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Review

Anxioselective anxiolytics: can less be more?

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Abstract

Benzodiazepines remain widely used for the treatment of anxiety disorders despite a side-effect profile that includes sedation, myorelaxation, amnesia, and ataxia, and the potential for abuse. γ-Aminobutyric acid$_A$ (GABA$_A$) receptor partial agonists, subtype-selective agents, and compounds combining both of these features are being developed in an attempt to achieve benzodiazepine-like efficacy without these potentially limiting side effects. This article reviews the nonclinical and clinical studies of "anxioselective" anxiolytics that target GABA$_A$ receptors and discusses potential mechanisms subserving an anxioselective profile.
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Keywords: Anxiolytic; Benzodiazepine; γ-Aminobutyric acid$_A$

Contents

1. Introduction

Anxiety is broadly defined as a state of unwarranted or inappropriate worry, often accompanied by restlessness, tension, distraction, irritability, and sleep disturbances. This disproportionate response to environmental stimuli can hyperactivate the hypothalamic–pituitary–adrenal axis and the autonomic nervous system, resulting in the somatic manifestations of anxiety, including shortness of breath, sweating, nausea, rapid heartbeat, and elevated blood pressure (Sandford et al., 2000). The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (2000) has classified anxiety disorders into

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multiple distinct conditions, including generalized anxiety disorder, acute stress disorder, obsessive–compulsive disorder, panic disorder, posttraumatic stress disorder, social and other specific phobias alone or in combination with the above disorders, as well as substance-induced anxiety disorders. Generalized anxiety disorder is the most common of the anxiety disorders, with a lifetime prevalence of approximately 5% (Wittchen and Hoyer, 2001). Every year, it is estimated that approximately 15 million people in the United States suffer solely from an anxiety disorder, with an additional 11 million suffering from anxiety as a comorbidity with at least one other psychiatric disorder (Greenberg et al., 1999). Moreover, anxiety has a lifetime prevalence of 1.5–3.5% of the population of the United States (Greenberg et al., 1999; Rice and Miller, 1998). Taken together, the total cost of anxiety disorders in the United States was estimated to be in excess of US$42 billion in 1990 (Greenberg et al., 1999; Rice and Miller, 1998). Therefore, anxiety disorders represent not only a significant public health issue, but place a substantial economic burden on society.

A number of novel compounds have either been developed or are currently in development for treating the different subclasses of anxiety. Some of these agents, such as the tricyclic antidepressants and β-adrenoceptor antagonists, found either limited use in treating specific disorders such as performance anxiety (e.g., β-adrenoceptor antagonist suppression of the sympathetic manifestations of anxiety), or have fallen out of favor for reasons of efficacy and/or safety. Currently, direct and indirect serotonin receptor agonists [e.g., serotonin-selective reuptake inhibitors (SSRIs) and buspirone] and benzodiazepines are most often prescribed for treating anxiety disorders, with the benzodiazepine receptor agonists remaining the preferred therapeutic modality (Atack, 2003; Stahl, 2002; Uhlenhuth et al., 1999; Varia and Rauscher, 2002). The ability of the benzodiazepines to enhance γ-aminobutyric acid (GABA) neurotransmission safely and rapidly is central to their effectiveness in treating anxiety disorders, especially generalized anxiety disorder and panic disorder (Stahl, 2002). Nonetheless, the use of benzodiazepines is limited by side effects associated with enhanced GABAergic neurotransmission, manifesting as sedation, muscle relaxation, amnesia, and ataxia. Moreover, a potential for abuse and dependence is associated with the long-term use of benzodiazepines. These therapeutic limitations and the societal burdens of anxiety provide the impetus for the development of new, anxioselective agents.

The concept of anxioselectivity is used here to describe anxiolysis in the absence of the side effects typically associated with benzodiazepines. While the historical target for anxioselective agents has been, and remains, the γ-aminobutyric acid$_A$ ($GABA_A$) receptors (Atack, 2003; Lippa et al., 1979b, 1982), other molecular loci have also been targeted, including metabotropic glutamate receptors (Schoepp et al., 1999), receptors for neurokinins and other peptides (Griebel, 1999; Millan et al., 2001), and serotonergic neurotransmission (Gorman and Kent, 1999; Riblet et al., 1982). However, none of these alternative targets has been shown to match either the efficacy or rapid onset of the benzodiazepines. This review will focus on the GABAergic mechanisms involved in achieving anxioselectivity, and summarize the current status of putative anxioselective agents.

2. GABAergic neurotransmission

GABA is the predominant inhibitory neurotransmitter in the central nervous system (CNS), with 30% of all synapses classified as GABAergic. The intrinsic inhibitory signal of GABA is transduced by a family of synaptic and extrasynaptic hetero-oligomeric proteins referred to as the $GABA_A$ receptors (Barnard et al., 1998; Korpi et al., 2002a,b). When GABA binds to its recognition site, the receptor complex is activated, causing a Cl^--permeant anion channel to open, allowing Cl^- to enter the neuron. This influx of Cl^- ions results in an inhibitory postsynaptic current that hyperpolarizes the neuron. The decrease in neuronal activity following activation of the $GABA_A$ receptor complex can rapidly alter brain function to such an extent that consciousness and motor control are impaired.

The $GABA_A$ receptors are also the molecular targets of a number of pharmacologic agents. In addition to the binding site for GABA [which can be occupied by pharmacological antagonists, such as bicuculline, and agonists, such as muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP)], there are binding sites for barbiturates (e.g., pentobarbital), neurosteroids (e.g., agonists such as allopregnanolone, antagonists such as pregnenolone sulfate), benzodiazepine-type molecules [e.g., agonists such as diazepam, antagonists such as flumazenil, and inverse agonists such as *N*-methyl-β-carboline-3-carboxamide (FG 7142)], and ionic regulators such as Zn^{2+} (Korpi et al., 2002a,b).

The identification of discrete receptors for benzodiazepines (Möhler and Okada, 1977; Squires and Braestrup, 1977) prompted efforts to determine whether specific receptor subtypes could subserve the multiple actions of benzodiazepines. Benzodiazepine receptor heterogeneity was first demonstrated both by physicochemical (multiphasic sensitivity to heat inactivation; Squires et al., 1979) and pharmacological (Lippa et al., 1979a) evidence. The latter study indicated that 3-methyl-6[3-trifluoromethyl)-phenyl]-1,24-triazolo[4,3-*b*] pyridazine (CL 218,872) bound with differential affinity to two populations of benzodiazepine receptors, referred to as types I and II (reviewed in Lippa et al., 1982). CL 218,872 possesses a high affinity for type I receptors, which are widely distributed but are concentrated, relative to the other subtypes, in the cerebellum. Type I receptors were hypothesized to mediate the anxiolytic and anticonvulsant actions of benzodiazepines. Additional complexity was

introduced by the designation of type II receptor subtypes (Lippa et al., 1982). The presence of the type II receptor was revealed by curvilinear saturation binding isotherms in cortical and hippocampal membrane preparations. CL 218,872 has a low affinity for type II receptors and was proposed to mediate the sedative and hypnotic actions of benzodiazepines (Lippa et al., 1982). While these observations provided the first substantive evidence for $GABA_A$ receptor heterogeneity, this classification is now of historical importance, as more than a dozen $GABA_A$ receptors have since been identified in the mammalian CNS (McKernan and Whiting, 1996). Furthermore, the pharmacological profiles of some benzodiazepine receptor ligands, such as the type I-selective hypnotic zolpidem (Arbilla et al., 1985), are inconsistent with the functions subserved by the subtypes as originally proposed (Lippa et al., 1982).

Subsequent studies of the molecular biology of $GABA_A$ receptors revealed a pentameric structure (Nayeem et al., 1994) comprised of distinct protein subunits falling into at least eight homologous families denoted as α_{1-6}, β_{1-4}, γ_{1-3}, δ, ε, π, θ, and ρ_{1-3} (Barnard et al., 1998). Most $GABA_A$ receptor subtypes are composed of α, β, and γ subunits, with the most likely stoichiometry of 2α, 2β, and 1γ subunits (Tretter et al., 1997) arranged as a γ–β–α–β–α pentamer (Minier and Sigel, 2004). The major (60%) $GABA_A$ receptor isoform in the adult mammalian brain consists of α_1, β_2, and γ_2 subunits ($GABA_{A1a}$[1]), which are found on a variety of neuron types (Wisden et al., 1992). Receptors containing α_2 or α_3 subunits each constitute 10–20% of the $GABA_A$ receptors in the brain, with α_2-containing receptors found in high abundance on hippocampal pyramidal neurons, as well as in the cortex, striatal spiny stellate neurons, and granule neurons in the olfactory bulb. Receptors containing α_3 subunits are also widely distributed to sites in the prefrontal cortex and neurons in cholinergic and serotonergic nuclei. While α_5-containing receptors are sparse throughout the CNS, they are found in relatively high densities in the adult hippocampus, but represent the primary form in neonatal brain (Laurie et al., 1992; Sieghart and Sperk, 2002; Skolnick et al., 1997). The α_1, α_2, α_3, and α_5 subunits are of particular importance to the concept of anxioselectivity, as the high-affinity binding sites for benzodiazepines (and related compounds) reside at the interface of the α_1, α_2, α_3, α_5, and γ subunits (Minier and Sigel, 2004).

While drug development efforts have largely targeted the α subunit, the γ subunit also influences both the potency and efficacy of benzodiazepines and other molecules that bind at the interface of these two proteins. The benzodiazepine recognition site spans the α and γ subunits, so that the γ subunit can dramatically affect both the affinity and efficacy of benzodiazepine-type molecules (Crestani et al., 1999; Graham et al., 1996; Pritchett et al., 1989). Among the three γ subunits, the γ_2 is most common, found in virtually every brain region (Sieghart and Sperk, 2002). Two splice variants of γ_2 subunits have been identified, with the long (L) variant containing an additional eight amino acids inserted in the intracellular loop between TM3 and TM4. The insert contains a consensus sequence for protein kinase C phosphorylation (Whiting et al., 1990). Potentiation of GABAergic activity by low concentrations of ethanol was proposed to be dependent upon the presence of the γ_{2L} subunit (Wafford and Whiting, 1992). The significance of this observation remains unclear, as investigators do not always report differences in ethanol sensitivity between $GABA_A$ receptor constructs containing the γ_{2L} and γ_{2S} subunits (Homanics et al., 1999; Kurata et al., 1993; Mihic et al., 1994). Nonetheless, the γ subunit can differentiate between benzodiazepine (e.g., diazepam, lorazepam) and nonbenzodiazepine agonists, such as CL 218,872 and zolpidem. Substituting a γ_1 subunit for a γ_2 subunit in an $\alpha_2\beta_1\gamma$ construct decreases agonist efficacy and reduces affinity up to 10-fold, while only ligand affinity is reduced in constructs containing γ_3 subunits (Dämgen and Lüddens, 1999; Graham et al., 1996). However, the efficacy of CL 218,872 is substantially increased at $\alpha_2\beta_1\gamma_1$ constructs without altering affinity (Wafford et al., 1993). Similarly, γ_3-containing $GABA_A$ receptors are zolpidem-insensitive, but CL 218,872 and zaleplon have relatively high affinities for $\alpha_{2,3}\beta_n\gamma_3$ constructs (Dämgen and Lüddens, 1999; Graham et al., 1996; Lüddens et al., 1994). These observations suggest that targeting γ_3-containing constructs of $GABA_A$ receptors may, despite their low abundance in the CNS relative to γ_2 subunits, be a useful approach for generating agents with novel pharmacologies.

3. Molecular genetic evidence of anxioselectivity

Important insights into the behaviors resulting from activation of $GABA_A$ receptors containing defined α subunits have been provided by investigations using transgenic mice, and in particular those with "knock-ins" of an α subunit with point mutations rendering the $GABA_A$ receptor insensitive to modulation by benzodiazepines. This is accomplished by replacing a histidine residue in the benzodiazepine binding region (residue 101 in the α_1 and α_2 subunits, residue 126 in the α_3 subunit, and residue 105 in the α_5 subunit, respectively) with an arginine residue, resulting in a significant loss in affinity for archetypic benzodiazepine receptor ligands such as diazepam (Benson et al., 1998; Wieland et al., 1992). This knock-in strategy does not appear to produce significant changes in subtype assembly (Low et al., 2000), as opposed to knockout animals (Kralic et al., 2002a), where alterations in $GABA_A$ receptor density or distribution have been observed (Kralic et al.,

[1] We have utilized IUPHAR nomenclature for the $GABA_A$ receptor (Barnard et al., 1998) whenever possible, so that: $GABA_{A1a}=\alpha_1\beta_n\gamma_2$; $GABA_{A1b}=\alpha_1\beta_n\gamma_3$; $GABA_{A2a}=\alpha_2\beta_n\gamma_2$, etc.

2002b; Korpi et al., 2002a,b). Moreover, the effects of agents acting at other loci on the $GABA_A$ receptor (e.g., neurosteroids, barbiturates) appear unaffected by these point mutations.

Mice expressing the α_1 histidine residue 101 (H101R) point mutation showed a substantial reduction in the sedative and amnestic effects of diazepam, and a partial loss of diazepam's (but a total loss of zolpidem's) anticonvulsant properties (Crestani et al., 2000; Low et al., 2000; McKernan et al., 2000; Rudolph et al., 2001). This is consistent with the actions of hypnotics such as zolpidem and zaleplon, which exhibit some selectivity for $GABA_{A1a}$ receptors (Crestani et al., 2001; Dämgen and Lüddens, 1999). Similarly, mice with a knock-in of a mutant α_5 subunit show enhanced cognitive function (Collinson et al., 2002), suggesting that the amnestic actions of benzodiazepine receptor agonists may be mediated through $GABA_A$ receptors containing this subtype—a hypothesis supported by the noötropic actions of α_5-selective inverse agonists (Chambers et al., 2003). Further, the anxiolytic actions of diazepam were no longer present in mice with this point mutation of the α_2 subunit (Low et al., 2000). In control mice, diazepam increases the amount of time spent in the lighted compartment of the light–dark box. However, α_2 knock-in mice treated with either diazepam or vehicle spent equal amounts of time in the lighted compartment. Furthermore, diazepam no longer increased the percentage of time spent in the open arms and the percentage of open arm entries in the elevated plus maze by α_2 knock-in mice. The α_2 subunit also appears to mediate, at least in part, the myorelaxant properties of benzodiazepine receptor agonists. Thus, diazepam had no effect on the ability of α_2 H101R knock-in mice to perform in the horizontal wire test—a measure of muscle tone (Crestani et al., 2001).

4. Pharmacological evidence of anxioselectivity

The above investigations suggest that the sedative and anxiolytic properties of benzodiazepine agonists can be differentiated and may be mediated through specific $GABA_A$ receptors. Nonetheless, the data obtained from studies of knock-in mice remain difficult to reconcile with data obtained from pharmacological investigations. 6-(3-Pyridyl)-5-(4-methoxyphenyl)-3-carbomethoxy-1-methyl-1*H*-pyridin-2-one was found to be a high-affinity inverse agonist at the $GABA_{A3a}$ receptor (Collins et al., 2002). This compound is a proconvulsant, and acted as an anxiogenic in the rats tested in the elevated plus maze, suggesting that the α_3 subunit is also involved in anxiety. Furthermore, α_3 partial agonists (the "TP" series) that are "GABA-neutral" (i.e., neither significantly enhance nor inhibit the actions of GABA, as observed with Ro 15-1788) at α_2 subunits have been synthesized, but show anxiolytic activity in several animal models (J. Atack, personal communication; McCabe et al., 2004). Additional insights have been provided by investigations using the α_1-selective antagonist β-carboline-3-carboxylate-*t*-butyl ester (β-CCT; Cox et al., 1995; June et al., 2003). β-CCT is 20–150 times more selective for $GABA_{A1a}$ than $GABA_{A2a}$, $GABA_{A3a}$, or $GABA_{A5a}$ receptors (Cox et al., 1995), and is "GABA-neutral" at $GABA_{A1a}$ receptors (June et al., 2003). Studies employing β-CCT have demonstrated that it antagonizes the anticonflict and anticonvulsant properties of diazepam and zolpidem, indicating that the α_1 subunit may participate in both the anxiolytic and anticonvulsant properties of these compounds. β-CCT also antagonized the sedative actions of diazepam and zolpidem, albeit at a dose 5–10-fold higher than that required to block the anticonflict actions, suggesting that the myorelaxant and amnestic actions of benzodiazepines and zolpidem may be mediated through other subunits (Belzung et al., 2000; Griebel et al., 1999a; Shannon et al., 1984). Based on observations from knock-in mice that $GABA_{A2}$ receptors may mediate benzodiazepine-induced myorelaxation (Crestani et al., 2001), it could be inferred that myorelaxation and anxiolysis are not dissociable properties. However, compounds such as 6-flouro-9-methyl-2-phenyl-4 -(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one (SL-651498, below) are full agonists (in electrophysiological assays) at $GABA_{A2}$ receptors, yet produce myorelaxation in animals only at doses one to two orders of magnitude higher than those required to produce anxiolysis (Griebel et al., 2003). This latter finding is consistent with the hypothesis that a partial activation of $GABA_{A2}$ receptors is required for an anxiolytic effect, while myorelaxation requires a larger activation (Atack, 2003; Reynolds et al., 2001).

In summary, there is currently no unifying hypothesis to explain the apparent discrepancies between studies using subtype-selective agents and those employing transgenic animals. If the above anomalies are ignored, studies using knock-in mice (Low et al., 2000; McKernan et al., 2000) indicate that a drug with high affinity and selectivity for $GABA_{A2}$ receptors would most closely mirror the profile of an ideal anxioselective. While no compounds that exhibit a marked $GABA_{A2}$ selectivity (i.e., at least a 10-fold greater affinity for $GABA_{A2}$ compared to $GABA_{A1}$, $GABA_{A3}$, or $GABA_{A5}$ receptors) have been disclosed, designing $GABA_{A2}$-selective compounds is a relatively new challenge (McKernan et al., 2000; Rudolph and Möhler, 2004). Further, the similarities among α_1, α_2, α_3, and α_5 subunits may make a 10-fold difference (defined here as pharmacologically relevant) in affinities among these subunits a difficult goal to achieve. A molecular $GABA_A$ receptor subunit-selective approach to anxioselectivity is elegant and conceptually attractive. However, there are compounds that do not exhibit a remarkable selectivity (i.e., <10-fold) among the benzodiazepine-sensitive $GABA_{A1}$, $GABA_{A2}$, $GABA_{A3}$, and $GABA_{A5}$ receptors, yet are "anxioselective" in animals (e.g., L-838417, McKernan et al., 2000; abecarnil, Stephens et al., 1993), and, in the case of ocinaplon, in humans (Atack, 2003;

Chilman-Blair et al., 2003; Czobor et al., 2003, 2004). Typically, such agents exhibit a lower efficacy than reference benzodiazepines at both native and recombinant $GABA_A$ receptors (Atack, 2003; Kostakis et al., 2003; Pribilla et al., 1993) and, in some instances, may be "GABA-neutral" (i.e., would act as an antagonist) at particular $GABA_A$ receptor isoforms (e.g., L-838417; McKernan et al., 2000).[2] Nonetheless, compounds such as SL-651498 (Griebel et al., 2003), L-838417 (McKernan et al., 2000), and ocinaplon (Krawcyzk et al., 2004) are as effective as benzodiazepines in models predictive of anxiolysis, and, in this sense, are full agonists. The least parsimonious explanation for these findings is that a relatively modest potentiation at one or more $GABA_A$ receptor subtypes results in anxiolysis, while "side effects" (e.g., sedation, muscle relaxation) require a more robust activation at either the same, or additional, $GABA_A$ receptor subtypes (Skolnick, 1991). For such compounds, side effects including muscle relaxation, sedation, and ataxia are reported only at doses that are significantly higher (>10-fold) than those required to produce anxiolysis (Atack, 2003; Griebel et al., 2003; Stephens et al., 1993).

Several of these very promising compounds have reached the clinic (notably, bretazenil and abecarnil) but have not exhibited the anxioselectivity predicted from their preclinical profiles (Ballenger et al., 1991; van Steveninck et al., 1996). Other compounds remain in development, and one (the pyrazolopyrimidine ocinaplon) exhibits anxioselectivity in the clinic. The following sections review the developmental history of these putative anxioselective agents.

5. Agents evaluated in the clinic

5.1. Bretazenil

Bretazenil is an imidazobenzodiazepine that does not exhibit $GABA_A$ receptor subtype selectivity in vitro (Griebel, 1999; Haefely, 1984; Martin et al., 1988). Biochemical and electrophysiological assays in vitro indicate that bretazenil is a partial agonist, with a maximal degree of cGMP inhibition of 25–50% in rodent cerebellum (compared to 75% for diazepam; Martin et al., 1988). GABA-gated Cl^- currents in recombinant $GABA_A$ receptors containing α_3 or α_5 subunits were enhanced to a maximum of 58% and 35% of that of flunitrazepam, respectively (Knoflach et al., 1993). Bretazenil is a potent anxiolytic and anticonvulsant, indicated by its ability to increase responses in punished drinking and food consumption tests, enhance time spent in the open arms of the elevated plus maze, and elevate audiogenic, pentylenetetrazol, and isoniazid-induced seizure latencies (Facklam et al., 1992; Griebel, 1999; Jones et al., 1994; Martin et al., 1993). Moreover, the doses required to produce benzodiazepine-like side effects, such as anterograde amnesia, hypolocomotion in the open field, and deficits in the rotarod, horizontal wire, and loaded grid tests, were as much as 10,000 times higher than the anxiolytic and anticonvulsant doses (Facklam et al., 1992; Griebel, 1999; Martin et al., 1993).

Phase I studies in humans demonstrated that bretazenil induced a typical "anxiolytic" electroencephalographic (EEG) spectrum profile similar to that of diazepam (Saletu et al., 1989). However, the EEG profile also indicated the presence of significant sedation at this dose (0.2 mg). Consistent with this EEG profile were substantial decrements in attention, numerical memory, psychomotor activity, wakefulness, and critical flicker fusion frequency. Bretazenil, at a dose of 0.5 mg, induced as much sedation as combinations of diazepam and ethanol, resulting in so many test subjects falling asleep that assessments of adaptive tracking and eye movement tests could not be completed (van Steveninck et al., 1996). The profound sedative actions of bretazenil in a clinical setting led to the discontinuation of its development, despite the remarkable dissociation of anxiolysis from sedative side effects described in preclinical studies.

5.2. Abecarnil

At the molecular level, abecarnil exhibits modest subunit selectivity. Abecarnil was found to have preferential affinity for the $GABA_{A1a}$ receptor (Griebel et al., 1999b; Ozawa et al., 1994; Smith et al., 2001; Stephens et al., 1990), with lower affinity for constructs containing α_3 and α_5 subunits. Abecarnil appears to be a full agonist at α_1 and α_3 subunit-containing constructs, but is a partial agonist at other receptor isoforms. Thus, abecarnil potentiates GABA-gated Cl^- currents to a maximum of approximately 50% relative to flunitrazepam at α_5 subunit-containing constructs of the $GABA_A$ receptor (Knoflach et al., 1993). Further, abecarnil maximally enhances GABA-receptor gated $^{36}Cl^-$ flux by 101%, 57%, 71%, and 43% relative to chlordiazepoxide at α_1, α_2, α_3, and α_5 subunit-containing constructs, respectively (Smith et al., 2001).

Although potent (2–250 times greater than diazepam), abecarnil showed varying degrees of efficacy compared to diazepam in punished conflict and neophobia tests (Dubinsky et al., 2002; Griebel et al., 1999b; Jones et al., 1994; Ozawa et al., 1994; Stephens et al., 1990). Specifically, the maximal efficacy of abecarnil in the mouse four-plate test (Jones et al., 1994) and punished drinking (Dubinsky et al., 2002; Ozawa et al., 1994) was comparable to diazepam, and was observed at doses that did not induce ataxia or myorelaxation. However, in the elevated plus maze, it was not clear if abecarnil was fully efficacious as an anxiolytic (as measured by the percentage of time spent in open arms, or number of open arm entries), as evidence of impaired motor performance was noted (decreased mean total arm

[2] This latter approach imparts a functional selectivity that may be more powerful than selectivity based solely on affinity.

entries) (Dubinsky et al., 2002; Griebel et al., 1999b; Jones et al., 1994). This variable efficacy extended to its anticonvulsant actions in rodent and primate models. Abecarnil potently inhibited seizures induced by metrazol, 3-mercaptopropionate, β-carbolines, picrotoxin, and kainate. Further, it blocked audiogenic seizures and kindling produced by chronic metrazol and FG 7142 administration. However, abecarnil had no effect on bicuculline, strychnine, quisqualate, and *N*-methyl-D-aspartate-induced seizures, or tonic convulsions induced by maximal electroshock in both rodent and primate models (Dubinsky et al., 2002; Turski et al., 1990). Generally, abecarnil was not observed to cause significant alterations in motor activity, with the anxiolytic and anticonvulsant activities of abecarnil typically manifested at doses 3–1000 times less than those inducing ataxia and myorelaxation (Turski et al., 1990; Stephens et al., 1990). However, Dubinsky et al., (2002) reported a significant overlap of the ED_{50} values for anxiolysis (1.3 mg/kg, punished drinking) and sedation (0.8 mg/kg, horizontal screen locomotion) in rats, as well as evidence of sedation at anxiolytic doses in the elevated plus maze. Together, these results suggest that abecarnil acts as a full agonist at receptors mediating anticonvulsant and anxiolytic effects ($GABA_{A1}$), but as a partial agonist at isoforms involved with muscle relaxation and ataxia ($GABA_{A2}$ and $GABA_{A3}$). In view of both its full agonist profile and high affinity for α_1 subunit-containing constructs, the low potency of abecarnil as a sedative/ataxic agent in animals stands in contrast to studies, with knock-in mice implicating the α_1 subunit as the sole mediator of the sedative effects of benzodiazepines.

Double-blind, placebo-controlled studies of abecarnil demonstrated an effect in patients with generalized anxiety disorder, with minimal effective doses of 3–9 mg/day (Ballenger et al., 1991; Pollack et al., 1997). However, this efficacy was not maintained above placebo levels throughout the trial, nor was this effect observed in a majority of trials. Drowsiness was frequently observed in subjects, especially after higher doses (7.5–30 mg/day) of abecarnil. In a separate study, normal subjects receiving either single or multiple doses of abecarnil reported dizziness, sedation, unsteadiness, inability to concentrate, and performance decrements in the symbol substitution task at doses two to four times higher than the effective anxiolytic doses (Duka et al., 1993). Despite these responses, visual analogue scale ratings did not reveal a sedative effect. Overall, questions regarding the duration of efficacy, high placebo responses, and lack of a clear-cut discrimination between the anxiolytic and sedative doses may have contributed to the cessation of development efforts.

5.3. Ocinaplon

In native $GABA_A$ receptors from rat cerebral cortex, ocinaplon inhibits [^{3}H]flunitrazepam binding with a low affinity (IC_{50}~2 μM) relative to benzodiazepines, and is less efficacious than diazepam at enhancing [^{35}S]*t*-butylbicyclophosphorothionate (TBPS) binding (Vanover et al., 1994). This latter property is characteristic of partial agonists, although agents (e.g., abecarnil; Knoflach et al., 1993) that appear to be partial agonists at all subtypes of $GABA_A$ receptor may exert full efficacy at one subtype and minimal efficacy at others. In the case of ocinaplon, the latter case is supported by observations of the homogenous displacement of [^{3}H]flunitrazepam from binding sites in cerebellar membranes, and heterogenous displacement from cortical membranes (Vanover et al., 1994). Electrophysiological studies in recombinant $GABA_A$ receptors demonstrated that the efficacy of ocinaplon varied dramatically among receptors containing α_1, α_2, α_3, or α_5 subunits expressed in *Xenopus* oocytes (Kostakis et al., 2003). In $\alpha_1\beta_2\gamma_2$ constructs, ocinaplon was as efficacious as diazepam in augmenting GABA-gated Cl^- currents, but was only 40–50% as efficacious as diazepam in α_2, α_3, and α_5 subunit-containing constructs. Substitution of a γ_3 subunit further reduced the efficacy of ocinaplon relative to diazepam across these $GABA_A$ receptors. Ocinaplon was between 5-fold and 35-fold less potent than diazepam in the eight recombinant isoforms examined.

Ocinaplon dose-dependently increased performance in models used to predict anxiolysis, including a punished responding paradigm in pigeons (Vanover et al., 1994), the thirsty rat conflict test, and with rats in the elevated plus maze (Krawczyk et al., 2004). The potency of ocinaplon was comparable to diazepam in these studies and sensitive to flumazenil, consistent with a $GABA_A$ receptor-mediated anxiolysis. Doses of ocinaplon greater than one order of magnitude higher than these anxiolytic doses were required to produce sedation and myorelaxation (Atack, 2003; Chilman-Blair et al., 2003). The difficulty of establishing ocinaplon as an interoceptive cue is consistent with its anxioselective profile. In pigeons, establishing ocinaplon as a cue required more than 100 sessions, on average, to reach criterion (Vanover et al., 1994), while benzodiazepines typically produced a rapid and stable discriminative stimulus.

Ocinaplon has been examined in two Phase II trials in patients with generalized anxiety disorder. The anxioselective profile demonstrated in preclinical studies was maintained in the clinic. The initial Phase II study was a double-blind trial comparing ocinaplon (total daily dose, 270 mg) to placebo for 4 weeks (Beer et al., 2002; Chilman-Blair et al., 2003; Czobor et al., 2003). A significant separation from placebo in scores on the Hamilton Anxiety Scale (HAM-A) was evident at 1 week. At 4 weeks, total reductions in HAM-A scores were 6.3 and 14.2 points in the placebo and ocinaplon groups, respectively. This nearly eight-point difference from placebo scores is larger than typically observed with benzodiazepines (Pande et al., 2003), and far exceeds the two- to three-point separation from placebo observed after 6–8 weeks of treatment with SSRIs (Pollack et al., 2001) and serotonin and norepinephrine reuptake

inhibitors (SNRIs) (Davidson, 2001). Ocinaplon was well tolerated and the incidence of side effects typically associated with benzodiazepines (sedation, dizziness) was not different from placebo. The second Phase II study was a 14-day, placebo-controlled, double-blind trial using two lower doses of ocinaplon (180 and 240 mg) (Czobor et al., 2004). A statistically significant improvement relative to placebo was observed after as little as 1 week of treatment with ocinaplon ($P=0.022$). Upon conclusion of the study, after 2 weeks of ocinaplon (120 mg/kg twice daily) treatment, patients averaged a 14-point decrease in HAM-A score, while placebo-treated subjects averaged only a nine-point decline ($P=0.02$). In general, the overall side-effect profile was unremarkable across the entire study population, with an absence of significant findings of sedation or dizziness commonly observed with benzodiazepine administration (Pande et al., 2003).

6. Anxioselective agents for which only preclinical data are available

6.1. L-838417

L-838417 is a pyridone with a unique efficacy profile. Radioligand binding assays indicate that it has uniformly high affinity among the diazepam-sensitive $GABA_A$ receptors (McKernan et al., 2000). However, not only is it a partial agonist at α_2, α_3, and α_5 subunit-containing constructs compared to diazepam (E_{max} 39–43%), but it is GABA-neutral at α_1 subunit-containing constructs as determined by modulation of GABA-gated Cl^- currents in recombinantly expressed $GABA_A$ receptors. Tests in vivo support the anxiolytic efficacy of orally administered L-838417, which increased the number of entries and the time spent in the open arms of the elevated plus maze, while reducing the amplitude of the response in the fear-potentiated startle at doses comparable to diazepam. However, no studies of its effects on conflict behavior have yet been published. In contrast, no evidence of ataxia or myorelaxation was noted in the rotarod or chain pull assays at doses 30 times the minimum effective dose for anxiolytic activity. Currently, there is no published information on the clinical efficacy of this agent (Atack, 2003).

6.2. Quinolone "compound 4"

The quinolone derivative, "compound 4" (7-chloro-1-ethyl-6-[(1,2,3,4-tetrahydro-1-napthylenyl)-amino]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid), was found to interact with a novel binding site on the $GABA_A$ receptor (Johnstone et al., 2004). Compound 4 had no effect on radioligand binding to the GABA or benzodiazepine binding sites, but allosterically inhibited [^{35}S]TBPS binding with an IC_{50}=1.1 μM and E_{max}=75%. Compound 4 potentiated GABA-gated currents elicited from human embryonic kidney cells expressing $\alpha_2\beta_2\gamma_2$ constructs with a minimum effective concentration of approximately 1 μM, but had no effect on currents elicited by GABA from $\alpha_1\beta_2\gamma_2$ constructs at concentrations up to 30 μM. The maximum potentiation produced by compound 4 at $GABA_{A2}$ receptors was approximately 50% of that produced by diazepam. Compound 4 appears to be anxioselective, increasing punished responses in the thirsty rat conflict test and decreasing the time spent on the dark side of the light–dark box with minimum effective doses of 10 mg/kg, i.p., for both tests. No disruption of rotarod performance was noted at doses up to 12 mg/kg, i.v., or 300 mg/kg, i.p. While compound 4 enters the CNS following parenteral administration, its oral bioavailability remains to be determined. Thus, compound 4 acts as an anxioselective partial agonist at the $GABA_{A2}$ receptor. Neither its effects on other $GABA_A$ receptors, nor the results of its clinical efficacy have been reported.

6.3. SL-651498

A research program designed to discover subtype-selective $GABA_A$ receptor agonists yielded the pyridoindole, SL-651498 (Griebel et al., 2003). This agent exhibits nanomolar affinity for $GABA_A$ receptors containing α_1, α_2, and α_3 subunits, with a 10-fold lower affinity for $GABA_{A5}$ receptors. In electrophysiological assays, SL-651498 acts as a full agonist at the α_2 and α_3 subunit-containing constructs and as a partial agonist at the α_1 and α_5 subunits. SL-651498 showed anxiolytic activity, as evidenced by performance in the elevated plus maze, light–dark box, and defense test battery at doses 3–100 times lower than those causing myorelaxation or ataxia (rotarod, grip strength), and it appeared to be less liable to induce cognitive impairment. SL-651498 did not impair spatial reference or working memory as measured in the Morris water maze and T-maze (Griebel et al., 2003). While rodents treated with SL-651498 were able to discriminate between familiar and novel objects in the object recognition task, there was an impairment of performance in the passive avoidance test. However, this may be due to the suppression of conditioned fear acquisition by the anxiolytic actions of SL-651498. This general lack of impairment of cognitive function by SL-651498 is consistent with its partial agonist activity at the α_5 subunit (Chambers et al., 2003; Crestani et al., 2002). While there is no published information on the results of its clinical trials, studies are being conducted on the efficacy of SL-651498 as a muscle relaxant. This indication would contradict the preclinical evidence provided for its potential utility as an anxioselective anxiolytic.

7. Conclusion

The benzodiazepines were the first truly safe and effective anxiolytics, and remain a mainstay of both

general and psychiatric practice because of their rapid and efficacious action. However, issues related to their sedation, ataxia, and abuse potential have led to the increasing use of SSRIs (despite their slow onset and marginal efficacy) for the treatment of generalized anxiety disorder. This review describes several of the approaches that have evolved to develop anxioselective agents acting through GABAergic mechanisms. Initially, such compounds (e.g., CL 218,872; Lippa et al., 1979a, 1982) were identified by in vivo screening (Lippa et al., 1979b) and emerged as tools to better understand $GABA_A$ receptor pharmacology. Over the past 15 years, advances in molecular biology—from the cloning of $GABA_A$ receptor subunits to the use of transgenic animals—have provided a framework to define and test these approaches. However refined the theory, the practical constraints encountered in drug development may limit these approaches. Such constraints range from synthesizing molecules that select and discriminate among closely related members of a gene family to the more pedestrian—but no less formidable—challenge of making compounds that are both safe and orally active.

Several of the compounds described in the review exhibited "anxioselectivity" in animals but failed in the clinic. Other molecules with anxioselective profiles may never be tested in the clinic because of obstacles encountered during development. To our knowledge, ocinaplon is the only molecule exhibiting an anxioselective profile in both animals and man. The anxioselective profile of ocinpalon should be viewed as a first step toward a better understanding of the molecular substrates of anxiety, and the partial fulfillment of the promise revealed by studies done more than 25 years ago that provided evidence for $GABA_A$ receptor heterogeneity.

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