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GABA-A receptors: a viable target for novel anxiolytics?

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Benzodiazepine (BZ) anxiolytics mediate their clinical effects by enhancing the effect of γ-aminobutyric acid (GABA) at the GABA-A receptor. Classical BZ full agonists such as diazepam, which maximally enhance the function of GABA-A receptors, are effective anxiolytics but carry unwanted side effects including sedation, dependence and abuse liability, limiting their utility. Although a second generation of 'partial agonist' BZs have been pursued, promising preclinical data, in terms of anxiolytic efficacy and decreased unwanted effects, have so far failed to translate to the clinic. Following the insights into GABA-A receptor subtypes mediating the effects of BZs, a third generation of 'receptor subtype-selective' BZ site ligands have been developed. However, it remains to be determined whether promising preclinical data are recapitulated in the clinic.

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Introduction

Anxiety disorders are a prevalent and disabling set of diseases which continue to represent a significant disease burden [1]. They can be categorized further into several distinct subgroups, including generalised anxiety disorder (the largest group), panic disorder, social anxiety and various phobias. For about 30 years from the 1960s, the gold standard treatment of anxiety disorders were the benzodiazepines (BZs), exemplified by drugs such as Valium (diazepam) from Hoffmann la Roche. BZs had an improved safety profile over the barbiturate drugs that they largely replaced, and had a rapid onset of efficacy much valued by the patient. However, BZs were not perfect drugs [2], and their sedative properties, cognitive impairing effects and, perhaps most importantly of all, dependence and abuse liability has generated a significant negative perception in the eyes of the regulatory agencies, prescribing clinicians and the general public. As such, in recent years, anxiety disorders have frequently been treated with the antidepressant selective serotonin reuptake inhibitors (SSRIs) [3]. This is in large part because the SSRIs lack the side effects that beset the BZs, and also because anxiety is often comorbid with depressive disorders. The major disadvantage of SSRIs is their speed of onset of efficacy. This is generally of the order of several weeks, and is undoubtedly an important disadvantage in treating the symptoms of anxiety. Thus, an unmet medical need and market opportunity exists if an anxiolytic could be developed which has a rapid onset of action but lacks the unwanted effects of existing BZs. The evolving science and our understanding of the diversity of the GABA-A receptor family might represent a path forward to such a goal.

GABA-A receptors

γ-aminobutyric (GABA) is the major inhibitory neurotransmitter in the mammalian brain [4] and its effects are mediated through two types of receptors, the ionotropic GABA-A and the metabotropic GABA-B (as discussed in other reviews in this issue). The GABA-A receptor is a member of the ligand-gated ion channel superfamily exemplified by the nicotinic acetylcholine receptor [5]. Its activation by GABA opens the intrinsic ion channel, enabling flux of chloride through the channel into the cell, and subsequent hyperpolarization. Since the initial cloning of complementary DNAs encoding subunits of the GABA-A receptor in 1987, our understanding of the complexities and diversity of this receptor have dramatically increased. There are 16 different subunits (α1–α6, β1–β3, γ1–γ3, δ, ε, π, θ), or indeed 19 if the so-called GABA-C subunits are included (ρ1−ρ3) [6]. These subunits coassemble as pentamers, with the basic template thought to be two α subunits, two β subunits and a γ, δ, ε, π or θ subunit. Variations undoubtedly exist [7]. Receptors certainly exist with more than one type of α or β subunit [8]. GABA-A receptor subtypes have distinct patterns of expression in the brain, and indeed different domains of expression within the neurone, which presumably underlies their function. The most quantitatively prevalent receptor subtypes are those containing a γ2 subunit, which might constitute in excess of 80% of all GABA-A receptors [9].

Pharmacological tools, particularly BZ site ligands, have been key to increasing our understanding of both the GABA-A receptor family and the mode of action of clinically prescribed BZs. BZs are allosteric modulators of the receptor; that is, they potentiate (or, indeed, for some BZs, inhibit) the activity of GABA but have no intrinsic activity themselves [10,11]. Currently, the clinically prescribed BZs are all considered to be 'full agonists'; they are defined

as giving a maximal potentiation of GABA. Other ligands for the BZ site are known which give only a partial potentiation as compared with the full agonists; these are defined as 'partial agonists'. This concept of partial versus full agonism is important and is discussed further below in the context of the development of novel anxiolytics. Only receptors with a γ2 subunit (and perhaps a small population with a γ3 subunit) have a BZ binding site, and only α1βxγ2, α2βxγ2, α3βxγ2 and α5βxγ2 (βx meaning any β subunit) have high affinity for the classical BZs (now referred to as 'nonselective BZs') such as diazepam, lorazepam and clonazepam [9]. A class of BZ ligands was also found to have higher affinity for α1-containing receptors; these are exemplified by the hypnotic zolpidem [12]. Defining the molecular pharmacology of GABA-A receptors led to the suggestion that certain receptor subtypes might be responsible for mediating specific effects of BZs. Indeed, the α2βxγ2 and the α3βxγ2 receptor subtypes have, using either subtype-selective compounds [13] (as discussed below) or genetically modified mice [13–15] (see also review by Rudolph and Möhler in this issue), been proposed to mediate the anxiolytic effects of the BZs.

From our understanding of the mode of action of BZs, and the diversity of the GABA-A receptor family, two concepts have emerged as approaches to develop GABA-A anxiolytics with improved profiles. The first, earlier concept is that BZs which are partial agonists might retain anxiolytic activity but have a much reduced propensity to cause the unwanted effects such as sedation and withdrawal. The second concept is that the various effects of BZs are mediated by different receptor subtypes, and that by specific modulation of those receptor subtypes that mediate the desirable effects of BZs, it might be possible to develop improved BZs.

BZ site partial agonists as anxiolytics

Around 15 years ago, the concept emerged that a BZ with lower efficacy at the GABA-A receptor than the classical full agonist BZs might maintain the beneficial anxiolytic properties but have a lower propensity to cause the unwanted side effects discussed above. In preclinical animal assays, it did indeed appear that compounds with this profile retained their anxiolytic activity but had decreased sedation, withdrawal and abuse liability. Examples of such compounds are bretazenil [16] and abecarnil [17]. Both compounds were evaluated in the clinic and, unfortunately, neither proved successful. Abecarnil did not achieve the required level of anxiolytic activity although it was sedative, whereas bretazenil was reported to not have a sufficient separation between anxiolytic and sedative effects. This flags up issues regarding the predictivity of preclinical assays, particularly sedation; humans are obviously more sensitive and aware of the sedative effects of drugs than are the species used in preclinical studies.

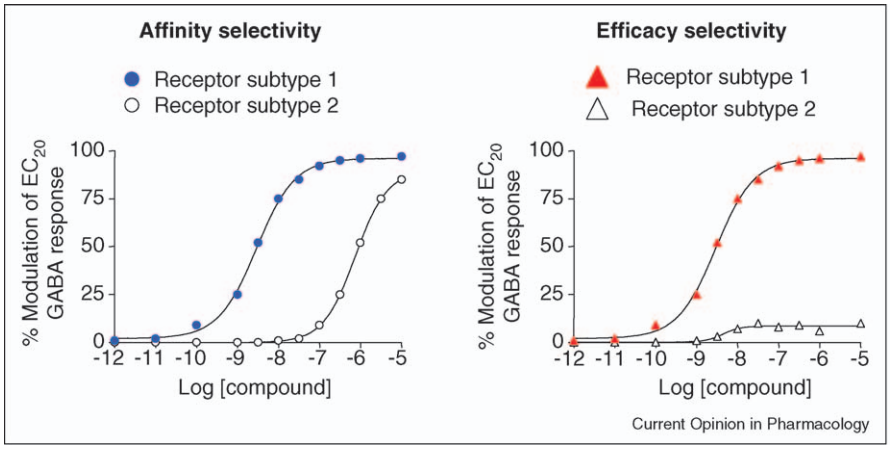
One compound currently in development which probably meets the definition of a GABA-A receptor BZ site partial agonist is ocinaplon. This compound, being developed by DOV Pharmaceuticals, is in Phase III clinical trials for generalized anxiety disorder (GAD) (although at the time of writing it has been reported that clinical development of ocinaplon is on hold owing to potential hepatic toxicity; http://www.dovpharm.com). In two Phase II clinical trials in GAD patients, DOV reported a significant reduction in anxiety, as measured by the Hamilton Anxiety Rating Scale clinical rating score. A recent paper [18•] documented the efficacy of this agent in GAD patients compared with placebo, using two different dosing regimens (60 mg t.i.d, 120 mg b.i.d.). Interestingly, only 1% of patients on the drug reported somnolence or sedation. These authors also described the pharmacological profile of ocinaplon. It is a low potency (micromolar EC_{50} at GABA-A receptors) compound which is essentially a full agonist at α1β2γ2 receptors and a partial agonist at α2β2γ2, α3β2γ2 and α5β2γ2 receptors. This profile is interesting because it would predict that, at clinically efficacious doses, ocinaplon should have hypnotic and sedative properties in humans as a result of its significant activity at α1 subunit-containing receptors [12–14]. The clinical data published by Lippa *et al.* [18•] do not reflect this profile, and the reason is currently unclear.

GABA-A receptor subtype-selective anxiolytics

There are two approaches to developing a receptor subtype-selective modulator (Figure 1). The most obvious approach is to develop a compound with binding selectivity — that is, with higher affinity for one receptor subtype than for another. For GABA-A receptors, the clear example is zolpidem, which, as discussed above, has higher affinity for α1 subunit-containing receptors. The alternative approach is to develop compounds with efficacy selectivity — that is, compounds which might bind with equal affinity to several receptor subtypes but will selectively modulate the activity of one or some of them. Given this approach, the potential opportunities to develop compounds with different efficacy profiles are, in theory, significant. Thus, at one extreme, one could consider developing a compound with absolute efficacy selectivity, potentiating the activity at only a single GABA-A receptor subtype and having no efficacy at any of the others. At the other extreme, in theory, one could develop a compound with a predefined spectrum of efficacies at the different receptor subtypes (e.g. an agent that is a full agonist at α1βxγ2, a weak partial agonist at α2βxγ2, a strong partial agonist at α3βxγ2 and a silent antagonist at α5βxγ2 receptor subtypes). The medicinal chemistry challenges of the latter strategy cannot be overstated!

To the authors' knowledge, no compounds with significant selective binding affinity for α2βxγ2 or α3βxγ2 (the two receptor subtypes which have been proposed to

Figure 1



Achieving GABA-A receptor subtype selectivity through selective affinity or selective efficacy. In the graph on the left, the hypothetical GABA-A modulator has greater than 200-fold higher affinity for receptor subtype 1, compared with receptor subtype 2, although at sufficiently high concentrations it reaches full potentiation at both subtypes. In the graph on the right, the hypothetical GABA-A modulator has approximately the same affinity (EC_{50}) at both receptor subtypes but exhibits subtype-selective efficacy, exhibiting full agonism at subtype 1 but minimal efficacy at subtype 2.

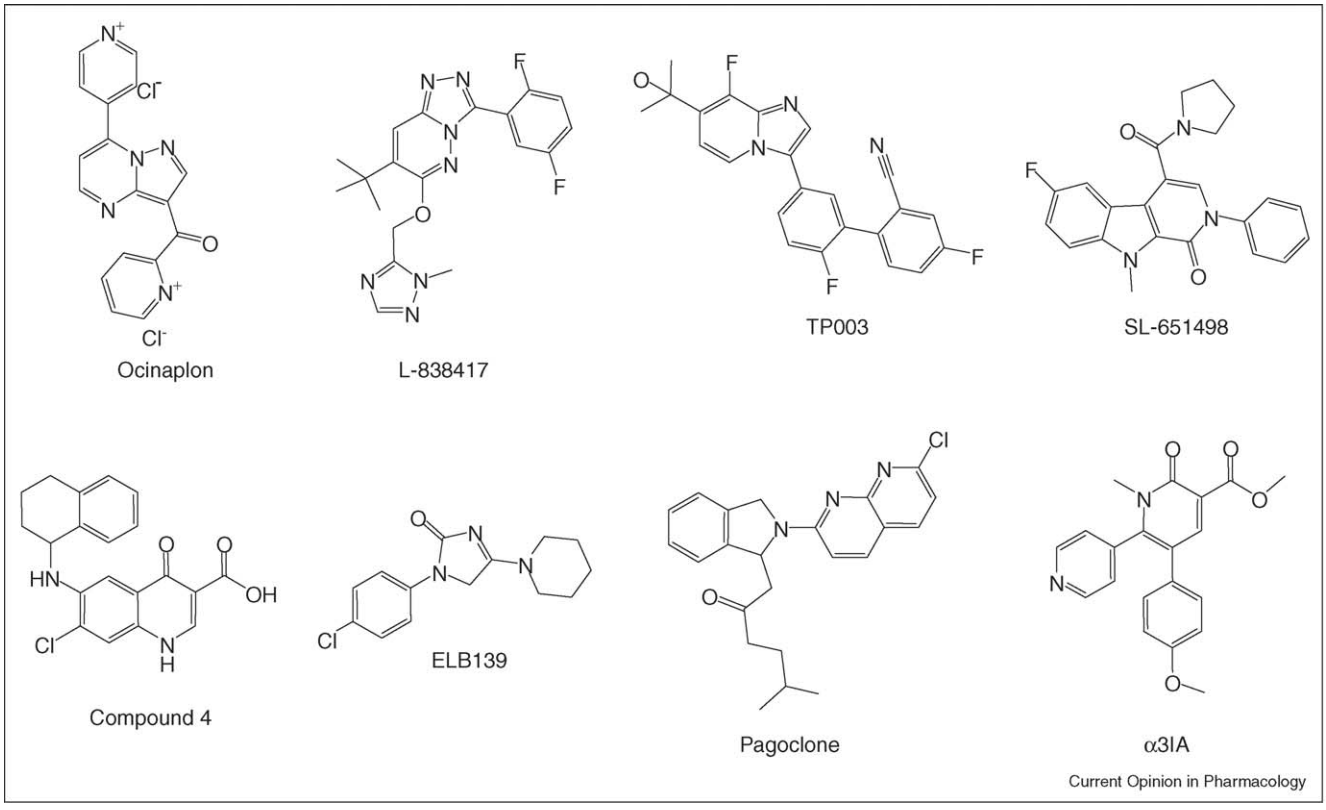
mediate the anxiolytic effects of BZs — see below) over $\alpha1\beta x\gamma2$ have been described. It is known that the BZ binding site is made up by contributions from both the α and $\gamma2$ subunits [19]. Thus, the inability to produce compounds with greater than 10-fold binding selectivity for $\alpha2$- or $\alpha3$-containing receptors does suggest that the BZ binding site determinants must be well conserved between $\alpha1$, $\alpha2$ and $\alpha3$ subunits. However, several compounds with subtype-selective efficacy have been described (Figure 2). The first of these was L-838417 [13]. This compound had nM (K_i) affinities (ranging from 2.3 to 0.7 nM) at recombinant human $\alpha1$-, $\alpha2$-, $\alpha3$- and $\alpha5$-(plus $\beta3$ and $\gamma2$)-containing receptors — that is, it was not binding selective. However, it exhibited significant efficacy selectivity; the maximum potentiation of an EC_{20} concentration of GABA was essentially zero at $\alpha1$-containing receptors, and 34%, 39% and 36%, respectively, at $\alpha2$-, $\alpha3$- and $\alpha5$-containing receptors (100% being the maximum potentiation achieved by the nonselective full agonist chlordiazepoxide). It is interesting to speculate how it is possible to achieve efficacy selectivity in the absence of binding selectivity; presumably, this reflects divergence of amino acid residues in the α subunits somewhere in the protein sequences responsible for the allosteric coupling of the BZ site, GABA site and the intrinsic ion channel.

L-838417 has been used as a pharmacological tool to investigate the behavioural effects mediated by BZs [13,20]. Using the elevated plus-maze test (an unconditioned anxiety model) and the fear-potentiated startle response (a conditioned anxiety model), it was found that L-838417 is a robust anxiolytic agent, with a 30-fold separation over doses needed to elicit a sedative effect [13]. Furthermore, L-838417 was found to be an effective non-sedating anxiolytic agent in primates, also exhibiting a much reduced potential for abuse compared with both full BZ nonselective agonists and zolpidem [20]. These data indicated that the $\alpha1$-containing GABA-A receptors primarily mediated the sedative effects of BZs, whereas the $\alpha2$-, $\alpha3$- or $\alpha5$-containing GABA-A receptors mediated the anxiolytic effects (most likely the $\alpha2$- and $\alpha3$-containing receptors). These conclusions were consistent with those reached using genetically modified mice [13–15] (see article by Rudolph and Möhler in this issue).

Atack *et al.* [21•] have described the preclinical activities of a GABA-A receptor inverse agonist (that is, an allosteric modulator that inhibits the activity of GABA at the receptor), referred to as $\alpha3$IA. This compound does show some limited affinity selectivity for $\alpha3$-containing receptors (EC_{50}s: 1300 nM at $\alpha1$-, 185 nM at $\alpha2$- and 70 nM at $\alpha3$-containing recombinant human GABA-A receptors), as well as some functional selectivity (maximum inhibition of EC_{20} concentrations of GABA being −31% at $\alpha1$-, −24% at $\alpha2$-, −45% at $\alpha3$- and −4% at $\alpha5$-containing recombinant human GABA-A receptors). These properties made $\alpha3$IA a useful pharmacological tool to probe the function of $\alpha3$-containing GABA-A receptors *in vivo*. When tested in rats, this compound was anxiogenic in the elevated plus-maze test, and also disrupted behaviour in a 'chain pulling' operant task, suggesting a 'lack of well-being'. The nonselective GABA-A receptor inverse agonist FG 7142 had the same effects in these assays. These data are consistent with an important role for GABA-A $\alpha3$-containing receptors in mediating the anxiolytic response of BZs.

A recent study from Dias *et al.* [22] has added further weight to the importance of $\alpha3$-containing receptors in

Figure 2



Structure of the key compounds discussed in this review.

the anxiolytic response. These authors describe the properties of the imidazopyridine TP003, which is perhaps the best characterized efficacy-selective compound to date. TP003 has equivalent high affinity at recombinant human GABA-A α1-, α2-, α3- and α5 (plus β3 and γ2 subunits)-containing GABA-A receptors, and minimal affinity at α4- and α6-containing receptors. In terms of selectivity, this compound appears to be uniquely selective, having significant efficacy only at α3-containing receptors (at which it is a full agonist). This compound was found to be efficacious in the rodent elevated plus-maze and stress-induced-hyperthermia models of anxiety, as well as a primate conditioned anxiety model, in the absence of any sedative activity. Consistent with data generated using α3IA, described above, these data strongly suggest an important role for α3-containing GABA-A receptors in the anxiolytic response to BZs. Interestingly, using genetically modified mice [15] (see paper from Rudolph and Möhler in this issue), it was found that α2-containing receptors, and not α3-containing receptors, mediated the anxiolytic effects of BZs. The reason for this apparent discrepancy remains unclear.

Sanofi Synthelabo have reported the properties of SL 651498, which appears to be a full agonist at α2- and α3-containing receptors and a partial agonist at α1- and α5-containing receptors [23]. In preclinical models, the compound appeared to have a significant separation of anxiolytic activity over sedative activity [23,24]. Similarly, in primate studies, SL 651498 was found to be an effective anxiolytic and myorelaxant, but did not induce ataxia, suggesting a favourable behavioural profile for a BZ site binding compound with the above receptor subtype activities [25]. The developmental status of this compound is unknown (at the time of writing, it does not appear in the Sanofi-Aventis pipeline; http://en.sanofi-aventis.com), although in the past it has been suggested as a treatment for both anxiety and muscle spasms.

In 2003, Johnstone *et al.* [26] described 'compound 4', a quinoline structure derived from the fluoroquinolone antibiotics such as norfloxacin. This compound appeared to have no efficacy at recombinant human α1β2γ2 receptors but significant efficacy at α2β2γ2 receptors. No data were provided regarding the effects at α3- or α5-containing receptors. Compound 4 was shown to have significant anxiolytic activity in the absence of sedative activity.

ELB139 is described as an α3-selective BZ agonist, and is thought to be in Phase III clinical trials for anxiety (http://www.elbion.de). A recent publication has described some properties of this compound [27]. It appears to be a

low-affinity BZ site ligand ($K_i \sim 2$ μM). Efficacy at GABA-A receptors in cultured neurones, as measured electrophysiologically, suggested that ELB139 is a partial agonist. No data were presented to support the notion that the compound is subtype selective. The compound has an anxiolytic profile in preclinical tests such as the elevated plus-maze test and the Vogel conflict test (a conditioned anxiety test), at lower doses than would be predicted from its *in vitro* affinity. The sedative potential of ELB139 was not directly determined.

Finally, pagoclone is a cyclopyrrolone compound, acting at the BZ site of the GABA-A receptor, which is reported to be well tolerated in humans and, indeed, to be efficacious in panic attacks [28]. *In vitro* studies indicate that it is a full agonist at α3-containing GABA-A receptors and a partial agonist at α1-, α2- and α5-containing receptors [29]. The partial agonism of this agent has been confirmed by positron emission tomography studies [30]. This compound was under development by Interneuron (now Indevus) together with Pfizer [31] but is now being developed by Indevus alone, as a treatment for stuttering (http://www.indevus.com).

Conclusions

An important unmet medical need, and a significant commercial opportunity, exists for a novel, fast-acting anxiolytic agent lacking the unwanted side effects of classical, full agonist, nonselective BZs. To date, the 'second generation' partial agonist approach has not achieved this goal, with encouraging preclinical data failing to translate into a clear clinical advantage; however, ocinaplon might paradoxically prove to be the exception, although further clinical data are required. A more recent approach, to develop receptor subtype-selective modulators, holds some promise but has yet to demonstrate translation of the encouraging preclinical data into the clinic.

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