Anxiolytics
ed. by M. Briley and D. Nutt

Subtype-selective benzodiazepine receptor ligands

Guy Griebel, Ghislaine Perrault and David J. Sanger

Sanofi-Synthelabo, 31 avenue Paul Vaillant-Couturier, 92220 Bagneux, France

Introduction

Introduced over 30 years ago, benzodiazepines (BZs) quickly became the most widely used of all psychotropic drugs. Their marked anxiolytic, hypnotic, anticonvulsant and muscle relaxant properties and their relative safety, rapidly elevated BZs to the treatment of choice for common and recurrent conditions such as anxiety states, tension and insomnia. However, in recent years, attitudes toward these compounds have greatly changed, and growing awareness and concern about dependence liability, withdrawal phenomena and short- and long-term side-effects has brought the long-term use of these compounds into question [1, 2]. BZs produce their pharmacological effects by allosterically and positively modulating the action of GABA at $GABA_A$ receptors at specific sites which are referred to as BZ/ω receptors [3–5]. The search for compounds chemically unrelated to BZs with more specific therapeutic actions and without the concomitant unwanted effects has led to the development of drugs that selectively bind to specific BZ/ω receptor subtypes and/or show different efficacies at BZ/ω receptors. For example, studies in animals showed that the non-selective BZ/ω receptor partial agonists bretazenil, imidazenil, Ro 19-5663, Ro 19-5686 and Ro 41-3696 displayed comparable or even greater efficacy in anxiety models than BZs, but produced less motor impairment [6–11]. Furthermore, following repeated treatment with Ro 19-5663, Ro 19-5686, Ro 41-3696 and the selective BZ/ω_1 receptor agonist zolpidem in rodents, there was no evidence for tolerance and physical dependence as was observed with most BZs [12, 13]. This article gives an overview of the main pharmacological findings with subtype-selective BZ/ω receptor agonists. The focus is on a review of the results obtained in animal studies, but clinical findings are also considered.

Benzodiazepine-sensitive $GABA_A$ receptors

Based on the finding that all BZs displaced the binding of [^{3}H]BZs in different brain regions in a monophasic manner, it was originally thought that there was a unique class of BZ receptors [14, 15]. However, the subsequent finding

that compounds structurally unrelated to BZs such as the triazolopyridazine CL218872, the imidazopyridine zolpidem or certain β-carbolines such as abecarnil, display different affinity for BZ receptors in the cerebellum than those in the hippocampus or other brain regions, suggested the existence of two BZ-receptor subtypes [4, 5, 16]. These were named BZ_1 and BZ_2 [3, 4], also designated as ω_1 and ω_2, respectively [5]. Subsequent cloning from cDNA libraries which has identified nearly twenty related $GABA_A$ receptor subunits in mammals, indicated that the $GABA_A$ receptor encompasses a heterogeneous population of multiple subunits. $GABA_A$ receptors have a pentameric form and each receptor is assembled from a combination of subunits from seven different sequence families (α_{1-6}, β_{1-4}, γ_{1-3}, ρ_{1-3}, ε_1, π_1 and δ_1) [17–20]. Hence, a new classification of the $GABA_A$ receptor subtypes based on subunit structure was proposed [21]. As an illustration, the BZ/ω_1 subtype is now referred to as $GABA_{A1a}$ receptor since its pharmacology mimics that of the co-expressed recombinants $\alpha_1\beta_n\gamma_2$ (Tab. 1). About 80% of all $GABA_A$ receptor subtypes contain the classical BZ/ω binding sites (for review, see [22]). The $GABA_{A1a}$ subtype is the most abundant, amounting to approximately 60% of the BZ/ω-sensitive $GABA_A$ receptors. It is expressed in numerous populations of GABAergic neurons, in particular in the cerebral cortex, basal forebrain, thalamus and cerebellum. The $GABA_{A2a}$,$GABA_{A3a}$ and $GABA_{A5a}$ subtypes are moderately abundant, each being associated with about 10% of $GABA_A$ receptors. They are most abundant in the olfactory bulbs and hippocampus (Tab. 1).

Table 1. Classification and distribution of the $GABA_A$ receptor subtypes containing the classical BZ/ω binding sites. Together, they represent about 80% of all $GABA_A$ recognition sites

$GABA_A$ receptor subtype	Subunit composition	Former classification	Regional preponderance
$GABA_{A1a}$	$\alpha_1\beta_n\gamma_2$	BZ/ω_1	Cerebral cortex, thalamus, cerebellum, basal forebrain
$GABA_{A2a}$	$\alpha_2\beta_n\gamma_2$	BZ/ω_2	Olfactory bulb, hippocampus, amygdala, striatum
$GABA_{A3a}$	$\alpha_3\beta_n\gamma_2$	BZ/ω_2	Olfactory bulb, amygdala, septum, thalamus
$GABA_{A5a}$	$\alpha_5\beta_n\gamma_2$	BZ/ω_2	Olfactory bulb, hippocampus, spinal trigeminal nucleus

Adapted from [21, 22]. n = 1–3.

Subtype-selective BZ/ω receptor ligands

A wide variety of ligands are now known to interact selectively with BZ/ω receptor subtypes, and the field is being researched with increased vigour in an effort to produce more selective agents. While there are BZ/ω receptor ligands

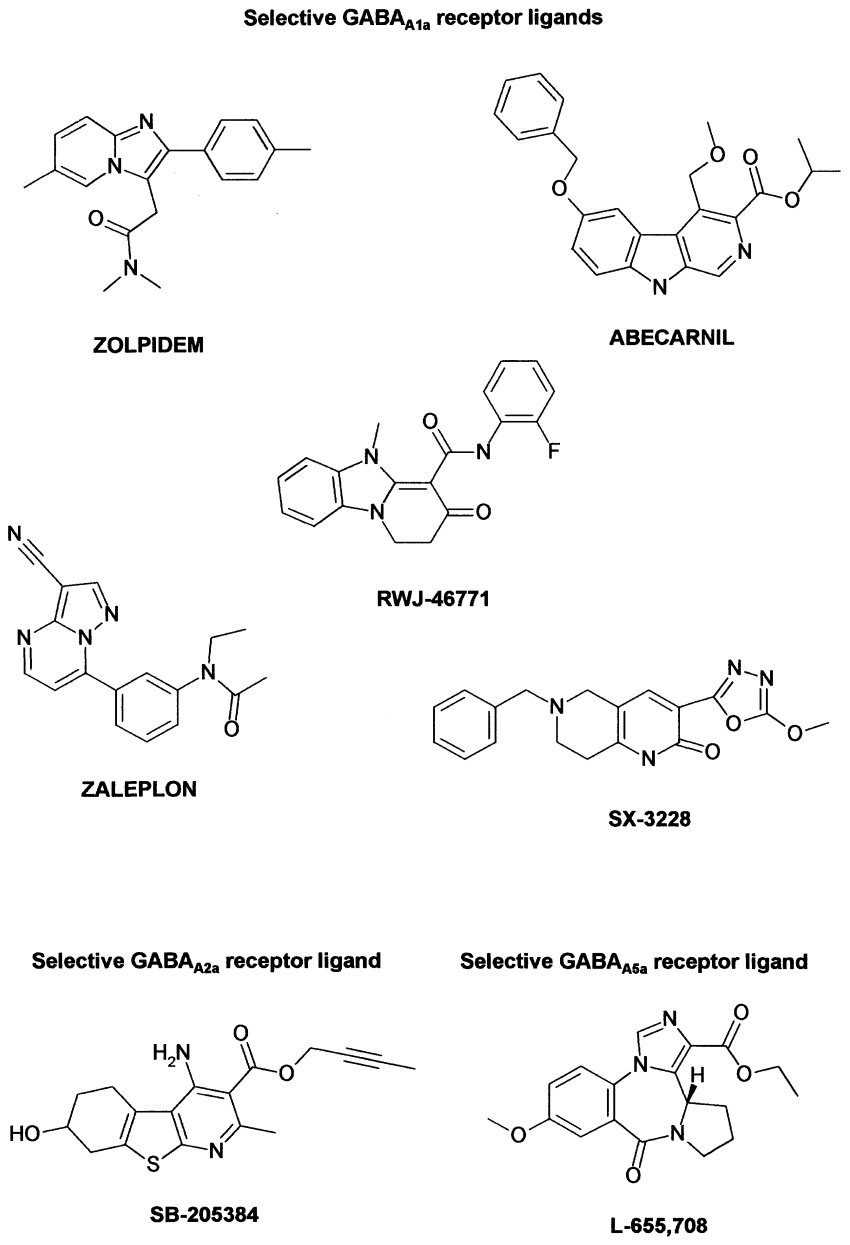

Figure 1. Examples of subtype-selective $GABA_A$ receptor ligands.

claimed in patents or shown to bind selectively for all BZ-sensitive $GABA_A$ receptor subtypes (Fig. 1), only compounds selective for the $GABA_{A1a}$ recep-

tor subtype have been studied extensively. As illustrated in Figure 1, these latter include compounds with greatly varying chemical structures. The most widely studied $GABA_{A1a}$ receptor ligands are presented in Table 2. They include the imidazopyridine zolpidem, the β-carboline abecarnil, the pyrido[1,2-a]benzimidazole RWJ-46771, the pyrazolopyrimidine zaleplon and the 1,6-naphthyridin-2(1H)-one derivative SX-3228. While some of these compounds are marketed (zolpidem, zaleplon) or pre-registered (abecarnil), others have been discontinued during the preclinical phase (RWJ-46771 and SX-3228). This article gives an overview of the main experimental findings with zolpidem, abecarnil, RWJ-46771, zaleplon and SX-3228. Their pharmacological profiles were compared to that of the non-selective $GABA_A$ receptor full agonist diazepam.

Table 2. Effects of diazepam and several compounds described as selective for the $GABA_{A1a}$ receptor subtype on the binding of [^{3}H] flumazenil to the native BZ/ω receptors in the rat cerebellum, a brain area enriched in $GABA_{A1a}$ receptors, and to the native BZ/ω receptors in the spinal cord, an area containing $GABA_{A2a}$, $GABA_{A3a}$ and $GABA_{A5a}$ sites

	IC_{50} (nM)		
	Cerebellum	Spinal cord	$GABA_{A1a}$ receptor selectivity
Diazepam[1]	19	12	0.6
Zolpidem[2]	14	130	9.3
Abecarnil[1]	1.4	4	2.9
RWJ-46771[1]	0.4	1	2.5
Zaleplon[3]	78	570	7.3
SX-3228[1]	8.9	58	6.5

[1][24], [2][23], [3]Schoemaker, personal communication.

In vitro binding to BZ/ω receptor subtypes

Experiments on the inhibition of [^{3}H] flumazenil binding to native BZ/ω receptor subtypes showed that zolpidem, abecarnil, RWJ 46771, zaleplon and SX-3228 were more potent in displacing [^{3}H] flumazenil binding to membranes from rat cerebellum, a brain area enriched in $GABA_{A1a}$ receptors, than from spinal cord, an area containing $GABA_{A2a}$, $GABA_{A3a}$ and $GABA_{A5a}$ sites, thereby indicating selectivity for the $GABA_{A1a}$ receptor subtype ([23, 24], Schoemaker, personal communication). In contrast, the classical BZ diazepam displaced [^{3}H] flumazenil binding in membranes from cerebellum and spinal cord non-selectively (Tab. 2). RWJ-46771 and abecarnil share similar high affinities (IC_{50}'s = 0.4 and 1.4 nM, respectively) for the $GABA_{A1a}$ receptor subtype. These values were slightly higher than those of SX-3228 and zolpi-

dem, and considerably higher than that of zaleplon. Among the $GABA_{A1a}$ compounds, zolpidem, zaleplon and SX-3228 are the most selective, whereas RWJ-46771 and abecarnil are only moderately selective for the $GABA_{A1a}$ receptor subtype.

Intrinsic efficacy of selective $GABA_{A1a}$ receptor ligands

While differential binding affinity represents one form of selectivity for receptors, it may not be the only factor, because receptor subtype specific differences in modulatory efficacy may also be possible [25]. The intrinsic efficacy of BZ/ω receptor ligands can be assessed *in vitro* by studying the potentiation of Cl currents induced by rapid application of GABA in transfected cells expressing different combinations of $GABA_A$ receptors. Classical BZs, such as diazepam, interact with nearly all receptor subtypes with high efficacy. In contrast, partial agonists, typified by bretazenil or imidazenil act with reduced efficacy compared to diazepam at all receptors [25–27]. Among the selective $GABA_{A1a}$ receptor ligands, zolpidem showed a greater efficacy than diazepam in potentiating the GABA response in recombinant cells expressing the α_1 subunit, whereas it displayed a lower intrinsic activity than diazepam in transfected cells containing $\alpha_3\beta_2,\gamma_2$ subunits, and no activity at $\alpha_5\beta_2,\gamma_2$ combination [28] (Tab. 3). Abecarnil was reported to exert full agonistic effects on $GABA_A$ receptors containing the α_1 and α_3 subunits, whereas it behaved as a partial agonist at receptors containing the α_2 and α_5 subunits [29, 30]. The maximal potentiation achieved with zaleplon at α_1-, α_3- and α_5-containing combination was similar to that obtained by diazepam (Granger, personal communication). RWJ-46771 has been described as a partial agonist. However, in tissue preparations from rat cerebral cortex, it produced a GABA shift value (i.e. 1.6) somewhat greater than that observed with BZ/ω receptor partial agonists (i.e. 1.0) and close to that of the BZ/ω receptor full agonist lorazepam (i.e. 1.7) [31]. No data on the effects of SX-3228 on the potentiation of GABA response

Table 3. Efficacies of diazepam and several compounds described as selective for the $GABA_{A1a}$ receptor subtype in modulating GABA-induced chloride flux in recombinant systems expressing subtypes of $GABA_A$ receptors or in cortical tissues (RWJ-46771)

	Efficacy in modulating GABA-induced Cl flux
Diazepam	High at the at α_1-, α_3- and α_5-containing combinations[1]
Zolpidem	High at α_1-combination, low at α_3-combination, no efficacy at α_5-combination[1]
Abecarnil	High at α_1 and α_3-combinations, low at α_5-combination[2,3]
RWJ-46771	High in tissues from cerebral cortex[4]
Zaleplon	High at the at α_1-, α_3- and α_5-containing combinations[5]
SX-3228	?

[1][28][2][29][3][30][4][31], [5]Granger, personal communication.

on transfected cells expressing different combinations of $GABA_A$ receptors have been published yet.

In vivo, the intrinsic efficacy of BZ/ω receptor ligands can be assessed by studying their ability to modify the latency to clonic seizures produced by isoniazid. Isoniazid inhibits glutamic acid decarboxylase, the enzyme that catalyses the synthesis of GABA from glutamic acid, thereby reducing the neuronal stores of GABA available for nerve impulse-mediated release of this transmitter [32]. The maximal delay in onset of isoniazid-induced seizures produced by a test compound may therefore be taken as an index of increased GABAergic function. It has been proposed as an *in vivo* measure of the intrinsic activity of BZ-ω receptor ligands at $GABA_A$ receptors [33]. Figure 2 shows that diazepam produced a larger increase in this measure than that seen with bretazenil, which is consistent with the well-acknowledged idea that diazepam shows higher intrinsic activity than bretazenil [24]. The selective $GABA_{A1a}$ receptor ligands showed different profiles in this test. Zolpidem and abecarnil produced a very large increase in the latency to clonic seizures produced by isoniazid, greater than those seen with diazepam and the other $GABA_{A1a}$ compounds [24, 34]. The efficacy of RWJ-46771 in increasing latency was slightly higher than that of diazepam and SX-3228, but considerably higher than that displayed by zaleplon [24]. Taken as a whole, these findings thus indicate that zolpidem and abecarnil may have greater intrinsic efficacy at $GABA_A$ receptors than RWJ-46771, diazepam, SX-3228 and zaleplon. It is noteworthy that

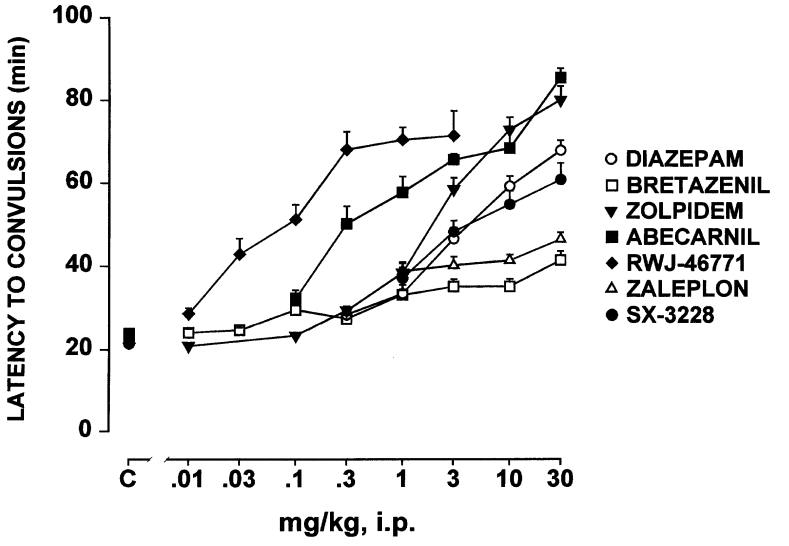

Figure 2. The anticonvulsant effects of diazepam, bretazenil and several selective $GABA_{A1a}$ receptor ligands against clonic seizures produced in mice by 800 mg/kg, s.c. of isoniazid. Data represent mean ± S.E.M. Adapted from [24, 34].

selective compounds with high efficacy in modulating GABA-induced chloride flux in recombinant systems also show high efficacy against isoniazid-induced convulsions, suggesting that this model is particularly sensitive to the action of selective $GABA_{A1a}$ receptor ligands.

Anxiolytic activity of selective $GABA_{A1a}$ receptor ligands

The anxiolytic-like properties of selective $GABA_{A1a}$ receptor ligands have been investigated in numerous studies. For example, abecarnil produced anxiolytic-like activities in several models in mice and rats, including the 4-plate test, the elevated plus-maze and the water-lick conflict test [35–38]. However, abecarnil was inactive in the free-exploration box, the light/dark test and in the defence test battery, three mouse models of anxiety [39, 40]. Table 4 summarises the effects obtained with zolpidem, abecarnil, RWJ-46771, zaleplon, SX-3228 and diazepam in five well-validated tests of anxiety under identical conditions. The tests include two conflict procedures (punished lever pressing and drinking tests in rats), two exploration models (elevated plus-maze in rats and light/dark test in mice) and a model based on defensive behaviours elicited in mice during confrontation with a natural threat (a rat) [24, 41].

Results showed that in the punished drinking test, all selective $GABA_{A1a}$ receptor ligands displayed anticonflict activity. In contrast, with the exception of SX-3228, selective compounds failed to modify punished responding in the lever pressing test. The anticonflict activity observed with the selective $GABA_{A1a}$ receptor ligands in the punished drinking test may have been contaminated by behavioural suppression as positive effects were observed at

Table 4. Minimal effective dose (MED) for anxiolytic-like activity and motor effects of diazepam and several selective $GABA_{A1a}$ receptor ligands

	MED (mg/kg, i.p.)				
	Rat			Mouse	
	Punished lever pressing	Punished drinking	Elevated plus-maze	Light/dark	Defence test Battery
Diazepam	2.5 (>5)	3	3 (>3)	4	1 (10)
Zolpidem	>3 (3)	3	1 (>1)	>3	10 (10)
Abecarnil	>1 (0.1)	3	0.3 (>3)	1	0.3 (0.3)
RWJ-46771	>0.3 (0.3)	1	>1 (1)	NT	0.03 (0.03)
Zaleplon	>3 (3)	1	0.3 (1)	10	3 (>10)
SX-3228	0.3 (1)	1	0.3 (0.3)	>0.3	0.03 (>1)

Motor effects (in brackets) were evaluated by measuring unpunished responding (Punished lever pressing), total arm entries (Elevated plus-maze) and line crossings (Defence test battery). NT: not tested. Adapted from [9, 11, 24, 39–41].

doses which impaired unpunished responding in the lever pressing procedure. One can assume that in the punished drinking test, motor deficits interfere less with responding than in the lever pressing model, so that anticonflict effects are still detectable. In the elevated plus-maze test, all drugs, except RWJ-46771, showed anxiolytic-like activity comparable to that of diazepam. Although the minimal effective dose for each compound was lower than that observed in the punished drinking test, anxiolytic-like activity appeared again at doses which were close to those producing impairment of motor activity as revealed by the data on the number of arm entries, a reliable measure of motor activity in this test. In the light/dark test, only abecarnil and zaleplon produced anxiolytic-like effects. However, it is important to note that the magnitude of the effects of zaleplon was small in comparison to diazepam. Moreover, in the case of abecarnil positive effects appeared at doses which also produced locomotor depression as indicated by results obtained in an actimeter which was run under identical test conditions. In the mouse defence test battery, only zaleplon and SX-3228 elicited anxiolytic-like activity at doses lower than those impairing motor activity. Moreover, like diazepam, zaleplon attenuated all defensive behaviours (e.g. flight, risk assessment, defensive threat and attack) recorded in this test battery.

Taken together, these data suggest that selective $GABA_{A1a}$ receptor ligands may have limited utility as anxiolytic agents and thus question the contribution of $GABA_{A1a}$ receptors in the anxiolytic activity of BZ ligands. A recent study using mice with point-mutated diazepam-insensitive $GABA_{A1a}$ receptors showed that they were still sensitive to the anxiolytic-like action of diazepam in the light/dark test, thereby indicating that different $GABA_A$ receptor subtypes may be involved in these effects [42]. However, the picture seems to be more complex. It was reported recently that the selective $GABA_{A1a}$ receptor antagonist β-CCT completely blocked the anxiolytic-like effects of diazepam in the light/dark test, suggesting that these effects were primarily mediated by $GABA_{A1a}$ receptors [43].

Alternatively, the lack of clear effects of the above-mentioned selective $GABA_{A1a}$ receptor ligands in anxiety models may be explained by the fact that their anxiolytic-like effects may have been confounded by decreases in locomotor activity. They all displayed high intrinsic efficacy as revealed by the findings from the isoniazid-induced convulsion test. Hence, it is possible that selective $GABA_{A1a}$ receptor ligands which behave as partial agonists at this receptor subtype may prove to produce fewer sedative effects, but retain anxiolytic properties. Clearly, further studies are needed before any definitive conclusion can be drawn on the contribution of selective $GABA_{A1a}$ receptor in the anxiolytic effects of BZ/ω receptor ligands and, therefore, on the anxiolytic potential of selective $GABA_{A1a}$ compounds.

The few clinical data available with abecarnil and zolpidem do not clarify the picture of the therapeutic potential of selective $GABA_{A1a}$ ligands as anxiolytics. One clinical study showed that zolpidem and the BZ triazolam displayed comparable efficacy in improving anxiety states of insomniac patients

[44]. In two dose-finding studies in subjects with generalised anxiety disorder (GAD), abecarnil demonstrated efficacy in global improvement ratings and on the Hamilton Anxiety Scale [45, 46]. Moreover, in a placebo-controlled study in patients with GAD, abecarnil was found as efficacious as the BZ alprazolam [47]. However, it is worth mentioning that the higher doses of abecarnil had a high incidence of CNS sedative adverse effects.

Central depressant effects of selective $GABA_{A1a}$ receptor ligands

Central depressant effects of traditional BZs generally seen as sedation, ataxia or myorelaxation are usually manifested at doses higher than those producing anxiolytic-like actions. For example, Figure 3 shows that diazepam impaired the performance of rats in the actimeter, the rotarod and the loaded grid tests (three models generally used to examine the sedative, ataxic and myorelaxant properties of psychoactive drugs, respectively) within a dose-range (3–10 mg/kg) which was slightly higher than that producing anxiolytic-like activity (2.5–3 mg/kg) (Tab. 4) [24]. Following the initial finding that the selective $GABA_{A1a}$ receptor ligand CL218,872 produced a motor deficit on an inclined plane in rats at doses much higher than those increasing punished responding in the Vogel conflict test [48], it was suggested that drugs with selectivity for $GABA_{A1a}$ receptors may have less propensity to produce ataxia and muscle relaxation than non-selective compounds. This idea was strengthened by results from several studies showing that the selective $GABA_{A1a}$ receptor ligands zolpidem, abecarnil and alpidem induced muscle relaxation and ataxia at doses much higher than those producing other pharmacological effects [49–52]. As illustrated by Figure 3, the overall profile of central-depressant effects of the selective $GABA_{A1a}$ compounds is quite different from that displayed by the non-selective BZ/ω receptor agonist diazepam. Unlike diazepam, which significantly impaired motor performance in the actimeter, rotarod and loaded grid tests at the same doses, zolpidem, abecarnil, RWJ 46771 and SX-3228 induced myorelaxation at doses which were 3 to 10 times higher than those needed to decrease exploratory activity. Overall, these findings suggest that the $GABA_{A1a}$ subtype is not primarily involved in mediating the myorelaxant effects of BZ/ω receptor agonists. Consonant with this view is a recent finding which showed that the selective receptor antagonist β-CCT did not block the myorelaxant effects of diazepam [43]. Moreover, it was reported that mice lacking a diazepam-sensitive α_1 subunit still displayed muscle relaxation following the administration of diazepam [42]. As yet it is unclear which $GABA_A$ receptor subtype mediates the myorelaxant effects of BZ/ω receptor agonists. Nevertheless, the fact that the relative proportion of $GABA_{A3a}$ and $GABA_{A5a}$ sites are particularly high in the spinal cord [22] might indicate that these receptors play a particularly important role in these effects.

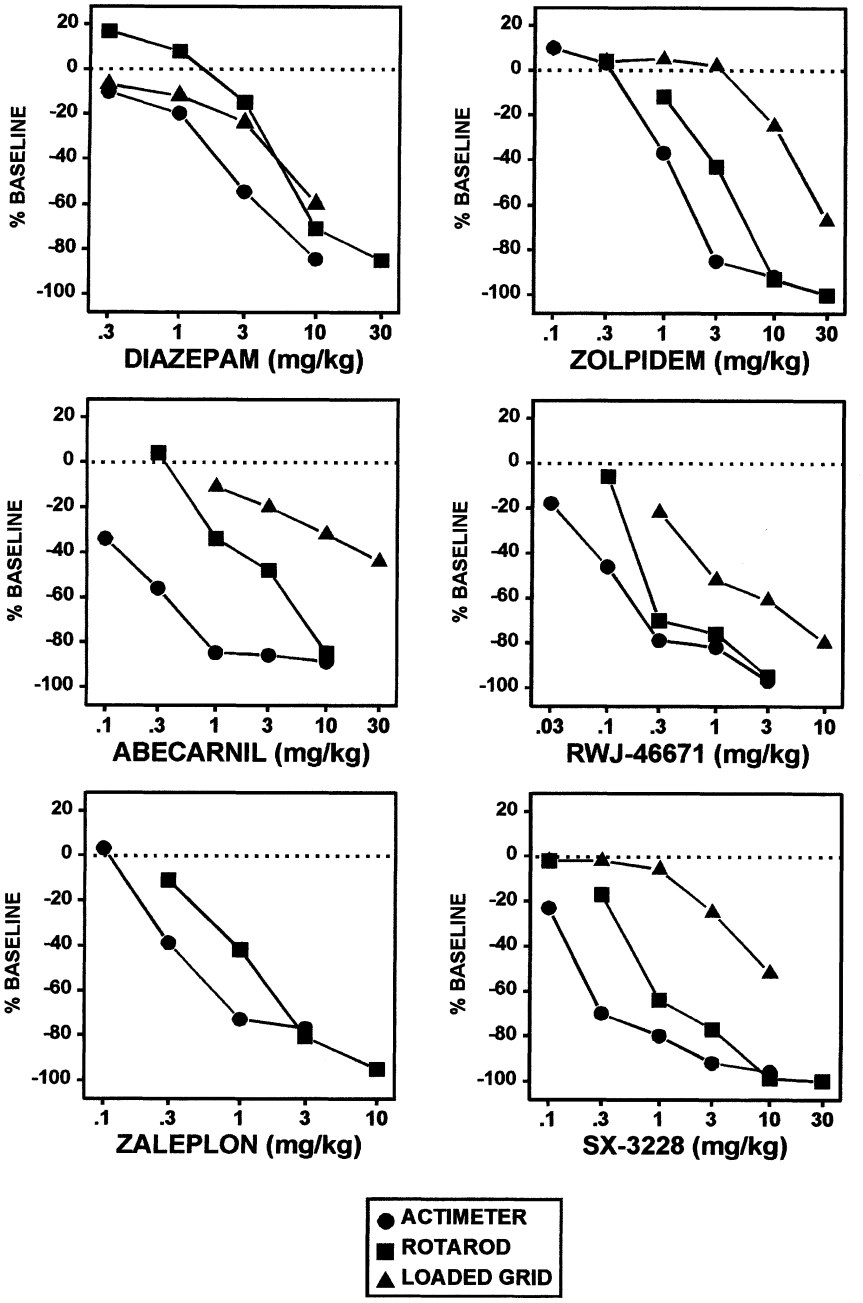

Figure 3. Effects of diazepam and several selective $GABA_{A1a}$ receptor ligands on tests measuring spontaneous locomotor activity (actimeter), ataxia (rotarod) and myorelaxation (loaded grid) in rats. Data are expressed as percentage of baseline levels. Adapted from [24].

Cognitive effects of selective $GABA_{A1a}$ receptor ligands

Anterograde amnesia is one of the troublesome adverse effects of the BZs, especially when they are used as tranquillisers. In animal procedures, BZs can give rise to actions indicating disturbances in learning [53]. Passive avoidance tests have been extensively used for studying the effects of BZs on learning and memory. These studies showed that administration of a BZ before the first trial produces a response deficit on the second trial, indicating a failure of acquisition. The selective $GABA_{A1a}$ receptor ligands zolpidem and alpidem have also been studied in passive avoidance tests in mice [13, 54]. Results showed that although both compounds disrupted the acquisition of conditioned fear, these effects occurred only at doses which greatly decreased locomotor activity, suggesting that a learning deficit may have been secondary to an action on motor performance. In contrast, diazepam disrupted learning at doses lower than those impairing motor activity. A comparative study of the effects of abecarnil and diazepam in a three-panel runway task has shown that the former drug impaired only working memory, whereas diazepam markedly impaired both reference and working memory. Moreover, the effects of abecarnil on working memory disappeared rapidly within 2 to 3 days of repeated treatment, whereas that of diazepam persisted during 14 days of repeated treatment [55].

Several clinical studies have described the effects of selective $GABA_{A1a}$ receptor ligands on various tests of memory. Zolpidem and alpidem have been found to produce impairments in a number of tests of recognition and recall, but these effects occurred at doses normally used for sleep-induction (zolpidem) or at doses higher than those recommended for the treatment of anxiety (alpidem) (for review, see [56]). In a study involving a comparison between abecarnil and lorazepam, Hege and colleagues [57] showed that these drugs impaired performance of healthy volunteers in tests of cognitive functions including memory encoding. However, abecarnil produced substantially less impairment than lorazepam. In another study, zaleplon and lorazepam were found to display similar impairment profiles in tests of cognitive functions, but recovery was rapid with zaleplon, whereas impairment induced by lorazepam persisted throughout the post-drug testing sessions [58]. In addition, zaleplon administered up to six times the dose normally used to induce sleep (ie 60 mg) did not affect the performance of healthy volunteers in a word recall test [59].

Taken together, both animal and clinical studies indicate that although selective $GABA_{A1a}$ receptor ligands may interfere with learning and memory processes, these effects usually occur at high and sedative doses.

Discriminative stimulus effects of selective $GABA_{A1a}$ receptor ligands

With BZ/ω receptor ligands, drug discrimination procedures provide additional information that assists in identifying receptor mechanisms involved in the

actions of these drugs. The stimulus effects of BZs has been analysed in great detail following early studies using chlordiazepoxide [60] and diazepam [61]. In general, there is complete cross substitution between different BZs. Figure 4 shows the effects of several selective $GABA_{A1a}$ receptor ligands in rats trained to discriminate chlordiazepoxide [62]. In contrast to results obtained with non-

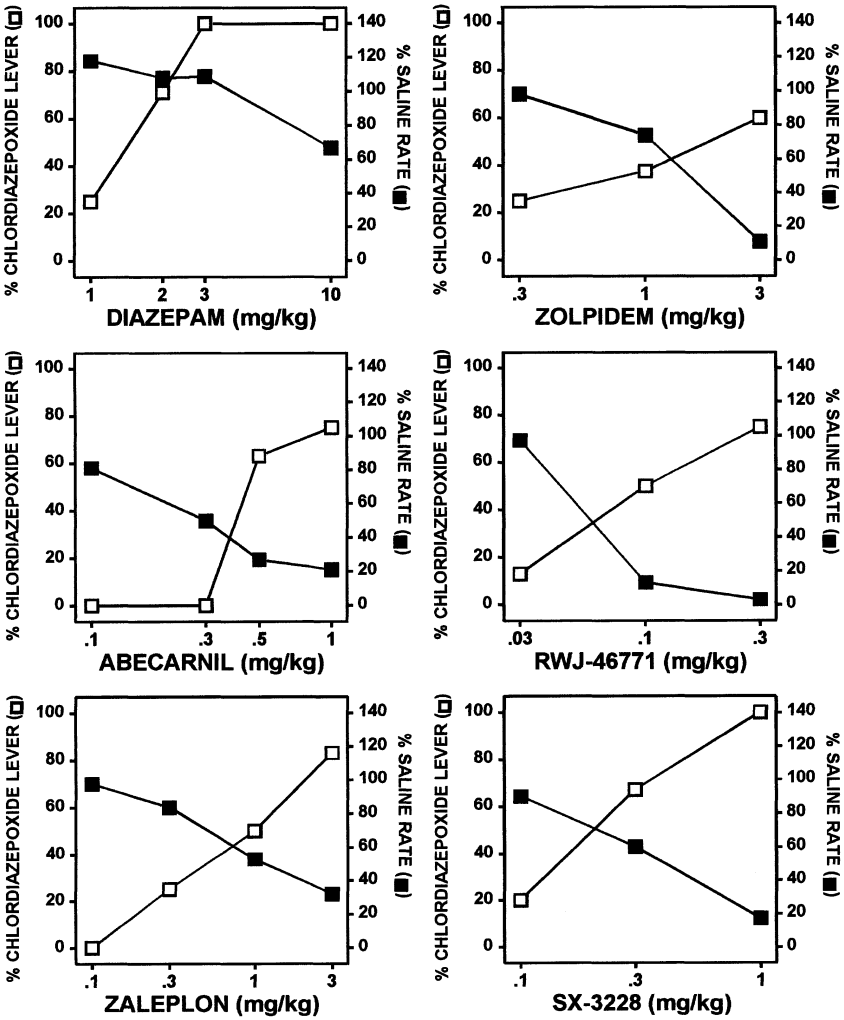

Figure 4. Effects of diazepam and several selective $GABA_{A1a}$ receptor ligands in rats trained to discriminate chlordiazepoxide (5 mg/kg) from saline. The results are shown as the percentage of animals responding on the chlordiazepoxide lever and the average response rate expressed as the percentage of response rates after saline injection. Adapted from [62].

selective compounds, these selective agents produced only partial substitution for the chlordiazepoxide cue and this activity was apparent only at sedative doses. These results show that this stimulus is mediated by $GABA_A$ receptor subtypes other than $GABA_{A1a}$. In line with this hypothesis, it has been demonstrated that there is a strong and significant correlation between drug lever responding following the administration of a variety of selective and non-selective BZ/ω receptor ligands, and *in vivo* displacement of [^{3}H]-flumazenil in the spinal cord, a region enriched in $GABA_{A5a}$ receptors ($r = 0.96$) [63]. The use of $GABA_{A5a}$ selective agents would provide more direct evidence that this receptor subtype is of relatively greater importance than the $GABA_{A1a}$ receptor in mediating the discriminative stimulus produced by chlordiazepoxide. Other experiments have used the $GABA_{A1a}$ selective agents zolpidem, alpidem, CL218,872 and zaleplon as training drugs in drug discrimination experiments with rats [62]. The results of these experiments showed differences between the effects of these compounds and non-selective agents suggesting that the discriminative cues may have been mediated by activity at the $GABA_{A1a}$ receptor subtype.

Effects of selective $GABA_{A1a}$ receptor ligands following repeated administration

Long-term administration of BZs is often associated with the development of tolerance. Drug tolerance has been defined as the process by which the effects of the same dose of a drug decrease with repeated administration. These effects are particularly well established for anticonvulsant and central depressant activities, but are not observed frequently in tests that assess anxiolytic-like activity [64]. Studies with the selective $GABA_{A1a}$ receptor ligands zolpidem, alpidem and zaleplon showed that these drugs did not give rise to tolerance to their anticonvulsant effects against isoniazid-, pentylenetetrazole- and/or bicuculline-induced convulsions in mice following repeated administration, while marked tolerance was observed with the BZs midazolam and diazepam [12, 34, 65]. Similarly, little tolerance was observed to the decrease in rates of operant responding produced in rats by zolpidem and CL 218,872, whereas clear tolerance was found with chlordiazepoxide, midazolam and triazolam [66–68]. Although in one study abecarnil was also found to produce little tolerance to its anticonvulsant effects against pentylenetetrazole-induced seizures following repeated administration for 10 days [55], in another study the drug was reported to lose anticonvulsant activity after 4 weeks of treatment [69]. The authors of the latter study discussed several possible reasons which may account for this discrepancy such as the use of an inadequate study design for obtaining predictable information on the tolerance of BZ/ω receptor ligands. Despite these latter findings, there is a great deal of evidence that chronic treatment with selective $GABA_{A1a}$ receptor ligands produces little or no tolerance to their anticonvulsant activity.

Numerous studies have documented withdrawal syndromes following abrupt discontinuation of long-term BZ treatment [64]. During withdrawal, the original anxiety symptoms often return in a more intense form. This phenomenon has also been described in laboratory research [56]. In animals, BZ-induced withdrawal signs can be quantified with a variety of behavioural and physiological measures and range from convulsions to subtle behavioural changes indicative of increased anxiety. Perrault et al. [12, 65] used increased sensitivity to convulsant drugs as a measure of physiological dependence to show that repeated treatment with the selective $GABA_{A1a}$ receptor ligands zolpidem and alpidem for 10 days did not modify sensitivity to convulsions induced by isoniazid, pentylenetetrazole or β-CCM. By contrast, in these studies, diazepam and midazolam produced increased sensitivity to all convulsant challenges. A similar lack of increased sensitivity to seizures induced by electroshock was observed following chronic zolpidem and CL218,872 [70]. Several studies with abecarnil have also found little or no evidence that repeated administration with this drug induces BZ-like dependence [71–74]. For example, Steppuhn and colleagues showed that mice withdrawn from repeated administration of abecarnil for 12 days displayed no anxiety and no changes in seizure susceptibility and muscle tone, unlike those treated chronically with diazepam which showed increased anxiety, muscle rigidity and seizures. In another study, chronic administration of abecarnil in baboons produced only transient signs of a mild withdrawal syndrome after drug discontinuation [72].

Clinical studies with zolpidem [75–79], alpidem [80] and zaleplon [81] have also indicated that a withdrawal syndrome does not occur with these drugs under conditions where such a syndrome is observed with some BZs. Although in two clinical trials with abecarnil there was no evidence for signs of withdrawal after drug discontinuation [47, 82], in another study, withdrawal symptoms emerged in patients who abruptly discontinued abecarnil (particularly at the higher dosage) but only in those receiving a longer duration of treatment [83].

Taken as a whole, these results with zolpidem, alpidem, abecarnil and zaleplon suggest that selective $GABA_{A1a}$ receptor ligands may give rise to little or no physiological dependence.

Final comment

It is clear from the above data that compounds which display selectivity for the $GABA_{A1a}$ receptor subtype offer several clinical advantages over traditional BZs. So far only compounds with high intrinsic efficacy at the $GABA_{A1a}$ receptor subtype have been described. Because of their high propensity to produce sedation, these compounds are useful in the clinical management of sleep disorders, but their utility in the treatment of anxiety disorders is limited. Selective compounds with partial agonistic activity at the $GABA_{A1a}$ receptor

subtype may circumvent the problem of sedation and may thus represent a valid alternative to agents currently used for the treatment of anxiety disorders.

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