits (protein) of humans (HS), mice (Mm). The program ClustalW2 was used to obtain this picture http://www.ebi.ac.uk/Tools/clustalw2/index.html.

gamma–alpha–beta–alpha–beta arrangement as viewed from the synapse [11]. The subunit stoichiometry of $GABA_A$ receptors was established by co-immunoprecipitation with isoform-specific antibodies or via the application of isoform-specific antibodies *in situ* [8]. Such studies have, for example, demonstrated that $GABA_A$ receptor comprising an $\alpha_1\beta_n\gamma_2$ structure are the most abundant in brain [8,9]. Most receptors in the population contain a single α isoform; however, in a minority of receptors, pairs containing $\alpha_1\alpha_2$, $\alpha_1\alpha_3$, $\alpha_1\alpha_5$, $\alpha_2\alpha_3$ and $\alpha_3\alpha_5$ have been detected. Co-occurrence of the α_6 subunit with the α_1 subunit in one cerebellar receptor has been observed, although not for all the α_6 subunits. For the γ subunits, co-occurrence of γ_2 with γ_3 was shown, as well as γ_{2L} with γ_{2S} [12]. The γ_1 subunit is restricted to very few brain areas were it may replace the γ_2 subunit and only a very limited number of receptors may contain the γ_3 subunit. Several studies imply a preferential association of α_2 with β_3 subunits and of α_1 with β_2 subunits. Indeed, clustering of α_1, α_6, β_2 and γ_2 subunit genes on the same chromosome (Table 1) may coordinate expression of these subunits [13].

In quantitatively minor receptor subtypes, the δ, ε and π subunits seem to be able to replace the γ subunit, whereas the θ subunit may be able to replace the β subunit. δ subunits are present in only 11% of all the receptors in total rat brain, but in 27% of those in cerebellum from which both $\alpha_6\beta_n\delta$ and $\alpha_6\beta_n\gamma_2$ subtypes can be isolated [14]. The δ subunit preferentially assembles with the α_6 subunit in cerebellum and with the α_4 subunit in forebrain [15]. Homozygous mice lacking the α_6 gene also lack the δ subunit in the cerebellar granule cells. Similarly, there is a decrease in the levels of the α_4 subunit the forebrain of the δ subunit deficient mice, whereas the levels of α_1 remain unchanged [16], providing additional evidence for the preferential association of the δ subunit with the α_4 or the α_6 subunit.

In situ hybridization [17] and immunocytochemistry [18] in rats have demonstrated that each of the subunits has a distinct regional and cellular distribution in brain. Most $GABA_A$ receptor subunits are diffusely distributed and concentrated in typical dendritic areas (e.g. in the hippocampus, cerebellum and olfactory bulb) but rarely seen in axonal fibre tracts. Subunits α_1, $\beta_{1\text{-}3}$ and γ_2 are most widespread, although differences in the overall distribution are noted. The α_2 subunit is preferentially located in the forebrain and the cerebellum. Subunits α_3, α_4, α_5, α_6, γ_1 and δ are mostly confined to specific brain areas: The α_6 subunit, for example, is only present in the granule cell layer of the cerebellum and in the cochlear nucleus, and the γ_1 subunit is found at appreciable

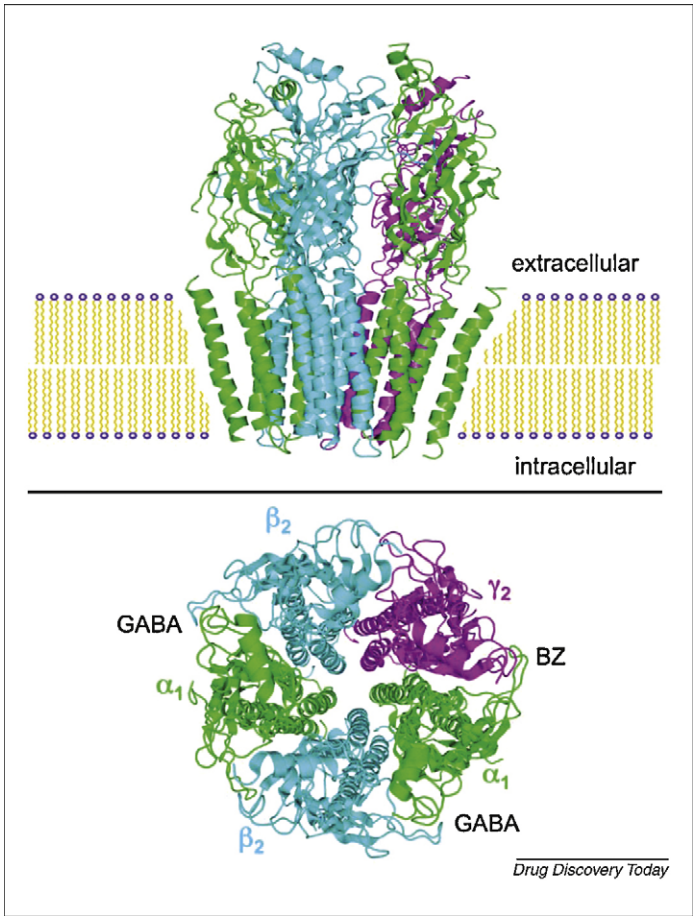

FIGURE 3

Structure of the nicotinic acetylcholine receptor (nAchR: PDB2BG9; http://www.rcsb.org) that is very similar to the $GABA_A$ receptor. Top: side view of the nAchR imbedded in a cell membrane. Bottom: view of the receptor from the extracellular face of the membrane. The subunits are labelled according to the $GABA_A$ nomenclature and the most prominent $GABA_A$ receptor subtype is displayed. The approximate locations of the GABA and benzodiazepine (BZ) binding sites are noted (between the α subunits and β subunits and between the α subunits and γ subunits respectively).

concentrations only in the central and medial nuclei of the amygdala, the pallidum, the septum, the hypothalamus, the *Substantia nigra*, pars reticulate, the superior colliculus and the inferior olive.

Clustering of $GABA_A$ receptors

After navigating their way through the secretory pathway, $GABA_A$ receptors are inserted in the plasma membrane where they can access inhibitory postsynaptic specializations or extrasynaptic sites, depending on their subunit composition [19]. Both the γ_2 subunit and the receptor-associated protein gephyrin are required to associate and cluster $GABA_A$ receptors to the postsynaptic membrane, although there is no evidence for a direct interaction between these proteins [20]. In brain, gephyrin is enriched at postsynaptic sites that contain $\alpha_{1\text{-}3}$, $\beta_{2\text{-}3}$ and γ_2 subunits [21]. Inhibiting gephyrin expression reduces $GABA_A$ receptor clustering at synaptic sites. Clustering in the absence of gephyrin also occurs, however, suggesting the existence of gephyrin-dependent and gephyrin-independent clustering mechanisms [19]. These observations suggest that the γ_2 subunit and gephyrin are interdependent components of the synaptic complex that allows postsynaptic clustering. Whether some receptor subtypes are actively distributed to extrasynaptic areas or are instead passively inserted into the membrane and lack the ability to cluster remains an active area of research [22].

It is thought that the majority of the δ-containing subtypes are located extrasynaptically, presumably as a result of different trafficking properties associated with this subunit [23]. Some γ_2-containing receptors can also be found extrasynaptically, such as the $\alpha_5\beta_3\gamma_2$ subtype, and it is currently unclear how the α_5 subunit overrides the γ_2 postsynaptic signal. Extrasynaptic receptors rely on spill over of transmitter from synapses, non-vesicular release, or the ambient levels of GABA controlled by the glia and GABA transporters. Ambient GABA is thought to be in the range of 300nM-3 μM, below the threshold for activation of many synaptic receptors. The $\alpha_4\beta\delta$ and the $\alpha_6\beta\delta$ receptor subtypes are particularly sensitive to GABA and exhibit comparatively little desensitization and are thus ideally suited for regulating neuronal excitability in response to ambient GABA [24]. Indeed, extrasynaptic receptors have been shown to mediate a non-desensitisizing or tonic inhibition, in contrast to the phasic inhibitory control exerted by synaptic receptors in response to pulsatile (transient) bursts of GABA [25].

TABLE 1

Chromosomal location of the $GABA_A$ receptor subunits in mice and men

$GABA_A$ R subunit	Chromosome Mm	Chromosome Hs
π	11A4	5q33-q34
γ_2	11A5	5q31.3-q33.1
α_1	11A5	5q34-q35
α_6	11A5	5q34
β_2	11A5	5q34
γ_1	5C3.1	4p12
α_2	5C3.1	4p12
α_4	5C3.2	4p12
β_1	5C3.2	4p12
α_5	7C	15q11.2-q12
β_3	7C	15q11.2-q12
γ_3	7C	15q12
ρ_1	4A5	6q13-q16.3
ρ_2	4A5	6q13-q16.3
δ	4E2	1p36.3
ε	XA7.3	Xq28
α_3	XA7.3	Xq28
θ	XA6	Xq28
ρ_3	16C1.3	3q11.2

Mm: Mus musculus, Hs: Homo sapiens.

Transgenic mice

As mammals show very high $GABA_A$ receptor subunit sequence identity (71-99% for mice *vs.* men), genetically engineered mice have been extensively studied to help clarify the physiological functions of $GABA_A$ receptors containing different subunits [26]. Table 2 summarizes the phenotypes of mice in which specific $GABA_A$ receptor subunits have been inactivated, including the consequence of these alterations for the function of synaptic and extrasynaptic receptors, and changes in behaviour. A caveat with such studies is that the deletion of specific $GABA_A$ receptor subunits in mice, may in some cases activate compensatory mechanisms,

TABLE 2

Overview of selected phenotypes of $GABA_A$ receptor subunit knockout (KO) mice

KO	Phenotype	Reference
α_1	Viable	[85]
	Loss of developmentally regulated decrease of decay time constants of synaptic GABA currents in cerebellar and hippocampal neurons	[79]
	Reduced sensitivity to midazolam, pentobarbital, and etomidate	
	Increased sensitivity to ketamine	[85]
	Increased locomotor-activating effects of ethanol	
	IPSCs are considerably shorter than in wild-types	
	Lower expression levels of functional β_2 subunit containing $GABA_AR$	
	Lower body weights (30%)	[84]
	Tremor	
α_3	Deficit in sensorimotor information processing = defect in prepulse inhibition and in the acoustic startle reflex	[54]
α_5	Improved spatial learning	[47]
	Decline in tonic currents	[71]
α_6	Up-regulation of 2-pore domain K^+ channel (TASK-1) mRNA expression level	[70]
	Ataxic phenotype in response to DZ	[78]
	Normal $GABA_A$ receptor δ subunit mRNA levels, but decreased δ subunit protein level	[77]
β_1	Increase in locomotor activity	
β_2	No remarkable phenotype	[84]
β_3	90% of the mice die within 24h after birth	[85]
	IPSCs arte considerably shorter than in wild-types	
	Lower expression levels of functional α_2/α_3 subunit containing $GABA_AR$	[76]
	Hyperactivity, hyperresponsiveness, poor performance on tasks of motor coordination, increased incidence of seizures, deficit learning and memory and cleft palate = mouse model for Angelman's syndrome	
	Anesthetic action of midazolam and etomidate is reduced	
γ_2	Almost all mice die in the first few days after birth	[75]
	Not responsive to BZD	
	Affected single-channel conductance and zinc-sensitivity	[74]
	Loss of $GABA_A$ receptor clusters is paralleled by loss of the synaptic clustering molecule gephyrin and synaptic GABAergic function	
	$\gamma_2^{+/-}$: reduced synaptic clustering, increased reactivity towards natural aversive stimuli and enhanced responses to trace fear conditioning = model for chronic anxiety	[73]
	γ_3 compensates for clustering reduction, but does not rescue the lethal phenotype	[73]
ρ_1	No $GABA_C$ receptor expression	[81]
	Visual processing in the mouse retina is altered	
δ	Occasional seizures and electroencephalographic abnormalities	[64]
	Severely reduced sensitivity to the behavioural effects of neuroactive steroids	
	Faster miniature IPSC decay time	[15]
	Altered pharmacology of forebrain $GABA_A$ receptors	
	Increase in γ_2 subunits and decrease of α_4 subunits	[83]
	Smaller tonic currents	
	Reduced anticonvulsant effects of alcohol	

such as, for example, an increase in γ_2 subunit levels in $\delta^{-/-}$ mice [15]. Remarkably, $\alpha_1{}^{-/-}$ and $\beta_2{}^{-/-}$ mice survive, while the $\beta_3{}^{-/-}$ phenotype is lethal. This may suggest that deletion of subunits expressed early postnatally is lethal, whereas deletion of subunits expressed later in development can be compensated for.

A series of mice containing point mutations (Table 3) have been employed to clarify the contribution of the different subtypes to the pharmacology of different drugs [27]. The rationale being that that point mutations within an allosteric, modulatory site for which there is no endogenous ligand would specifically prevent the modulation by defined drugs while leaving the physiological function of the receptor intact [28]. With regard to the BZD binding site on $GABA_A$ receptors, α subunits containing a histidine (H) residue at a conserved position (α_1-H101, α_2-H101, α_3-H126 and α_5-H105) can bind diazepam, whereas α subunits containing an arginine (R) at the corresponding position in the protein (α_4-R99 and α_6-R100) are unable to bind diazepam [29]. When the H101 residue in the α_1 subunit is replaced by an arginine residue, the resulting recombinant receptor becomes diazepam-insensitive. Similar observations were made on receptors containing α_2, α_3 and α_5 subunits [30]. Using gene-targeting technology, mice harbouring a subtle point mutation (N265 M or N265S) in the second transmembrane region of the β3 subunit or β2 subunit were generated [31,32]. In these mice, it was possible to differentiate the anaesthetic and sedative properties of etomidate and propofol, with, for example, the noxious-evoked movements in response to the intravenous anaesthetics etomidate and propofol being reduced in the β_3N265 M mice, whereas the subanaesthetic sedative properties were much reduced in the β_2N265S mice.

Pharmacological properties

$GABA_A$ receptors can be allosterically modulated by benzodiazepines, barbiturates, steroids, anaesthetics, convulsants, and many other drugs, the number of which is constantly increasing [33,34].

TABLE 3

Overview of selected phenotypes of $GABA_A$ receptor subunit knockin (KI)

KI	Phenotype	Reference
α_1(H101R)	Mediation of sedative action of DZ	
	Mediation of anterograde amnesic action of DZ	[82]
	Mediation of anticonvulsant action of DZ (partial)	
	Compensatory up-regulation of α_2 and α_3 subunits → Increased sensitivity to anxiolytics	[45]
α_2(H101R)	Mediation anxiolytic action of DZ	[86]
	Mediation of myorelaxant action of DZ (partial)	
α_3(H126R)	Mediation of myorelaxant action of DZ (partial)	[80]
α_5 (H105R)	Improved trace fear conditioning learning paradigm	[72]
	Mediation of myorelaxant action of DZ (partial)	
α_6 (R100Q)	Naturally occurring in alcohol-non-tolerant rats (ANT)	[88]
	Increased behavioural motor-impairment	[87]
	Increased alcohol sensitivity of α_6 (R100Q)$\beta_3\delta$ tonic currents	
	Increased enhancement of tonic currents by ethanol in cerebellar granule cells from α_6 (R100Q) rats	
β_2 (N265S)	Loss of etomidate-induced sedation	[32]
β_3 (N265M)	Anaesthetic actions of etomidate and propofol reduced	[31]

These compounds do not interact directly with the GABA binding site but exert their actions by binding to allosteric sites at $GABA_A$ receptors that influence the binding properties of other binding sites present on these receptors and so modulate GABA-induced chloride ion-influx (Table 4).

GABA binding site of the $GABA_A$ receptor

Several mutagenesis studies on recombinant $GABA_A$ receptors have identified the GABA binding site at the interface of an α and a β subunit (Figure 3) [35]. Given that the amino acids that comprise the GABA binding site are relatively conserved across the different α and β subunits, it is not surprising that although agonist binding affinity does vary across subtypes, these differences are relatively modest [36]. Although it does not affect binding affinity to a large extent, the α subunit does have an appreciable effect on efficacy. For example, in $\beta_3\gamma_2$-containing receptors, the α subunit has a large effect upon potency with, for example, GABA agonists

TABLE 4

Pharmacology of $GABA_A$ receptors

Site	GABA	Benzodiazepine	TBPS/picrotoxin	Steroids
Endogenous agonist	GABA			Progesterone metabolites
Agonists	Muscimol	Diazepam Flunitrazepam Clonazepam Bromazepam Lorazepam		Alphaxolone Ganaxolone
Antagonists	Bicuculline Gabazine	β-CCE Flumazenil	TBPS Picrotoxinin	
Inverse agonist		DMCM		
Partial agonists	Imidazole-4-acetic acid Piperidine-4-sulfonic acid 4-PIOL Subtype selective agonists: THIP (Gaboxadol) (δ)	Bretazenil Subtype selective agonists: - TPA-023 (α_2,α_3) - L838417 ($\alpha_2\alpha_3$) - Zolpidem (α_1) - NS11394 (α2/α3)		
Partial inverse agonists		FG-7142 Subtype selective inverse agonists: - α_5IA(α_5) - L-655708(α_5) - α_3IA - RO4938581 (α_5) - RO154513 (weak α_5)		
Subunits involved	α and β	$\alpha_{1,2,3 \text{ and } 5}$, β and γ_2	Channel pore	$\alpha_{4 \text{ and } 6}$, β and δ

β-*CCE*: Beta-carboline ethylcarboxylate; *THIP*: 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol;*4-PIOL*: 5-(4-peridyl)isoxazol-3-ol; *TBPS*: *t*-butylbicyclophosphorothionate; α_5*IA*: α_5 inverse agonist; *FG-7142*: β-carboline; *L838-417*: 7-*tert*-butyl-3-(2,5-difluoro-phenyl)-6-(2-methyl-2H-[1,2,4]triazol-3-ylmethoxy)-[1,2,4]triazolo[4,3-b]pyridazine; α_3IA: α_3 inverse agonist: 6-(4-pyridyl)-5-(4-methoxyphenyl)-3-carbomethoxy-1-methyl-1H-pyridin-2-one; *DMCM:* methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate. Agonists enhance the inhibitory effect of GABA, antagonists have no *in vivo* effects, the bind the receptor as a competitor and can be used as an antidote in a BZD overdose for example. Inverse agonists reverse the action of GABA at the $GABA_A$ receptor. Although compounds acting at the BZD site are traditionally known as agonists, antagonists or inverse agonists, strictly speaking they should be described as positive-, neutral- or negative allosteric modulators, respectively.

being more potent at α_6-containing than α_1-containing receptors.

Interestingly, although not playing a direct role in agonist (GABA) binding, the nature of the 'fifth' $GABA_A$ receptor subunit can play a marked role in agonist binding. More specifically, when the pharmacology of αβγ-containing and αβδ-containing receptors was compared with (non-physiological) αβ receptors, introduction of a γ subunit in the αβγ receptor impaired the agonist response but this is not seen in the αβδ subtype [37]. Consequently, αβδ receptors have a higher agonist affinity relative to the αβγ subtype and, since they have a slow rate of desensitization they are generally considered to be extrasynaptic and contribute to tonic inhibition [38]. The differences in agonist efficacy between γ and δ subunit-containing $GABA_A$ receptors is further demonstrated by the selectivity of 4,5,6,7-tetrahydroisoxazolo-[4,5-c]pyridin-3-ol (THIP, Gaboxadol) for δ-containing $GABA_A$ receptors compared with γ-containing $GABA_A$ receptors [37,39,40]. Interestingly, although Gaboxadol has an effect on sleep in preclinical species, probably mediated via the relay neurons of the ventrobasal thalamus [40,41] development of this compound was stopped in Phase III. Nevertheless, δ subunit-containing $GABA_A$ receptors remain a novel therapeutic target.

Benzodiazepine binding site of the $GABA_A$ receptor

Benzodiazepines

Benzodiazepines (BZD) such as diazepam, are one of the strongest anticonvulsive, sedative-hypnotic, and anxiolytic compounds in clinical use. Although, as with so many psychopharmaceuticals, the benzodiazepines were discovered by serendipity, it is now known that these drugs produce allosteric changes that enhance the action of GABA on $GABA_A$ receptors by increasing the GABA-induced frequency of opening of the chloride channels, increasing the apparent affinity of the receptor for GABA. The BZD tranquilizers represented a major advance in psychopharmacology in the 1960 s and 1970 s. For a period of almost 10 years diazepam (Valium®) was the biggest selling prescription drug in the world and many structural analogues were introduced. BZDs are, however, sedative and, more importantly, they produce physical dependence such that significant withdrawal symptoms are observed on treatment cessation. In addition, BZDs can be a drug of abuse. This inspired the development of subtype-selective agonists, which might retain the beneficial effects of an anti-anxiety and anti-convulsant profile, while no longer being sedative, ataxic or having dependence liability [42].

The benzodiazepine binding site

BZDs and compounds interacting with the BZD binding site of $GABA_A$ receptors can modulate ongoing GABAergic activity but cannot elicit chloride ion influx in the absence of GABA and thus exhibit an extremely low degree of toxicity [43]. Compounds that enhance the action of GABA are called allosteric agonists or positive allosteric modulators. These exhibit anxiolytic, anticonvulsant, muscle relaxant and sedative-hypnotic effects. Compounds that allosterically reduce GABA-induced chloride flux are called inverse agonists or negative allosteric modulators. These compounds have opposite effects to those of the agonists, for example, are anxiogenic, proconvulsant, and enhance vigilance and learning and memory. A third class of compounds binds to the BZD recognition site but does not produce allosteric changes (Table 4). These compounds in most cases do not elicit behavioural effects on their own, but prevent interaction of agonists or inverse agonists with these receptors and are therefore called allosteric antagonists [34]. Even when occupying all available binding sites, antagonists have virtually no physiological effect, an observation often used as evidence that there is no endogenous ligand for the BZ binding site. Seventy-five percent of all $GABA_A$ receptors have a classical benzodiazepine binding site and such receptors contain β, γ_2 and either an α_1, α_2, α_3 or α_5 subunit in a 2:2:1 stoichiometry with the benzodiazepine site occurring at the interface between the α and γ_2 subunit [8].

The agonist or inverse agonist efficacy of a compound can be either similar at the different $GABA_A$ receptor subtypes (non-selective compounds) or can differ between these subtypes (subtype-selective efficacy). Thus, a compound can be a full agonist at one type of receptor and exhibit different degrees of partial agonist or inverse agonist activity at other receptor subtypes [reviewed by 44]. The α subunit is the main determinant of the variability of the benzodiazepine site's affinity and efficacy. Receptors composed of $\alpha_4\beta\gamma_2$ or $\alpha_6\beta\gamma_2$ subunits differ drastically in pharmacology from each other. Most of the classical benzodiazepines, such as diazepam, flunitrazepam, or clonazepam do not interact with these so-called diazepam-insensitive receptors [34]. Imidazobenzodiazepines, such as Ro-15-4513 or flumazenil, however, do interact with these receptors, but also with the $\alpha_1\beta\gamma_2$, $\alpha_2\beta\gamma_2$, $\alpha_3\beta\gamma_2$ and $\alpha_5\beta\gamma_2$ subtypes. Remarkably, flumazenil has a partial agonist effect on these $GABA_A$ receptor subtypes.

$GABA_A$ receptor subtype selective modulators at the benzodiazepine site

The use of transgenic mice and/or BZD ligands with either subtype selective affinity or efficacy in conjunction with behavioural tests has contributed to our understanding of the physiological and pharmacological role of the different α subunit-containing subtypes [45–48]. The $GABA_A$ receptor α_1-containing subtype mediates the sedative effect of BZDs, whereas the α_2 and α_3 containing-subtypes are responsible for the anxiolytic effects [45]. The localization of the α_2 and α_3 subunits in the amygdala and cortical regions is consistent with this anxiolytic phenotype. L-838,417, TPA-023 and NS11394 are examples of non-sedating anxiolytics [45,49,50]. Marked interspecies variations in the pharmacokinetics of L-838,417 as well as metabolic issues limited its further development [48]. TPA-023, however, is essentially an antagonist at the α_1 and α_5 subunits, and a low efficacy partial agonist at the α_2 and α_3 subtypes. In addition to anxiolytic-like efficacy, TPA023 had anticonvulsant activity in a mouse pentylenetetrazole seizure model [49]. Moreover, these non-sedating anxiolytic properties were also observed in primates [51] and relative to the non-selective BZ lorazepam, this compound also has much reduced abuse potential in baboons [52] and clearly has a pharmacodynamic effect in man that is distinct from lorazepam [53]. Yee *et al.* [54] suggested that α_3- selective agonists may constitute an effective treatment for sensorimotor gating deficits in various psychiatric conditions. Overactivity of the dopaminergic system in the brain is believed to contribute to the symptomatology of schizophrenia and morphological studies have shown that the dopaminergic system receives GABAergic inhibitory input mainly via α_3-containing $GABA_A$ receptors [55]. In this regard, TPA023 (also

known as MK-0777) has recently been shown to demonstrate a tendency towards cognitive improvement in schizophrenia [56]. Inverse agonists are anxiogenic-like, increase vigilance and are either convulsant or proconvulsant, preventing their use in humans [57]. A compound possessing inverse agonism at the $GABA_A$ receptor subtype responsible for the cognition-enhancing effects but devoid of the anxiogenic and convulsant/proconvulsant properties would, however, be of clinical utility. $GABA_A$ receptors containing an α_5 subunit, are preferentially localized in the hippocampus [18], suggesting a role in hippocampally mediated functions, such as learning and memory [58]. More specifically, it has been hypothesised that a compound that is an inverse agonist selective for the α_5 subtype may enhance hippocampally based cognitive functions. In this regard, several cognition enhancing compounds have been described, such as L-655,708 [59], α_5IA [60] and RO4938581 [61]. In rodents, Dawson *et al.* [62] have shown that α_5IA enhances the performance in hippocampus-dependent cognitive tests and is devoid of anxiogenic-like behaviour and (pro)convulsant, kindling or motor-impairing activities. In man α5IA attenuated the ethanol-induced impairment of memory in healthy volunteers [63].

Interaction of steroids with $GABA_A$ receptors

Several steroids, such as the anaesthetic alphaxalone, or the sedative-hypnotic, anxiolytic, and anticonvulsant 3α-hydroxylated, 5α-, or 5β-reduced metabolites of progesterone (allopregnanolone) and deoxycorticosterone enhance GABA-stimulated chloride conductance at nanomolar concentrations, whereas at >1 μM concentrations, these compounds produce direct opening of the $GABA_A$ receptor-associated ion channel, like barbiturates [34,38]. This points to the existence of at least two different steroid binding sites on $GABA_A$ receptors. Mihalek *et al.* [64] showed a reduced response to neuroactive steroids in $\delta^{-/-}$ mice, revealing a potential role for δ-containing $GABA_A$ receptor subtypes in modulating behavioural responses to endogenous neuroactive steroids. For instance ganaxolone is a potent positive allosteric modulator of the GABA(A) receptor. This synthetic analogue of allopregnanolone has robust anticonvulsant effects in a variety of animal models of epilepsy, is orally active and lacks hormonal side effects [65]. Unlike diazepam, anticonvulsant tolerance does not develop following chronic therapy. In general, ganaxolone has a favourable safety profile and is now in phase II clinical trials for promising treatment of catamenial epilepsy (i.e. seizures exacerbation in relation to the menstrual cycle).

Other interactions with the $GABA_A$ receptor

In this paragraph, only a few additional compounds will be discussed, primarily to emphasize the pharmacological richness of the $GABA_A$ receptor, with more complete reviews being available elsewhere [5,34]. The convulsants *t*-butylbicyclophosphorothionate (TBPS) and picrotoxinin non-competitively block GABA-gated chloride flux by binding to one or more sites located within or close to the chloride channel [33]. The degree of TBPS binding in the presence of GABA seems to closely reflect the functional state of $GABA_A$ receptors [44,66]. Sedative-hypnotic barbiturates, such as pentobarbital, phenobarbital, or secobarbital enhance the action of GABA by increasing the average channel open duration but have no effect on channel conductance or opening frequency. At concentrations >50 μM, barbiturates are able directly to open the $GABA_A$ receptor associated ion channels in the absence of GABA, at still higher concentrations they change desensitization of receptors, suggesting the existence of several sites of interaction of barbiturates with $GABA_A$ receptors [34].

Despite the fact that ethanol is the most widely used psychoactive agent, its actions on brain functions are poorly understood. Several types of receptors and channels have been shown to be functional altered by ethanol, which include glutamate, serotonin, glycine, and $GABA_A$ receptors [67]. It was suggested that ethanol at low concentrations primarily acts via extrasynaptic receptors composed of $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$ [67]. The specificity of the effects of ethanol on $GABA_A$ receptors remains, however, controversial [68,69].

Conclusion

The multitude of $GABA_A$ receptor subunits assemble in a restricted and non-random way to generate a series of specific $GABA_A$ receptor subtypes. The different pharmacological properties of these receptor subtypes provide the potential for rational drug therapy in a variety of disorders in which dysfunction of GABAergic neuronal systems is implicated, including epilepsy, anxiety disorders, Huntington's disease, Angelman/Prader-Willi syndrome, schizophrenia and fragile X syndrome [2,19]. Compounds that selectively modulate specific subtypes of the $GABA_A$ receptor, and are devoid of the side effects of the classical BZD, are currently in various stages of clinical trials. Hence, the emerging understanding of the physiological functions of distinct populations of $GABA_A$ receptor, based on a convergence of mouse genetics (knockout and knockin mice), pharmacology (subtype-selective compounds) or pathophysiology (neuropathological changes or genetic disease associations) studies, provides the rational basis for hypothesis-driven drug discovery efforts. Consequently, the $GABA_A$ receptor is re-emerging as an attractive target for drug therapy by dealing with the underlying cause of these diseases.

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