GABA RECEPTORS

Ian L Martin and Susan M J Dunn

Ian Martin is Professor of Pharmacology in the School of Life and Health Sciences at the University of Aston, Birmingham B4 7ET, UK. Susan Dunn is a Senior Lecturer in the Department of Pharmacology at the University of Bristol, University Walk, Bristol BS8 1TD, UK. They have common interests in GABAergic transmission in the central nervous system and their research is focused on the structure-function relationships of the Group 1a ligand-gated ion channel receptors.

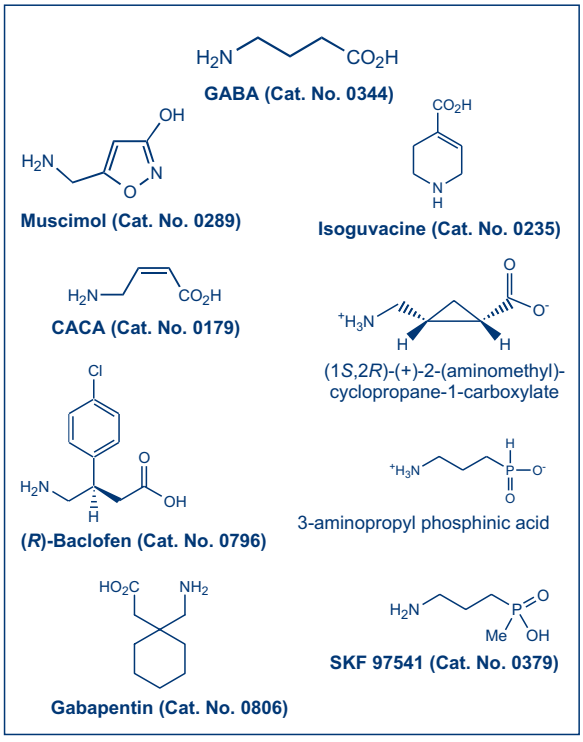
Historical Perspective

GABA is the major inhibitory amino acid transmitter of the mammalian central nervous system and it is present in some 40% of all neurones. Most of the early studies, carried out with iontophoretic application of GABA in the CNS, indicated that it generally produced inhibitory hyperpolarizing responses on neurones,[1] which were blocked competitively by the alkaloid bicuculline.[2] The hyperpolarizing response is due to an increase in the chloride conductance of the neuronal membrane allowing chloride ions to flow down their electrochemical gradient into the cell.[3,4] However, in the late 1970s, Bowery and his colleagues, in attempts to identify GABA receptors on peripheral nerve terminals, noted that GABA application reduced the evoked release of noradrenaline in the rat heart and that this effect was not blocked by bicuculline. This action of GABA was mimicked by baclofen, 4-amino-3-(4-chlorophenyl) butanoic acid (Figure 1), a compound that had no effect on chloride conductance in central neurones. The new receptor was named $GABA_B$ to differentiate it from its more familiar cousin, which was termed $GABA_A$.[5,6] The $GABA_C$ receptor had a rather more difficult birth. In an attempt to discover which conformation of GABA was responsible for activating the receptor, Johnson and his colleagues[7] synthesised a number of conformationally restricted analogues of GABA and noted that *cis*-4-aminocrotonic acid (CACA), which has a partially folded conformation (Figure 1), depressed the firing of cat spinal neurones in a bicuculline insensitive manner. These depressant effects could not be reproduced by baclofen,[8] suggesting a pharmacology distinct from that of either the $GABA_A$ or $GABA_B$ receptors. The receptor became known as $GABA_C$. The DNAs that encode these receptor proteins have now been identified, providing not only a facile means for their molecular characterisation but also a significant stimulus for our attempts to understand their physiological importance.

The $GABA_A$ Receptors

The $GABA_A$ receptors are widely distributed within the mammalian CNS and exhibit a differential topographical distribution.[9] Systematic modification of the natural agonist demonstrated that $GABA_A$ receptors can be activated by a number of compounds (Figure 1) such as muscimol, isoguvacine, 3-aminopropane sulphonic acid, piperidine-4-sulphonic acid and 4,5,6,7-tetrahydro-[5,4-c]-pyridin-3-ol,[10] many of which were subsequently used as radioligands.[11] At equilibrium the binding of GABA agonists is heterogeneous with a high affinity component (K_d values of 10-20 nM) and one or more low-affinity sites with dissociation constants in the range of 100 nM to 1 µM.[12] The presence of even lower affinity sites (K_d values about 50 µM) has been inferred from

Figure 1. Structures of selected GABA receptor agonists

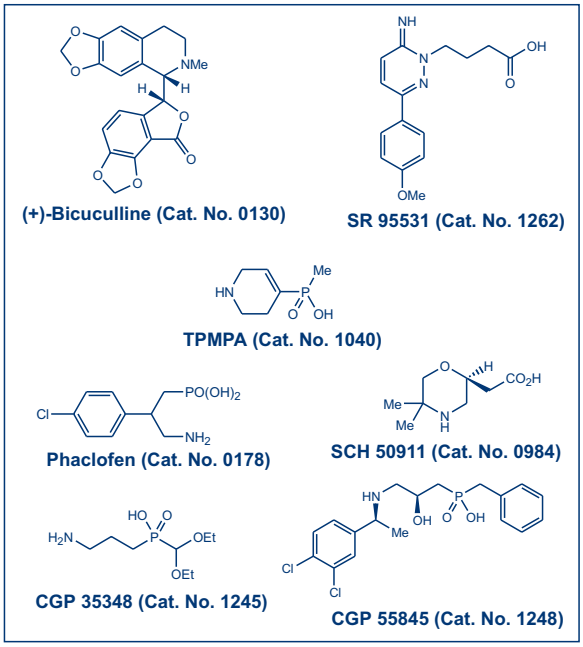


(Bold text denotes compounds available from Tocris)

the higher concentrations of GABA that are required to activate the channel. There has been much speculation about the role of these sites in receptor function. Whether the sites are physically distinct or represent allosteric conformations of the same site remains an intriguing question.[13]

Electrophysiological studies demonstrated that the activation of the receptor resulted in increased chloride conductance of the cell membrane[3,4] with the concentration-response curve exhibiting positive cooperativity, consistent with the presence of at least two agonist binding sites on the receptor molecule.[14-16] The agonist induced current decreased on continued exposure to high agonist concentrations[17-19] suggesting that these receptors undergo desensitisation. Biophysical characterisation of the receptor, initially using noise analysis of neurones in primary culture, provided the first estimates of mean single channel conductance and average channel open times.[20] The latter varied with the nature of the activating agonist.[21] Development of single channel recording techniques[22] provided further detail on the stochastic nature of the single channel events with the demonstration of multiple single channel conductances: 44, 30, 19 and 12 pS.[23] The 30 pS conductance is the most prevalent with distinct open time states, varying from 0.5 to 7.6 ms. The distribution of these states is agonist concentration dependent, with longer open times predominating at higher agonist concentration.[24,25] The competitive antagonist bicuculline appears to reduce conductance through the channel by reducing not only the opening frequency but also the mean open time.[24] Other competitive antagonists, such as the pyridazinyl GABA derivative SR 95531 (Figure 2), are available. In addition, the receptor can be blocked non-competitively by picrotoxin and by a number of bicyclophosphates.[26] Penicillin also decreases the channel open probability in a manner that is compatible with open channel block.[27]

Figure 2. Structures of selected GABA receptor antagonists



(Bold text denotes compounds available from Tocris)

The purification of the receptor protein in the early 1980s[29] provided the opportunity to raise monoclonal antibodies to the receptor, which were later used to study the fine anatomical detail of receptor distribution.[28] Subunit purification, and elucidation of their partial amino acid sequences was crucial in their eventual molecular cloning. Only two receptor subunits (α and β) were initially identified at the protein level and, in the mid 1980s these were cloned. When co-expressed from their encoding nucleic acids in *Xenopus* oocytes, these subunits produced a receptor that responded to $GABA_A$ receptor agonists and antagonists in the expected manner.[30]

Subsequent studies have revealed a multiplicity of protein subunits that have been divided into seven classes, according to similarities in their deduced amino acid sequence. Within these classes there are further subdivisions into subunit isoforms, some of which exhibit alternate splice variants. In man, six α, three β and three γ subunit isoforms are presently known, together with single representatives of the δ, ε, π and θ classes. Additional isoforms in other species are known for some of these classes.[31] Within a single subunit class the sequence homology is about 70% but between classes this falls to around 30%. Deduced amino acid sequences of subunits from all of the families indicate that each has a long amino terminus of about 200 amino acids which contains a signature cysteine-cysteine loop, common to the receptor family, prior to the first of four hydrophobic segments of about 20 amino acids, which are termed TM1-TM4. TM2 is thought to form a major part of the lining of the ion channel as it crosses the cell membrane, while between TM3 and TM4 there is a large intracellular loop, which is the most divergent part of the sequence within the sub-family. The $GABA_A$ receptors have been purified from the pig brain and imaged, after negative staining, in the electron microscope.[32] The receptor has a diameter of about 8 nm in the plane of the membrane and consists of five protein subunits arranged pseudosymmetrically around the integral ion channel; it appears very similar to images of the nicotinic acetylcholine receptor.

Despite the plethora of receptor subunits, it appears that there are a limited number of combinations expressed *in vivo*.[33] A separate gene encodes each subunit and *in situ* hybridisation, together with immuno-histochemical studies, have revealed a distinct distribution for these gene products,[33,34] consistent with the idea that they each serve a defined physiological role. The recognition and functional characteristics of the individual $GABA_A$ receptor subtypes are defined by their constituent subunits and while ectopic expression studies continue to explore this diversity, the authentication of particular $GABA_A$ receptor subtypes *in vivo* remains a significant task. However, it is clear that the most common $GABA_A$ receptor in the mammalian CNS consists of two copies each of the α1 and β2 subunits together with a single γ2 subunit.[35] It is this receptor that appears to mirror the pharmacological and biophysical characteristics

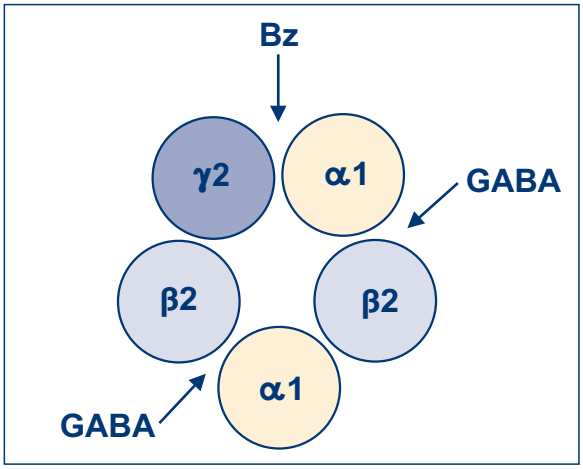
of the $GABA_A$ receptors characterised previously in whole animals and in primary cultures of central neurones.

Weiss and colleagues[36] used site-directed mutagenesis to identify residues in the $GABA_A$ receptor that are involved in channel activation. They identified residues in the β subunit that appear to be important for low affinity agonist recognition. Based on homology with other members of the receptor family, and the currently available evidence from labelling and mutagenesis studies, it appears likely that the activation sites for GABA are located at the β-α interface(s). Recent work has identified residues in the β subunit that may be involved in high affinity agonist binding and this has led to the suggestion that these site(s) may lie at the α-β and γ-β interfaces.[13] The current consensus is that a benzodiazepine modulatory site lies at the interface between α and γ subunits.[37] Much of the evidence for binding site locations is somewhat indirect, involving mutational studies to selectively disrupt the sites. However, our ability to interpret these data has recently been significantly enhanced by the crystallisation of an acetylcholine binding protein which bears a marked structural similarity to the extracellular domain of this receptor family.[38] This protein, though not a ligand-gated ion channel, displays remarkable homology within its ligand binding domains. The crystal structure thus provides a valuable physical template against which the mutational information may be interpreted. Figure 3 shows the subunit arrangement of the $GABA_A$ receptor that is consistent with the currently available ligand recognition site data.

The Benzodiazepines

The benzodiazepines, because of their therapeutic importance, have been a major focus of $GABA_A$ receptor research since the discovery of saturable, high affinity binding sites for [^{3}H]diazepam in the CNS.[39,40] Agonist activation of the $GABA_A$ receptor is augmented by the anxiolytic benzodiazepines, causing a parallel leftward shift of the GABA concentration-response curve. All the overt effects of the benzodiazepines: sedative,

Figure 3. Viewing the $GABA_A$ receptor from the extracellular space, the orientation of the subunits within the pentamer together with the location of the benzodiazepine (Bz) and low affinity GABA sites shown is in accord with all the mutagenesis data that is available to date



anxiolytic, anticonvulsant, muscle relaxant and amnesic, are produced via the $GABA_A$ receptors. However, not all the $GABA_A$ receptors that exist in the brain recognise the benzodiazepines. The particular α subunit isoform present within an individual $GABA_A$ receptor subtype is the primary determinant of benzodiazepine recognition (Table 1). If the α1 subunit of the most common $GABA_A$ receptor is replaced with α4 or α6, the receptor fails to recognise the benzodiazepines. It is now clear from both biochemical and mutational analysis that this insensitivity is due primarily to a single amino acid substitution: an arginine residue in α4 and α6 subunits replaces histidine 101, which is present in α1, α2, α3 and α5 subunits.[42,43] While receptors containing α1, α2, α3 or α5 together with a β and a γ subunit are all recognised by the 'classical' benzodiazepines, several agents are able to distinguish the receptors on the basis of their a subunit isoform composition. The first of these compounds was the triazolopyridazine CL218, 872 ,[44] which is related to the recently introduced hypnotic 'Zaleplon' (Figure 4), while certain β-carboline-3-carboxylic acid esters

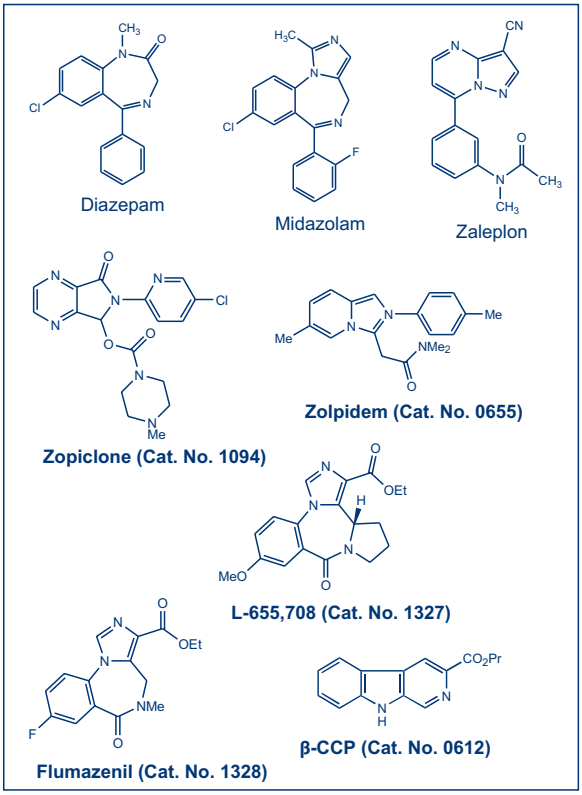
Table 1. Approximate binding affinities of benzodiazepine site ligands for $GABA_A$ receptor subtypes.

Compound	α1βγ2	α2βγ2	α3βγ2	α4βγ2	α5βγ2	α6βγ2
Diazepam	16.1	16.9	17.0	> 10,000	14.9	> 10,000
Clonazepam	1.3	1.7	2.0	–	–	> 10,000
Triazolam	1.8	1.2	3.0	–	1.2	–
Bretazenil	1.2	1.2	1.3	–	2.4	–
Ro15-1788 (Flumazenil)	1.0	1.1	1.5	107	0.4	90
Ro15-4513	2.6	2.6	1.3	–	0.24	6.5
CL218872	130	1820	1530	> 10,000	490	> 10.000
β-CCM	1.7	6.5	4.1	–	27	2050
Zolpidem	17	291	357	–	> 15,000	–
L-655,708	48.5	27.4	24.5	–	0.45	83.2

(Bold text denotes compounds available from Tocris)

Values are quoted in nM. Information abstracted from references 41, 89 and 90. Note that the determinations were carried out with either β2 or β3 subunit isoforms, which do not have a pronounced effect on the affinity of benzodiazepine site ligands.

Figure 4. Structures of selected benzodiazepine site ligands



(Bold text denotes compounds available from Tocris)

allowed a similar distinction (Figure 4).[45] Zolpidem (Figure 4), currently the most widely prescribed hypnotic in the USA, is also able to distinguish $GABA_A$ receptors on the basis of their α subunit isoform composition: it has a high affinity for those receptors which contain α1, a lower affinity for those receptors which contain α2 or α3 and a very low affinity for those receptors which contain α5.[46] Again zolpidem does not recognise receptors which contain α4 or α6 subunits.[47] A nomenclature for these receptors with distinct affinities for the 'subtype selective' ligands was developed prior to the cloning era: α1 containing receptors proved to have the Bz1 phenotype, while those that contained α2, α3 or α5 subunits exhibited Bz2 pharmacology. Other nomenclatures have appeared during the intervening years culminating, most recently, in the classification of $GABA_A$ receptors by a combination of molecular and pharmacological characteristics;[48] time will tell if the scientific community finds it acceptable.

Perhaps one of the most interesting phenomenological observations to appear from studies of the benzodiazepine interaction with the $GABA_A$ receptors has been the development of the inverse agonist concept. The classical benzodiazepines are anxiolytic, sedative/hypnotic, anti-convulsant and muscle relaxant but one of the first non-benzodiazepine ligands discovered, which was able to displace the benzodiazepines from their binding sites, was ethyl β-carboline-3-carboxylate (β-CCE). This compound has effects which are diametrically opposed to those of the classical benzodiazepines, i.e. it is pro-convulsant. It was termed an inverse agonist with the classical benzodiazepines being then classified as agonists.[49,50] Indeed, electrophysiological experiments with the inverse agonists *in vitro* show that they shift the GABA concentration-response curve to the right, thus decreasing the potency of the natural transmitter. While the agonist benzodiazepine site ligands increase channel opening frequency, the inverse agonists decrease it.[51] It is now clear that within this series of β-carbolines, whereas the ethyl ester is pro-convulsant and thus a partial inverse agonist, the propyl ester is essentially devoid of efficacy and thus an antagonist.[52] At the other end of the scale a methyl ester analogue, 6,7-dimethoxy methyl β-carboline-3-carboxylate (DMCM), is overtly convulsant and thus a full inverse agonist.

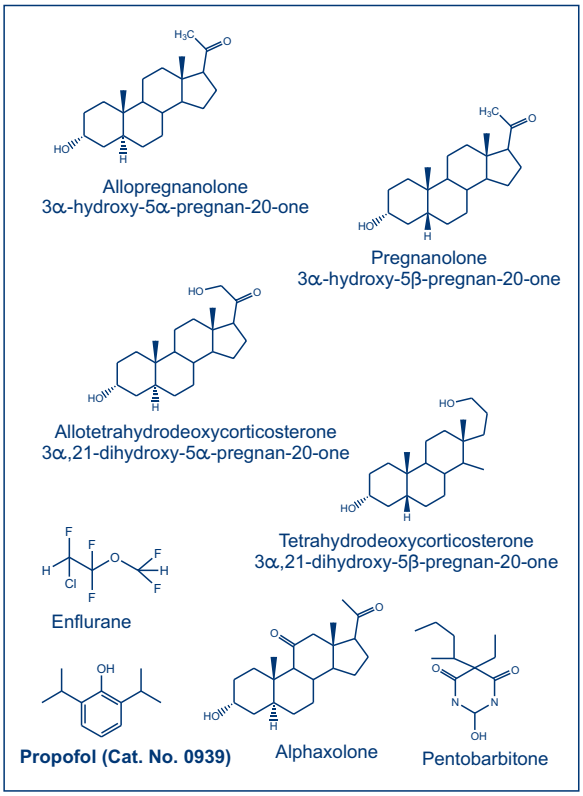
Attempts to delineate the functional importance of individual $GABA_A$ receptor subunits using the gene knockout technology have proved less than fruitful with the β3 and γ2 proving neonatally lethal[53,54] while the α6 knockout mouse displayed no overt phenotype.[55] However, a knock-in approach, which makes use of the histidine/arginine exchange of the α subunits mentioned above, has demonstrated that particular $GABA_A$ receptor subtypes mediate distinct aspects of benzodiazepine pharmacological profile: those receptors containing the α1 subunit are responsible for the sedative effects while those containing the α2 subunit are responsible for their anxiolytic effects.[56] This knowledge will undoubtedly prove valuable in the development of agents with a restricted pharmacological profile and a recent review of the patent literature suggests that this approach is well advanced.[57]

Other Allosteric Sites

The barbiturates also produce many of their effects by interaction with the $GABA_A$ receptors of the mammalian CNS. Like the benzodiazepines, they shift the GABA concentration-response curve to the left but unlike the agonist benzodiazepines, the barbiturates also increase the maximum response. They clearly interact with a distinct allosteric site; the barbiturates augment the GABA mediated current by increasing the average channel open time but have little effect on channel opening frequency.[58,59] Whereas the benzodiazepines require the presence of a γ subunit within the $GABA_A$ receptor oligomer to exert their effects[60] this is not the case for the barbiturates. It is also clear that the barbiturates, at high concentrations, are able to open $GABA_A$ receptor channels directly, which also distinguishes them from the benzodiazepines.[61,62]

In addition to allosteric sites for the benzodiazepines and barbiturates, the $GABA_A$ receptors also exhibit high affinity recognition sites for certain steroids. The observation that alphaxalone, the synthetic steroid general anaesthetic, was able to cause stereoselective potentiation of $GABA_A$ receptor mediated responses in cuneate nucleus slices from rat brain[63] was subsequently confirmed in voltage clamp studies conducted in both neuronal and adrenomedullary chromaffin cells.[64,65] The

Figure 5. Structures of selected steroids, barbiturates and anaesthetics that interact with the $GABA_A$ receptor



(Bold text denotes compounds available from Tocris)

progesterone metabolites 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and 3α-hydroxy-5β-pregnan-20-one (pregnanolone) together with 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone) are even more potent than alphaxalone (Figure 5). It is now clear that these steroids facilitate GABA mediated responses via recognition sites distinct from both the barbiturates and the benzodiazepines. Mechanistically the active steroid analogues appear to produce their effects by increases in both channel open time and opening frequency; like the barbiturates the steroids directly activate $GABA_A$ receptor mediated channels at high concentrations.[66] The neuroactive steroids show limited $GABA_A$ receptor subtype specificity. However, it appears that in those receptors containing the α4 subunit, GABA currents are inhibited by the naturally occurring pregnanolone and alphaxalone while they are stimulated in other $GABA_A$ receptors. The δ subunit also appears to markedly increase the efficacy of both pregnanolone and alphaxalone in α4β3δ receptors compared to those comprised of α4β3γ2 subunits.[67] These observations are particularly interesting in view of the plasticity of both α4 and δ subunit expression associated with pregnancy,[68,69] observations which may prove to have a bearing on the mood changes associated not only with premenstrual stress but also those experienced in postpartum depression.

There is now general consensus that the $GABA_A$ receptor plays a significant role in general anaesthesia. These receptors fulfil the necessary criteria being sensitive to clinically relevant concentrations of the anaesthetic agents together with exhibiting the expected stereospecific effects.[70] The volatile anaesthetics, the halogenated ethers and alkanes together with chloroform and diethyl ether are differentiated from the intravenous anaesthetics such as the barbiturates, propofol and etomidate by their mechanism of action, but both groups appear to facilitate GABA mediated inhibition at the $GABA_A$ receptor. While there remains a good deal of work to do, studies over the past decade have provided a significant body of evidence to address the sites on the receptor protein which form the targets for interaction with the anaesthetics. Not surprisingly these agents appear to bind to hydrophobic pockets within the protein although differences have been identified with, for example, a specific aspartate residue within TM2 being required for etomidate sensitivity but of no consequence to the activity of pentobarbitone, propofol or the anaesthetic steroids.[71]

Although the majority of studies have focussed on the $GABA_A$ receptor it is clear that certain anaesthetics, such as ketamine, nitrous oxide and xenon do not produce their effects through this receptor but probably by inhibition of the *N*-methyl-D-aspartate receptor. It is also clear that many of the anaesthetics interact with other ligand gated ion channels, in addition to the NMDA receptor, with pronounced effects being seen on the neuronal nicotinic acetylcholine receptors, particularly those containing the α4 subunit, and the $5\text{-}HT_3$ receptor. Undoubtedly the potential target population will expand as these studies progress.[72]

$GABA_C$ receptors

In addition to the $GABA_A$ receptors there is a distinct class of ligand gated ion channels that are activated by GABA; referred to as the $GABA_C$ receptor.[8] The natural agonist GABA is about an order of magnitude more potent at the $GABA_C$ receptors than at the most common of the $GABA_A$ receptors. The $GABA_C$ receptors are activated by *cis*-aminocrotonic acid (CACA), which is not recognised by either the $GABA_A$ or $GABA_B$ receptors, suggesting that they recognise the partially folded conformation of GABA (Figure 1). $GABA_C$ receptors are not blocked by bicuculline and do not recognise the benzodiazepines, barbiturates or the neuroactive steroids but, like $GABA_A$ receptors are blocked by picrotoxin, while 1,2,5,6-tetrahydropyridine-4-yl methyl phosphinic acid (TPMPA; Figure 2) appears to inhibit $GABA_C$ receptors selectively. Pharmacologically they are thus quite distinct. However, molecular cloning studies have revealed that this pharmacological profile is remarkably similar to that exhibited by the ρ subunits when expressed ectopically.[73] Two homologous ρ subunits, ρ1 and ρ2, have been identified in man and these can be expressed as homomers or heteromers, but do not co-assemble with any of the $GABA_A$ receptor subunits. The DNAs are encoded on chromosome 6 in man, distinct from the clusters of $GABA_A$ receptor subunit genes which are found on chromosomes 4, 5, 15 and X; the ρ subunits are between 30 and 38% homologous

to the $GABA_A$ receptor subunits at the amino acid level. In the important TM2 region of the sequence, they show greater homology to the glycine α subunits than to any of the $GABA_A$ receptor subunits. It is assumed that they form pentameric assemblies, similar to the other members of the ligand gated ion channel family, which enclose a chloride selective channel. The single channel conductance of the homomeric or heteromeric receptors composed of ρ subunits is smaller (around 7 pS) than that exhibited by the $GABA_A$ receptors (25-30 pS) and the gating kinetics are quite distinct, with both the activation and deactivation time constants being very slow. These ρ homomers or heteromers also appear to be remarkably resistant to desensitisation in the presence of high concentrations of the agonist. While it is clear that the homomeric and heteromeric combinations of the ρ subunits display distinct biophysical and recognition characteristics[74] they do generally mirror, quite closely, the $GABA_C$ receptors that have been characterised in retinal bipolar cells from a number of species. Discussions continue with regard to the nomenclature: do we use sequence homology to classify proteins or their functional and recognition characteristics.[75] This question will not easily be resolved: a man with one watch knows what time it is but a man with two watches is never quite sure!

$GABA_B$ Receptors

GABA also activates metabotropic $GABA_B$ receptors, which are widely distributed within the central nervous system and also in peripheral autonomic terminals.[5] Their activation causes an inhibition of both basal and forskolin stimulated adenylate cyclase activity together with a decrease in Ca^{2+} and an increase in K^+ conductance in neuronal membranes. The receptors are activated by baclofen, used in the treatment of spasticity, (*R*)-baclofen being the active isomer (Figure 1). There is evidence that $GABA_B$ receptor agonists may be useful in the treatment of pain and to reduce the craving for drugs of addiction. There is limited information on the therapeutic potential of $GABA_B$ receptor antagonists but there is support for the idea that they may prove valuable in the treatment of absence epilepsy and as cognition enhancers.[76]

$GABA_B$ receptors have now been identified by expression cloning using the high affinity ligand CGP-64213.[77] Functional receptors are formed only after heterodimerization of $GABA_{(B1)}$ and $GABA_{(B2)}$ (previously known as GBR1 and GBR2) by interaction through their C-termini, the first time that this form of 1:1 stoichiometry has been identified within this family.[78-81] Both subunits are members of the 7-transmembrane receptor family that show over 30% sequence homology to the metabotropic glutamate receptors. A number of splice variants have been identified for both $GABA_{(B1)}$ and $GABA_{(B2)}$.[82] The mRNA encoding $GABA_{(B2)}$ is found exclusively in neurones but $GABA_{(B1)}$ is found in both neurones and glia. Immunoprecipitation studies suggest that $GABA_{(B1)}$ and $GABA_{(B2)}$ are always found as heterodimers although the paucity of mRNA for $GABA_{(B2)}$ in the striatum has led to suggestions that further subunits remain to be identified.[82] There have been suggestions that the two most widely studied splice variants $GABA_{(B1a)}$ and $GABA_{(B1b)}$ my be differentially located within the cell, the former being pre-synaptic while the latter is found post-synaptically. The large deletion at the N-terminus which occurs in splice variant $GABA_{(B1b)}$ is consistent with a differential subcellular localisation and it will be interesting to see the whether or not this is region specific.

Although a significant number of phosphinic acid derivatives have been synthesised as $GABA_B$ receptor agonists few exceed the potency of (*R*)-baclofen and none has proved useful in the

Table 2. Comparative pharmacology of GABA receptors

Compound	$GABA_A$	$GABA_B$	$GABA_C$	Reference
GABA	Agonist	Agonist	Agonist	
Muscimol	Agonist	Inactive	Partial agonist	6, 8, 91
Isoguvacine	Agonist	Inactive	Antagonist	6, 8
THIP	Agonist	Inactive	Antagonist	6, 8
P4S	Agonist	Inactive	Antagonist	6, 8
TACA	Agonist	Inactive	Agonist	8
CACA	Inactive	Inactive	Partial agonist	8
(*R*)-Baclofen	Inactive	Agonist	Inactive	6, 8
Bicuculline	Antagonist	Inactive	Inactive	6, 8
Picrotoxin	Antagonist	Inactive	Antagonist	6, 8
CGP 35348	Inactive	Antagonist	Inactive	76
CGP 54626	Inactive	Antagonist	Inactive	76
CGP 64213	Inactive	Antagonist	Inactive	76
SCH 50911	Inactive	Antagonist	Inactive	76
TPMPA	Inactive	Inactive	Antagonist	8, 86, 87

(Bold text denotes compounds available from Tocris)

differentiation of the distinct receptor subtypes. However, recently it has been reported that gabapentin selectively activates the $GABA_{(B1a,2)}$ heterodimer coupled to inwardly rectifying potassium channels expressed in *Xenopus* oocytes but this did not occur with $GABA_{(B1b,2)}$ or $GABA_{(B1c,2)}$.[83] While the early specific $GABA_B$ receptor antagonists suffered from a limited potency with phaclofen, for example, displaying an affinity of only 100 μM, a number of selective, high affinity and systemically active antagonists are now available, although most rely on the phosphinic acid moiety (Figure 2). It is undoubtedly only a matter of time before subtype specific antagonists will become available.

Like the metabotropic glutamate receptors, both the $GABA_{(B1)}$ and $GABA_{(B2)}$ subunits possess a large extracellular N-terminal tail which has been modelled by homology on the bacterial periplasmic binding protein motif characterised by two globular domains connected by a hinge region. It has been suggested that agonist activation relies on the closure of the two globular domains subsequent to agonist binding: the venus fly-trap model.[84] A limited number of mutagenic studies have been carried out to delineate the residues important in agonist and antagonist recognition[85] but the nature of the interaction between the two proteins within the heterodimer, in both ligand recognition and their liaison with the G-proteins integral to receptor function remains to be investigated.

The $GABA_B$ receptors remain somewhat forlorn. Their ubiquitous distribution within the CNS, their proven importance in the modulation of transmitter release and the late inhibitory postsynaptic potential promise a good deal as targets for pharmacological intervention. However, this has been compromised, until recently, by the paucity of potent, systemically active and selective ligands. The molecular cloning of their constituent cDNAs coupled with the discovery that they function as heterodimers has now engendered a new urgency to research in this arena and the future promises to be exciting.

Conclusions

Interest in the receptors for GABA, the major inhibitory transmitter in the CNS, has been developed, with varying degrees of enthusiasm, over the past 40 years. We now have agonists and antagonists which allow us to differentiate, experimentally at least, between responses mediated by the three pharmacologically distinct receptor families with which they interact (Table 2). The information base is most extensive for the $GABA_A$ receptors, driven largely by observations that these proteins are the targets for a number of drugs with significant clinical importance. The expansion continues with the conviction that this almost bewildering complexity can be harnessed for the next generation of pharmacological agents with a more restricted profile of activity. The $GABA_B$ receptors remain the rather poor cousins in the sense that their potential is not yet realised. The developments over the past 7 years or more have delivered a promissory note that is producing significant investment and many hold real conviction in their future. Surveys of the literature would suggest that the $GABA_C$ receptors have generated rather more interest from the nomenclaturists than is warranted. However, recent evidence that their distribution is more diverse than previously thought[88] could provide a further extension to the potential inhibitory control of the central nervous system.

References

1. **Krnjevic and Schwartz** (1967) Exp.Brain Res. ***3*** 320. 2. **Curtis** *et al* (1970) Nature ***226*** 1222. 3. **Curtis** *et al* (1968) Exp.Brain Res. ***5*** 1. 4. **Kelly** *et al* (1969) Exp.Brain Res. ***7*** 11. 5. **Bowery** *et al* (1981) Eur.J.Pharmacol. ***71*** 53. 6. **Hill and Bowery** (1981) Nature ***290*** 149. 7. **Johnston** *et al* (1975) J.Neurochem. ***24*** 157. 8. **Johnston** (1996) TiPS ***17*** 319. 9. **Palacios** *et al* (1981) Brain Res ***222*** 285. 10. **Krogsgaard-Larsen** *et al* (1984) in Actions and interactions of GABA and the benzodiazepines, p109, Publ Raven. 11. **Stephenson** (1988) Biochem.J. ***249*** 21. 12. **Fisher and Olsen** (1986) in Benzodiazepine/GABA chloride channels: structural and functional properties, p241, Alan R. Liss. 13. **Newell** *et al* (2000) J.Biol.Chem. ***275*** 14198. 14. **Dichter** (1980) Brain Res. ***190*** 111. 15. **Sakmann** *et al* (1983) J.Neural.Transm.suppl. ***18*** 83. 16. **Bormann and Clapham** (1985) Proc.Natl.Acad.Sci.USA ***82*** 2168. 17. **Adams and Brown** (1975) J.Physiol. ***250*** 85. 18. **Akaike** *et al* (1987) J.Physiol. ***391*** 219. 19. **Mathers** (1987) Synapse ***1*** 96. 20. **Barker** *et al* (1982) J.Physiol. ***322*** 365. 21. **Barker and Mathers** (1981) Science ***212*** 358. 22. **Hamill** *et al* (1981) Pflugers Arch. ***391*** 85. 23. **Bormann** *et al* (1987) J.Physiol. ***385*** 243. 24.**Macdonald** *et al* (1989) J.Physiol. ***410*** 479. 25.**Twyman** *et al* (1990) J.Physiol. ***423*** 193. 26.**Bowery** *et al* (1976) Br.J.Pharmacol. ***57*** 435. 27.**Twyman** *et al* (1992) J.Physiol. ***445*** 97. 28.**Richards** *et al* (1987) J.Neurosci. ***7*** 1866. 29. **Stephenson and Barnard** (1986) in Benzodiazepine/GABA chloride channels: structural and functional properties, p261, Alan R. Liss. 30. **Schofield** *et al* (1987) Nature ***328*** 221. 31. **Hevers and Luddens** (1998) Mol.Neurobiol. ***18*** 35. 32. **Nayeem** *et al* (1994) J.Neurochem. ***62*** 815. 33. **Whiting** *et al* (1996) Int.Rev.Neurobiol. ***38*** 95. 34. **Wisden** *et al* (1992) J.Neurosci. ***12*** 1040. 35. **Farrar** *et al* (1999) J.Biol.Chem. ***274*** 10100. 36. **Amin and Weiss** (1993) Nature ***366*** 565. 37. **Smith and Olsen** (1995) TiPS ***16*** 162. 38. **Brejc** *et al* (2001) Nature ***411*** 269. 39. **Squires and Braestrup** (1976) Nature ***266*** 732. 40. **Mohler and Okada** (1976) ***267*** 65. 41. **Sieghart** (1995) Pharmacol.Rev. ***47*** 181. 42. **Duncalfe** *et al* (1996) J.Biol.Chem. ***271*** 9209. 43. **Wieland** *et al* (1992) J.Biol.Chem. ***267*** 1426. 44. **Squires** *et al* (1979) Pharmacol.Biochem.Behav. ***10*** 825. 45. **Nielsen and Braestrup** (1980) Nature ***286*** 606. 46. **Pritchett and Seeburg** (1990) J.Neurochem. ***54*** 1802. 47. **Hadingham** *et al* (1993) Mol.Pharmacol. ***43*** 970. 48. **Barnard** *et al* (1998) Pharmacol.Rev. ***50*** 291. 49. **Tenen and Hirsch** (1980) Nature ***288*** 609. 50. **Cowan** *et al* (1981) Nature ***290*** 54. 51. **Barker** *et al* (1984) in Actions and interactions of GABA and the benzodiazepines, p203, Publ. Raven. 52. **Smiechen** *et al* (1993) in Anxiolytic β-carbolines: from molecular biology to the clinic, p7, Springer Verlag. 53. **Culiat** *et al* (1995) Nature Genet. ***11*** 344. 54. **Gunther** *et al* (1995) Proc.Natl. Acad.Sci.USA ***92*** 7749. 55. **Nusser** *et al* (1999) Eur.J.Neurosci. ***11*** 1685. 56. **Ruddolph** *et al* (2001) TiPS ***22*** 188. 57. **Martin and Lattman** (1999) Exp.Opin.Ther.Patents ***9*** 1347. 58. **Study and Barker** (1981) Proc.Natl.Acad.Sci.USA ***78*** 7180. 59. **Twyman** *et al* (1989) Ann.Neurol. ***25*** 213. 60. **Pritchett** *et al* (1989) Nature ***338*** 582. 61. **Nicoll** *et al* (1975) Nature ***258*** 625. 62. **Simmonds** (1981) Br.J.Pharmacol. ***73*** 739. 63. **Harrison and Simmonds** (1984) Brain Res. ***323*** 287. 64. **Cottrell** *et al* (1987) Br.J.Pharmacol. ***90*** 491. 65. **Harrison** *et al* (1987) J.Neurosci. ***7*** 604. 66. **Twyman and Macdonald** (1992) J.Physiol. ***456*** 215. 67. **Adkins** *et al* (2001) J.Biol.Chem. ***276*** 38934. 68. **Smith** *et al* (1998) Nature ***392*** 926. 69. **Smith** *et al* (1998) J.Neurosci. ***18*** 5275. 70. **Franks and Lieb** (1998) Toxicol.Lett. ***100*** 1. 71. **Belelli** *et al* (1997) Proc.Natl.Acad.Sci. USA ***94*** 11031. 72. **Thompson and Wafford** (2001) Curr.Opin. Pharmacol. ***1*** 78. 73. **Enz and Cutting** (1998) Vision Res. ***38*** 1431. 74. **Enz and Cutting** (1999) Eur.J.Neurosci. ***11*** 41. 75. **Bormann** (2000) TiPS ***21*** 16. 76. **Bowery and Enna** (2000) J.Pharmacol.Exp. Ther. ***292*** 2. 77. **Kaupmann** *et al* (1997) Nature ***368*** 239. 78. **Jones** *et al* (1998) Nature ***396*** 674. 79. **Kaupmann** *et al* (1998) Nature ***396*** 683. 80. **White** *et al* (1998) Nature ***396*** 679. 81. **Kuner** *et al* (1999) Science ***283*** 74. 82. **Billinton** *et al* (2001) TiNS ***24*** 277. 83. **Ng** *et al* (2001) Mol.Pharmacol. ***59*** 144. 84. **Galvez** *et al* (1999) J.Biol.Chem. ***274*** 13362. 85. **Galvez** *et al* (2000) Mol.Pharmacol. ***57*** 419. 86. **Murata** *et al* (1996) Bioorg.Med.Chem.Lett. ***6*** 2071. 87. **Raggozino** *et al* (1996) Mol.Pharmacol. ***50*** 1024. 88. **Schmidt** *et al* (2001) J.Neurosci. ***21*** 691. 89. **Quirk** *et al* (1996) Neuropharmacology ***35*** 1331. 90. **Hadingham** *et al* (1993) Mol. Pharmacol. ***43*** 970. 91. **Zhang** *et al* (2001) TiPS ***22*** 121.

GABA Receptor Compounds available from Tocris

$GABA_A$ Receptor Compounds

Agonists

0344	GABA	Endogenous agonist
0235	Isoguvacine	Specific $GABA_A$ agonist
0289	Muscimol	Potent $GABA_A$ agonist
0381	Quisqualamine	Weak $GABA_A$ agonist
0181	TACA	$GABA_A$ agonist. Also GABA-T substrate and GABA uptake inhibitor
0807	THIP	$GABA_A$ agonist
0180	ZAPA	Agonist at 'low affinity' $GABA_A$ receptor. More potent than GABA/muscimol

Antagonists

0130	(+)-Bicuculline	Potent $GABA_A$ antagonist
0109	(-)-Bicuculline methobromide	Water soluble $GABA_A$ antagonist
0131	(-)-Bicuculline methochloride	Water soluble $GABA_A$ antagonist
1128	Picrotoxin	$GABA_A$ receptor antagonist
1262	SR 95531	Selective, competitive $GABA_A$ receptor antagonist

Other

0311	CHEB	Convulsant barbiturate
0881	Chlormethiazole	Potentiates $GABA_A$ receptor function
0505	Dihydroergotoxine	Binds with high affinity to $GABA_A$ receptor Cl^- channel
1471	Etomidate	GABA mimetic and GABA modulatory agent
1295	Loreclezole	Subtype-selective $GABA_A$ receptor modulator
0830	Primidone	Potentiates $GABA_A$ receptor function

Benzodiazepines and Related Compounds

0865	1-Amino-5-bromouracil	Agonist at benzodiazepine-$GABA_A$ site
0405	β-CCB	Inverse agonist, putative endogenous ligand
0612	β-CCP	Inverse agonist
0456	Chlormezanone	Skeletal muscle relaxant
0554	FG-7142	Inverse agonist
0658	FGIN-1-27	Potent, specific ligand for mitochondrial DBI receptor
0659	FGIN-1-43	Potent, specific ligand for mitochondrial DBI receptor
1328	Flumazenil	Benzodiazepine antagonist
0770	GBLD 345	High affinity agonist
1327	L-655,708	Selective for α5-containing $GABA_A$ receptors
R1327	[^{3}H]-L-655,708 ☢	Radiolabelled form of (1327)
0670	PK-11195	Antagonist at peripheral BDZ receptors
0655	Zolpidem *	Benzodiazepine agonist
1094	Zopiclone	Benzodiazepine agonist

$GABA_B$ Receptor Compounds

Agonists

0417	(*RS*)-Baclofen	Selective $GABA_B$ agonist
0796	(*R*)-Baclofen	Active enantiomer
0344	GABA	Endogenous agonist
0379	SKF 97541	Extremely potent $GABA_B$ agonist

Antagonists

1245	CGP 35348	Brain penetrant, selective $GABA_B$ antagonist
1247	CGP 46381	Brain penetrant, selective $GABA_B$ antagonist
1246	CGP 52432	Potent, selective $GABA_B$ antagonist
1088	CGP 54626	Potent, selective $GABA_B$ antagonist
R1088	[^{3}H]-CGP 54626 ☢	Radiolabelled form of (1088)
1248	CGP 55845	Potent, selective $GABA_B$ antagonist
0245	2-Hydroxysaclofen	Selective $GABA_B$ antagonist, more potent than saclofen (0246)
0178	Phaclofen	Weak, selective $GABA_B$ antagonist
0246	Saclofen	Selective $GABA_B$ antagonist
0984	SCH 50911	Selective, competitive, orally active $GABA_B$ antagonist

Other

1513	CGP 7930	Positive modulator at $GABA_B$ receptors
1514	CGP 13501	Positive modulator at $GABA_B$ receptors

$GABA_C$ Receptor Compounds

Agonists

0179	CACA	Partial $GABA_C$ agonist
0344	GABA	Endogenous agonist
0815	Imidazole-4-acetic acid	Partial $GABA_C$ agonist
0289	Muscimol	Partial $GABA_C$ agonist
0181	TACA	$GABA_C$ agonist

Antagonists

R1301	[^{3}H]-P4MPA ☢	$GABA_C$ antagonist radioligand
0379	SKF 97541	Potent $GABA_B$ agonist. Also $GABA_C$ antagonist
0807	THIP	$GABA_C$ antagonist
1040	TPMPA *	Selective $GABA_C$ antagonist
0180	ZAPA	$GABA_C$ antagonist

Inhibitory Amino Acid Uptake Inhibitors

0206	β-Alanine	Distinguishes GABA transporters
1296	CI 966	Selective inhibitor of GAT1
0234	Guvacine	Specific GABA uptake inhibitor
0236	(±)-Nipecotic acid	GABA uptake inhibitor
0768	Riluzole	GABA uptake inhibitor. Also glutamate release inhibitor
1081	SKF 89976A	Potent GABA uptake inhibitor. Penetrates blood brain barrier
0181	TACA	GABA uptake inhibitor. Also $GABA_A$ agonist and substrate for GABA-T

Miscellaneous GABA/Glycine Receptor Compounds

0806	Gabapentin	Anticonvulsant. Increases brain GABA
0538	*trans*-4-Hydroxycrotonic acid	GHB receptor ligand
1260	Ivermectin	Modulates glutamate/GABA-activated Cl^- channels
0386	3-Methyl-GABA	Activator of GABA amino-transferase
0780	NCS-382	Antagonist of γ hydroxybutyric acid
0939	Propofol	Potentiates $GABA_A$ receptor-mediated inhibition and inhibits NMDA receptor-mediated excitation
0808	Vigabatrin	GABA-T inhibitor

***Local regulations may restrict the sale of these products in certain territories. Please consult your local Tocris Cookson office or distributor for further details.**

GABA Receptors, Tocris Reviews No. 20, March 2002

Published and distributed by Tocris Cookson, Bristol, UK

Editors: Samantha Manley, Ph.D., Natalie Barker, B.Sc.
Design and Production: Jane Champness

GABA[Rev](0302)

Tocris Cookson Ltd.
Northpoint Fourth Way
Avonmouth Bristol BS11 8TA UK
Tel: + 44 (0)117 982 6551
Fax: + 44 (0)117 982 6552
customerservice@tocris.co.uk
technicalsupport@tocris.co.uk

Tocris Cookson Inc.
16144 Westwoods Business Park
Ellisville MO 63021 USA
Toll Free Tel: (800) 421-3701
Toll Free Fax: (800) 483-1993
tocris@mo.net