

# Development of Subtype Selective GABA<sub>A</sub> Modulators

By Gerard R. Dawson, PhD, Neil Collinson, PhD, and John R. Atack, PhD

## FOCUS POINTS

- $\gamma$ -aminobutyric acid type A (GABA<sub>A</sub>) receptors are widely distributed in the brain, however, the subunit composition of the receptor differs between brain regions.
- Non-selective benzodiazepines- (BZs) type drugs, such as diazepam, have beneficial effects in a number of disorders including epilepsy, insomnia, and anxiety. However, they also have many side effects including daytime somnolence, muscle relaxation, and cognitive disruption.
- GABA<sub>A</sub> receptor subtypes mediate specific effects of BZs such as their sedative or anti-anxiety efficacy.
- Compounds modulating specific GABA<sub>A</sub> receptor subtypes via the BZ-binding site can retain the beneficial effects of non-selective BZs without specific side effects leading the way to more efficacious drugs.

## ABSTRACT

Drugs modulating  $\gamma$ -aminobutyric acid (GABA) transmission via the benzodiazepine (BZ) site on the  $\gamma$ -aminobutyric acid type A (GABA<sub>A</sub>) receptor have been in widespread use for more than 40 years to treat anxiety, epilepsy, and sleep disorders. These drugs have been shown to be safe, well tolerated, and effective although the mechanism by they produce a myriad of pharmacologic effects remains elusive. In recent years it has been discovered that, although the GABA<sub>A</sub> receptor is widely distributed in the brain, the substructure and composition of the receptor differs from between brain regions. Termed "GABA<sub>A</sub> receptor subtypes" their discovery leads to speculation that different subtypes may mediate specific effects of BZs such as anxiety or sedation. The phenotypic analysis of transgenic knock-in and knock-out mice in which particular GABA<sub>A</sub> receptors were rendered insensitive to the effects of BZ while others

were unaffected confirmed this speculation. Subsequently, subtype-specific GABA<sub>A</sub> ligands were developed that, for example, retained the anxiolytic effects of BZs but were devoid of their sedative effects. Therefore, it may be possible to develop effective anxiolytic compounds that have a much reduced side-effect profile compared with existing drugs.

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## INTRODUCTION

$\gamma$ -aminobutyric acid (GABA) is accepted as the principal inhibitory neurotransmitter in the vertebrate central nervous system (CNS). The widespread distribution of GABA and the fact that virtually all neurons are sensitive to its inhibitory effect suggests that its function is ubiquitous in the brain. Depending upon the brain region, it is estimated that 20% to 50% of all central synapses use GABA as their transmitter.<sup>1</sup> GABA is formed from glutamate by the action of glutamic acid decarboxylase and is inactivated by a transamination reaction in which the amino group is transferred to  $\alpha$ -oxoglutaric acid (to yield glutamate) in a reaction catalysed by GABA-transaminase.

Within the CNS, GABA exerts its effects via interactions with bicuculline-sensitive, ionotropic GABA type A (GABA<sub>A</sub>) receptor and the baclofen-sensitive metabotropic GABA type B receptors. The ionotropic GABA type C receptors, which are comprised of proteins that are related to GABA<sub>A</sub> receptor subunits,<sup>2</sup> are found primarily in the retina.<sup>3</sup> GABA<sub>A</sub> receptors are ligand-gated chloride ion channels which in addition to their agonist (GABA) binding sites also contain recognition sites for a number of pharmacologically and clinically important drugs, such as benzodiazepines (BZs), barbiturates, steroids, anaesthetics, and anticonvulsants.<sup>1</sup> Many of these drugs exert at least part of their clinically relevant effects by interaction with discrete allosteric binding sites on GABA<sub>A</sub> receptors.<sup>4</sup> Because of its

Dr. Dawson is senior director, Dr. Collinson is research biologist, and Dr. Atack is senior investigator at Merck Sharp & Dohme Research Laboratories in Essex, England.

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Please direct all correspondence to: John R. Atack, PhD, Neuroscience Research Centre, Merck Sharp & Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, England; E-mail: j\_atak@merck.com

therapeutic relevance the GABA<sub>A</sub> receptor, and more especially the BZ binding site, has been the focus of considerable attention and the elucidation of the molecular structure and physiological functions of different GABA<sub>A</sub> receptor populations has formed the basis for strategies to develop drugs which selectively modulate particular subtypes.

### STRUCTURE, COMPOSITION AND SUBUNIT STOICHIOMETRY OF GABA<sub>A</sub> RECEPTORS

Initial evidence for heterogeneity of GABA<sub>A</sub> receptors was based on pharmacologic analyses. Thus, by using radiolabelled BZ site ligands, it was shown that BZ binding sites were not a homogeneous population. As a result, the existence of two BZ receptor subtypes was postulated on the basis of the different binding profiles of CL 218,872, type I having a high affinity for CL 218,872 and type II having a low affinity for CL 218,872.<sup>5</sup>

Biochemical evidence of BZ binding heterogeneity came from photoaffinity labelling studies using [<sup>3</sup>H]flunitrazepam gel electrophoresis which showed that labelled protein separated into several polypeptide bands—results interpreted as indicating receptor heterogeneity.<sup>6</sup> The purification of the GABA<sub>A</sub> receptor from bovine brain provided a major step towards the molecular cloning of the receptor.<sup>7</sup> Oligonucleotide probes were subsequently designed and two complementary DNA (cDNA) sequences were initially identified and were referred to as  $\alpha_1$  and  $\beta_1$ . Further screening of cDNA revealed a third sequence sharing 79% identity with  $\alpha_1$  and only 34% with  $\beta_1$  and was thus referred to as  $\alpha_2$ .<sup>8</sup> The screening of brain cDNA libraries using probes based on sequences conserved between the initially identified subunits subsequently identified a family of GABA<sub>A</sub> receptor subunit genes. These polypeptides are classified by the relative degree to which they share amino acid sequences. Within a subgroup, such as the  $\alpha$  subunits, there is 70% to 80% sequence identity, whereas between subgroups, such as between the  $\alpha$  and  $\beta$  subunits, there is only 30% to 40% sequence identity.<sup>9</sup>

The GABA<sub>A</sub> receptor is a member of a superfamily of ligand-gated ion channels, which includes the serotonin type 3 receptor, the nicotinic acetylcholine receptor and the glycine receptor.<sup>10</sup> Similar to the other members of this receptor family, GABA<sub>A</sub> receptors seem to be heterooligomeric assemblies of five subunits.<sup>11,12</sup> To date, 16 GABA<sub>A</sub> receptor subunits have been cloned from the mammalian nervous system:  $\alpha_{1-6}$ ,  $\beta_{1-3}$ ,  $\gamma_{1-3}$ ,  $\delta$ ,  $\epsilon$ ,  $\pi$ ,  $\theta$ , with an additional three related genes,  $\rho_{1-3}$ , encoding

GABA<sub>C</sub> receptors.<sup>2,13</sup> The subunits of the GABA<sub>A</sub> receptor could theoretically be assembled into thousands of potential pentameric assemblies. Studies using various expression systems have shown that a combination of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunit variants are required for the expression of fully functional GABA<sub>A</sub> receptors.<sup>14</sup> Indeed, most GABA<sub>A</sub> receptors are composed of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits.<sup>2,15</sup> Subunit-specific antibodies along with quantitative immunoprecipitation have allowed the determination of the subunit composition, stoichiometry and relative abundance of native GABA<sub>A</sub> receptor subtypes to be determined and demonstrated that relatively few of the theoretically possible subunit combinations occur in vivo.<sup>12,15-18</sup>

The most abundant subunits as determined by immunoprecipitation experiments are  $\alpha_1$ ,  $\beta_2$ , and  $\gamma_2$ , representing 60% to 90% of GABA<sub>A</sub> receptors in adult rat brain.<sup>15,16,19-21</sup> The  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_5$ ,  $\beta_3$ , and  $\delta$  subunits are less abundant and represent 15% to 30% of GABA<sub>A</sub> receptors in the rat brain<sup>19,21-24</sup> while the remaining subunits ( $\alpha_4$ ,  $\alpha_6$ ,  $\beta_1$ ,  $\gamma_1$ ,  $\gamma_3$ ) are least abundant, each representing <10% of GABA<sub>A</sub> receptors.<sup>26-28</sup> Taken together, these studies show that the majority of native GABA<sub>A</sub> receptors are comprised of two  $\alpha$ , two  $\beta$ , and one  $\gamma$  subunit<sup>10,29</sup> with the actions of 'classical' BZ agonists, such as diazepam, being exerted via receptors containing  $\beta$ ,  $\gamma_2$  and either an  $\alpha_1, \alpha_2, \alpha_3$ , or  $\alpha_5$  subunits.<sup>1,15</sup>

Less prevalent receptors appear to be composed of  $\alpha\beta\delta$ ,  $\alpha\beta\epsilon$ ,  $\alpha\beta\pi$ , or  $\alpha\beta$  subunits.<sup>10,15</sup> Thus, the  $\delta$ ,  $\epsilon$ , and  $\pi$  subunits seem to be able to replace the  $\gamma$  subunit in GABA<sub>A</sub> receptors,<sup>30-33</sup> whereas the  $\theta$  subunit may be able to replace a  $\beta$  subunit in these receptors.<sup>34</sup> The  $\rho$  subunits that constitute GABA<sub>C</sub> receptors do not appear to be able to combine with other classes of GABA<sub>A</sub> receptor subunits<sup>35</sup> but instead form homo- and heterooligomeric channels with each other.<sup>35</sup>

Since members of the GABA<sub>A</sub> receptor subunit family are related to the nicotinic acetylcholine receptor,<sup>10</sup> the structure of the GABA<sub>A</sub> receptor has been modeled<sup>29,36</sup> using the acetylcholine binding protein as a template.<sup>37</sup> These data, along with studies by Minier and Sigel<sup>38,39</sup> have shown the major GABA<sub>A</sub> receptor populations possess an  $\alpha\beta\alpha\beta\gamma$  arrangement of subunits as viewed from the extracellular side of the receptor. GABA binding sites occurring at the interface of  $\alpha$  and  $\beta$  subunits and the BZ binding site occurring at the interface of  $\alpha$  and  $\gamma$  subunits. Figure 1 shows a schematic illustration of a GABA-gated ion channel with the BZ and GABA binding sites indicated.

## ANATOMICAL AND FUNCTIONAL HETEROGENEITY OF GABA<sub>A</sub> RECEPTOR SUBTYPES IN VIVO

The structural complexity and variety of GABA<sub>A</sub> receptors is compounded by the anatomical heterogeneity of GABA<sub>A</sub> receptor subunits. Thus immunocytochemical and mRNA in situ hybridization has been used to demonstrate that different GABA<sub>A</sub> receptor subunits have discrete neuroanatomical localizations, which suggests that different receptor populations possess discrete physiological functions.<sup>28,40,41</sup> For example, the major GABA<sub>A</sub> receptor subtype is assembled from the subunits  $\alpha_1\beta_2\gamma_2$  with only few brain regions showing no expression of this receptor (granule cell layer of the olfactory bulb, reticular nucleus of the thalamus and spinal cord motoneurons).<sup>29,41</sup> Receptors containing the  $\alpha_2$  or  $\alpha_3$  subunit are in less abundant and are highly expressed in brain regions where the  $\alpha_1$  subunit is present at low levels.  $\alpha_2$  and  $\alpha_3$  subunits are frequently coexpressed with the  $\beta_3$  and  $\gamma_2$  subunits, which is particularly evident in hippocampal pyramidal neurons ( $\alpha_2\alpha_3\gamma_2$ ) and in cholinergic neurons of the basal forebrain ( $\alpha_3\beta_3\gamma_2$ ). The ligand binding

profile of these receptors is different from that of  $\alpha_1\beta_2\gamma_2$  by having a lower displacement potency for ligands with non-BZ structures such as the triazolopyridazine CL 218,872 and the imidazopyridine zolpidem.<sup>42-44</sup>

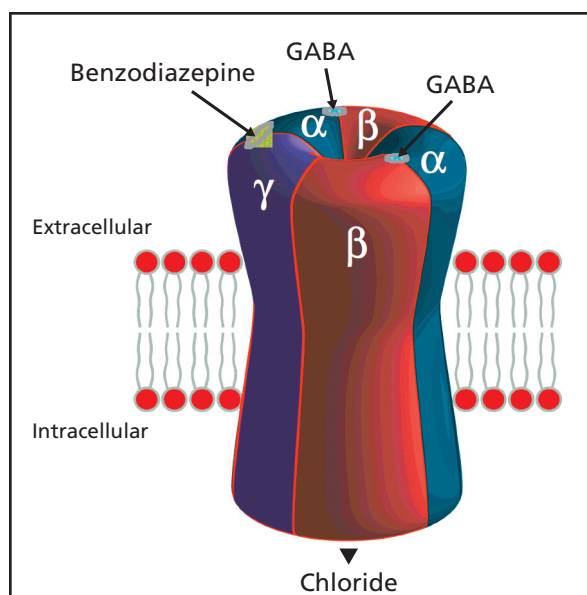
The results from these experiments showed that the  $\alpha_1$  subunit displayed a ubiquitous distribution and that there was consistent co-expression of  $\alpha_1$  and  $\beta_2$  subunits, which often co-localized with the  $\gamma_2$  subunit. In contrast, the  $\alpha_6$  subunit was only expressed in the cerebellum. Co-localization of the  $\alpha_2$  and  $\beta_3$  subunits was also apparent and predominated in the amygdala, hippocampus, and hypothalamus. Also of interest was the expression of the  $\alpha_5$  subunit which was restricted predominantly to the hippocampus, and that it appeared to co-localize with  $\beta_1$  mRNA. This hippocampus-specific distribution has been confirmed using radioligands selective for  $\alpha_5$  GABA<sub>A</sub> compared with GABA<sub>A</sub>  $\alpha_1$ , GABA<sub>A</sub>  $\alpha_2$ , or GABA<sub>A</sub>  $\alpha_3$  receptors.<sup>26,45,46</sup>

The normal and pharmacologically mediated functions of GABA receptors has been investigated in mice in which particular GABA receptor subunits are absent or have been mutated to alter their physiological and/or pharmacologic responsiveness.<sup>47,48</sup> Mice in which point mutations have been introduced into the  $\alpha$  subunit have been particularly useful in establishing which GABA<sub>A</sub> receptor subtypes mediate specific components of the pharmacologic response produced by diazepam.

### THE BENZODIAZEPINE BINDING SITE

For more than 40 years, BZs have been in widespread use as drugs to treat anxiety, epilepsy and sleep disorders. The so-called "classical" BZs are characterised structurally by a 1,4-BZ core structure to which a 5-phenyl group is attached, such as diazepam, chlordiazepoxide, clonazepam, triazolam, alprazolam, and flunitrazepam. BZs are in widespread use and have been shown to be safe, well-tolerated and effective treatments as either anxiolytics or hypnotics. Aside from their acute, sedating effects when used as anxiolytics, the main issue regarding BZ use is their dependence liability and withdrawal symptoms following discontinuation.<sup>49,50</sup>

The initial clinical use of BZs preceded any understanding of their mechanism of action. Indeed, it was not until some 15 years after the initial introduction of chlordiazepoxide in clinical practice that BZs were proposed to mediate their effects by facilitating GABAergic neurotransmission<sup>55,52</sup> and shortly thereafter it was demonstrated that they did indeed bind to high-affinity sites in the brain.<sup>5,53</sup> Colloquially referred to as "benzodiazepine receptors," these binding sites are in fact an integral part of the GABA<sub>A</sub>



**FIGURE 1.** A schematic representation of the GABA<sub>A</sub> receptor complex. Five subunits form a transmembrane chloride-gated ion channel. The receptor complex has binding sites for numerous molecules including the inhibitory neurotransmitter GABA and BZs. The locations of the GABA and BZ binding sites on the receptor (which occur at the interfaces of  $\alpha$  and  $\beta$  and  $\alpha$  and  $\gamma$  subunits, respectively) are also shown.

GABA= $\gamma$ -aminobutyric acid; GABA<sub>A</sub>= $\gamma$ -aminobutyric acid type A.  
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receptor complex. When a BZ receptor agonist binds to the GABA<sub>A</sub> receptor it positively enhances the effect of endogenous GABA when it binds to its site on the receptor. In the absence of GABA binding to its receptor BZs are without effects.

With the introduction of radioligand binding assays for BZ receptors<sup>5</sup> the search for endogenous BZs started, but despite many years searching none have been identified. However, many BZ site ligands have been identified including some that negatively modulated the effects of GABA, (eg, FG 7142 and methyl-6,7-dimethoxyl-4-ethyl- $\beta$ -carboline-3-carboxylate). Termed “inverse agonists,” these compounds had the opposite effects to agonists and were anxiogenic and proconvulsant in animals. To complete the spectrum, BZ site antagonists have been identified. These compounds, the prototypic example of which is flumazenil, are not only without intrinsic activity, further supporting the idea that no endogenous ligands for this recognition site exist, but can also block the modulating effects of both agonists and inverse agonists.<sup>54,55</sup> Recombinant GABA<sub>A</sub> receptors have added enormously to our understanding of the molecular basis of the BZ binding site.<sup>1,15</sup> Recombinant expression of GABA<sub>A</sub> receptor subunits have shown that an  $\alpha$ , a  $\beta$ , and a  $\gamma$  subunit is required to form a GABA<sub>A</sub> receptor with a BZ binding site receptor.<sup>42,43</sup> Further studies confirmed that the conformation of the  $\alpha$  subunit is rendered BZ-sensitive due to its interaction with the  $\gamma_2$  subunit. That is, the binding site for BZ ligands straddles the  $\alpha$  and  $\gamma$  subunit. GABA<sub>A</sub> receptors containing the  $\alpha_4$  or  $\alpha_6$  subunit are relatively insensitive to BZs.<sup>56,57</sup> Strikingly, this lack of sensitivity to BZs is due to a single amino acid residue in the  $\alpha$  subunit. In the BZ-insensitive  $\alpha_4$  and GABA<sub>A</sub>  $\alpha_6$  receptors, the histidine residue which confers BZ binding sensitivity in  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  receptors is replaced by arginine.<sup>58</sup> This molecular switch—from histidine to arginine—provided the basis of a set of experiments with transgenic knock-in mice and diazepam that began to unravel the role of GABA<sub>A</sub> receptor subtypes in the rich pharmacology of BZ ligands.

Subsequent studies using cell lines confirmed that mutating histidine 101 to an arginine ( $\alpha 1H101R$ ) rendered the receptor insensitive to diazepam, but it was otherwise indistinguishable from the wild-type receptor, that is GABA continued to bind normally to the mutated receptor and have the normal functional effects.<sup>59</sup> A number of groups<sup>60,61</sup> have exploited this observation and generated genetically modified mice in which the  $\alpha 1H101R$  mutation was introduced. These so-called knock-in mice have a

mutation knocked into, rather than knocked out, of the receptor as a means of investigating its function. In these animals diazepam continued to mediate its effects through the  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  subtypes, but not through the  $\alpha_1$  subtype. This allows the effects of diazepam at this subtype to be inferred from the behavior of the knock-in mice compared with wildtype mice when both are dosed with diazepam. The  $\alpha 1H101R$  knock-in mice bred normally and compared with wild-type mice, showed no differences in weight, balance, feeding, core body temperature, grip strength, or startle reflex, indeed they appeared identical to the wild-type mice. However, when the  $\alpha 1H101R$  knock-in mice were given diazepam (3–30 mg/kg PO) and their behavior assessed in a range of assays, a different picture emerged. In a rotarod assay diazepam significantly impaired the ability of wild-type mice to walk on the revolving rod at doses  $\geq 5$  mg/kg, while the  $\alpha 1H101R$  mice remained unaffected by diazepam at doses  $\geq 10$  mg/kg. These data indicated that the sedative/motor impairing effects of diazepam are mediated primarily through the  $\alpha_1$  subtype.<sup>61</sup> This finding revealed the intriguing possibility that although the sedative/muscle relaxant effects of BZs were mediated via the  $\alpha_1$  subtype the anxiolytic effects of BZs may be mediated via the  $\alpha_2$ ,  $\alpha_3$ , or  $\alpha_5$  subtype. Further experiments with  $\alpha_2$  and  $\alpha_3$  knock-in mice confirmed this possibility.<sup>62</sup>

### SUBTYPE SELECTIVE COMPOUNDS

The molecular basis of the heterogeneity of CL 218,872 binding to native GABA<sub>A</sub> receptors, initially described as BZ I and BZ II receptors<sup>63</sup> became apparent with the cloning and expression of recombinant GABA<sub>A</sub> receptors.<sup>64</sup> Thus, the high-affinity BZI binding corresponded to binding to  $\alpha_1\beta\gamma_2$  receptors whereas CL 218,872 bound with lower affinity to  $\alpha_2\beta\gamma_2$ ,  $\alpha_3\beta\gamma_2$ , and  $\alpha_5\beta\gamma_2$  receptors; the combined population of which represented the native BZ2 receptors.<sup>43</sup> Furthermore, although, like CL 218872, zolpidem has higher affinity for GABA<sub>A</sub>  $\alpha_1$  receptors,<sup>64</sup> it could differentiate subtypes within the lower-affinity BZ2 receptor population. Hence, zolpidem has moderate affinity for  $\alpha_2$  and  $\alpha_3$  but essentially no affinity for GABA<sub>A</sub>  $\alpha_5$  receptors.<sup>43,44</sup> The novel pharmacology of GABA<sub>A</sub>  $\alpha_5$  receptors has permitted compounds, such as L-655708 and RY-80, with around 50-fold selectivity for GABA<sub>A</sub>  $\alpha_5$  compared with GABA<sub>A</sub>  $\alpha_1$ , GABA<sub>A</sub>  $\alpha_2$ , or GABA<sub>A</sub>  $\alpha_3$  receptors<sup>26,45,65</sup> to be identified. Although compounds with 10–20-fold selectivity for  $\alpha_1$  receptors (CL 218,872 and zolpidem) or around 50-fold selectivity for  $\alpha_5$  receptors (L-655708, RY-80) have been described,

there are no reports of compounds with similar degrees of binding selectivity for  $\alpha_2$  and/or  $\alpha_3$  receptors. In order to identify compounds which selectively activate  $\alpha_2$  and/or  $\alpha_3$  receptors, and might therefore prove to be non-sedating anxiolytics, a selective efficacy approach was adopted to identify L-838417. The basis of this strategy is that although a compound might bind with equivalent affinity at the BZ binding site of different GABA<sub>A</sub> receptor subtypes, if it possesses antagonist efficacy at a particular subtype (eg, the “sedation” subtype [ie,  $\alpha_1$  receptors]), then the functions of that GABA<sub>A</sub> receptor population should remain unaltered. On the other hand, agonist efficacy at other subtypes (eg, the “anxiolytic” subtypes [ie,  $\alpha_2$  and/or  $\alpha_3$  receptors]) would result in a selective modulation of those receptors in vitro and in vivo. This strategy was also extended to the identification of  $\alpha_5$  efficacy-selective compounds.

### L-838417

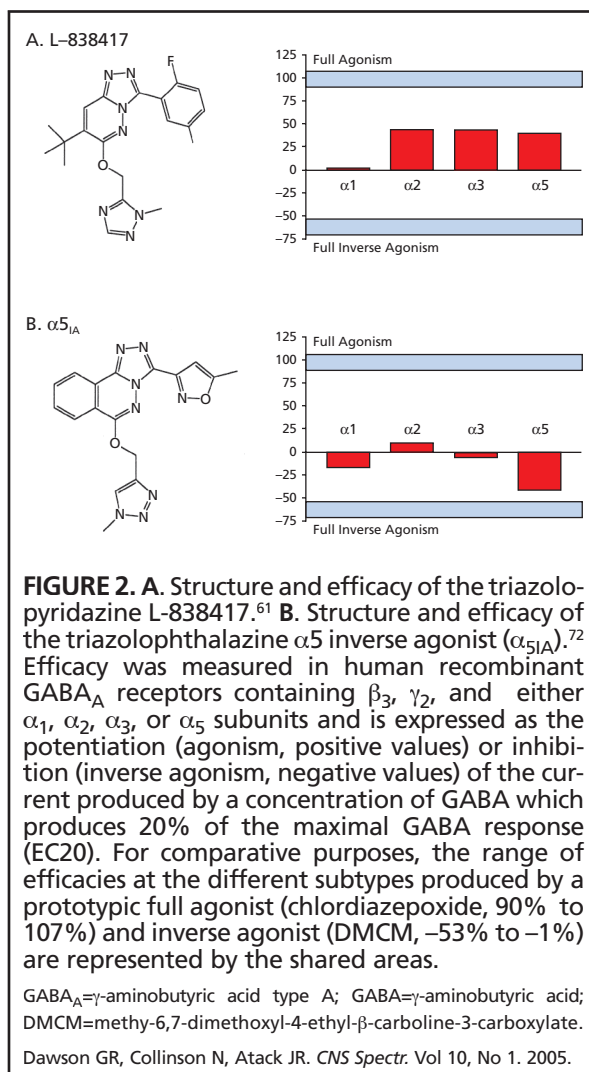
L-838417 has essentially equivalent nanomolar affinity for GABA<sub>A</sub>  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  receptors (0.7–2.3 nM), but much lower affinity at receptors containing an  $\alpha_4$  or  $\alpha_6$  subunit.<sup>61</sup> However, it is an antagonist at  $\alpha_1$  receptors and a partial agonist at the  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  subtypes (Figure 2A). As a result, L-838417 would be expected to exert no functional effects via this subtype, which based on the hypnotic properties of the  $\alpha_1$ -selective compound zolpidem<sup>66</sup> and the loss of diazepam-induced sedation in  $\alpha_1$  knock-in mice<sup>60,61</sup> has clearly been shown to be involved in sedation. Consequently, the in vivo effects produced by this compound can be attributed to its action at the  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  subtypes. A second compound, TP023, has a similar profile in that it binds with equal affinity for all BZ sensitive GABA<sub>A</sub> receptors but is a partial agonist albeit lower than L-838417 at  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  subtypes.

The in vivo activity of L-838417 was evaluated in wild-type mice and in several well characterized BZ-sensitive behavioral models in rats. L-838417 (30 mg/kg) did not impair mouse motor performance as measured using the rotarod. L-838417 was also evaluated in a more sensitive operant chain-pulling test in rats. Compounds such as FG 8205, which have a partial  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  efficacy profile similar to L-838417 but with an additional  $\alpha_1$  partial agonism and the full non-selective BZ agonist diazepam significantly impair performance in this model.<sup>67</sup> By comparison, L-838417 (0.3–30 mg/kg PO) was without effect at doses that occupied >95% of BZ-sensitive binding sites. In mice, L-838417 retained anticonvulsant activity in seizures induced chemically (by pentylenetetrazole) or audio-

genically at doses that occupied <50% of BZ binding sites. L-838417 induced anxiolytic-like activity in a rat the elevated plus maze and in fear-potentiated startle at doses that occupied <50% of the BZ binding sites. These observations in rodents have recently been extended to primates.<sup>68</sup> TP023 also has a robust anxiolytic effect in a range of rodent and primate assays designed to detect anxiolytic activity. Interestingly, neither compound induces overt signs of sedation or ataxia in either rodents or primates.

### $\alpha_5$ IA

In addition to compounds which have efficacy selective for the proposed anxiety ( $\alpha_2$  and/or  $\alpha_3$ ) compared with the sedation ( $\alpha_1$ ) subtypes of GABA<sub>A</sub> receptor, a compound ( $\alpha_5$ IA) with inverse agonist efficacy selective for the  $\alpha_5$  subtype has also been identified (Figure 2B). Based on its predominantly hippocampal localization<sup>26,28,40,41,45,46</sup> as well as data



from knock-out or knock-in mice<sup>69,70</sup> it would appear that the  $\alpha_5$  subtype plays a role in certain aspects of cognition.<sup>71</sup> In vivo,  $\alpha_5$ IA enhanced performance in a delayed matching to position version of the Morris water maze but was devoid of the proconvulsant and anxiogenic liabilities associated with the non-selective partial (FG 7142) or full (methyl-6,7-dimethoxy-4-ethyl- $\beta$ -carboline-3-carboxylate) inverse agonists.<sup>72</sup>

## CONCLUSION

While BZ site agonists have been proven over several decades to be safe and efficacious drugs, one of the key issues that have limited their utility is their multiple actions. Gene targeting approaches have been used to demonstrate that the  $\alpha_1$  subtype is mainly responsible for the sedative/motor effects of non-selective BZs, such as diazepam, while other subtypes are responsible for anxiolytic activity. The localization of  $\alpha_2$  and  $\alpha_3$  subtypes in amygdala and cortical regions would be consistent with these subtypes, rather than the hippocampally located  $\alpha_5$  subtype, being responsible for mediating the anxiolytic properties of BZs.<sup>69,70</sup> In the future, the combined use of both molecular genetic and pharmacological approaches should help clarify further the physiological roles of receptor subtypes.

The novel approach of identifying subtype-selective compounds based on selective efficacy rather than the more traditional and intuitive selective affinity approach has been exemplified preclinically not only using the non-sedating anxiolytic L-838417 but also the cognitive enhancer  $\alpha_5$ IA and it is hoped that this approach might generate GABA<sub>A</sub> subtype-selective drugs with increased clinical specificity. **CNS**

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