

# Amino Acid Transmitter Systems

Catherine Belzung, Guy Griebel, Florence Dubois-Carmagnat and Jean Michel Darves-Bornoz

## INTRODUCTION

Amino acids correspond to a wide range of compounds, including precursors of catecholamine and serotonin synthesis (tyrosin and tryptophan respectively) as well as neurotransmitter systems such as the excitatory amino acids glutamate and aspartate and the inhibitory amino acids  $\gamma$ -aminobutyric acid (GABA) and glycine. As few studies revealed anxiolytics acting on excitatory amino acids via the *N*-methyl-D-aspartate (NMDA) complex and/or the metabotropic glutamate (mGlu) receptors, only a short paragraph will be devoted to such agents. Furthermore, data suggesting abnormalities in excitatory amino acid systems in some anxiety disorders will be mentioned. On the other hand, we will focus on anxiolytic drugs acting on GABAergic neurotransmission, as most anxiolytics act via GABA-related mechanisms, first focusing on the mechanisms underlying the anxiolytic action of some anti-anxiety agents such as benzodiazepines (BZs) and second describing the use of such compounds in the clinic, in an attempt to link the pharmacology with neurochemical changes that have been observed in these disorders.

## ANXIOLYTIC AGENTS ACTING ON EXCITATORY AMINO ACID NEUROTRANSMISSION

The anti-anxiety-like action of compounds acting on excitatory amino acid neurotransmission has mainly been investigated in pre-clinical studies, using animal models of anxiety. Indeed, because of multiple side effects of such compounds (ataxia, myorelaxation, impairment of learning and memory), such agents cannot be proposed as potential anxiolytic drugs in the clinic.

Glutamate and aspartate bind to two types of receptors: the ionotropic receptors (AMPA, kainite and NMDA receptors) and the metabotropic glutamate receptor. Among the ionotropic receptors, solely the NMDA receptor has been proposed as a potential target for anxiolytic agents in pre-clinical studies.

### Ligands of the NMDA Receptor

The NMDA complex consists of various binding sites, including a glutamate recognition site, a polyamine site, a glycine site, a phencyclidine site (channel site) and a  $Zn^{2+}$  site. Low doses of the non-competitive NMDA antagonist MK-801 or of the competitive NMDA antagonists AP5, AP7 and CPP elicited anxiolytic behaviour in several animal tests (see Chojnacka-Wójcik and Klodzinska (2001) for a recent review). Similar effects were observed with antagonists (7-CIKYN and 5,7-CIKYN) and partial agonists (HA-966, ACPC and *D*-cycloserine) of the Glycine<sub>B</sub> receptors; however, none of these agents crosses the blood-brain barrier.

Finally, discrepant results were obtained with antagonists of the polyamine site (ifenprodil and eliprodil) as these compounds are endowed with anxiolytic properties in some, but not all animal models of anxiety.

### Ligands of the Metabotropic Glutamate Receptor

The mGlu receptors are a family of eight receptors designated mGlu1 through mGlu8, which can be divided in three groups based on the similarity of the amino acid sequence, pharmacology and second messenger coupling. The first group, which consists of mGlu1 and mGlu5, is positively coupled with phospholipase C and is sensitive to *trans*-ACPD as well as quisqualate. The second group, which includes mGlu2 and mGlu3, is negatively coupled to adenylate cyclase and is sensitive to *trans*-ACPD but not quisqualate. The third group, consisting of mGlu4, mGlu6, mGlu7 and mGlu8, is negatively coupled to adenylate cyclase and does not respond to *trans*-ACPD and quisqualate but rather binds specific compounds. Antagonists of Group I mGlu receptors such as S-4C3HPG or (S)-4CPG, as well as an antagonist of the mGlu1 (CPCCOEt) or of the mGlu5 (MPEP) elicited anxiolytic effects in pre-clinical models. Furthermore, LY-354740, an agonist of Group II mGlu receptors, displayed anxiolytic activity in several models of anxiety. In fact, as Group II mGlu receptors are localized presynaptically, their receptor agonists may inhibit glutamate release, so that they are parallel to the effects of Group I mGlu receptors antagonists. Finally, regarding Group II mGlu receptors ligands, the issue remains to be clarified as discrepant results have been obtained.

## ANXIOLYTIC AGENTS ACTING ON GABAERGIC NEUROTRANSMISSION

### Early Anxiolytics Acting via a GABAergic Mechanism

#### *Ethanol*

There is evidence suggesting that ethanol, a compound usually termed as alcohol, has been used in prehistoric times. It can therefore be considered as the first anxiolytic compound. Mead, a fermentation product of honey, is considered as the oldest alcoholic beverage; it seems that it existed in the paleolithic age, about 8000 B.C. Alcoholic beverages are known to produce relaxation, elevation of mood, anxiolysis and disinhibition in response to social constraints. At higher doses it induces sedation. Most alcohol users drink occasionally (75% of the population of the USA). However, 15% of users are considered alcoholics. This highlights the great abuse potential of this compound.

### Barbiturates

In 1864, Adolph von Baeyer synthesized barbituric acid (malonylurea). The name of this drug is said to be linked to the presence, on the day of the experiment, of Baeyer in a tavern in which officers were celebrating the Day of St. Barbara, their patron saint. Barbituric acid was devoid of clinical potency but it led to the development of barbiturates after 1903, the date of the synthesis of barbital that became rapidly popular. These compounds rapidly took a dominant place because they facilitated sleep and produced relaxation. However, the bioavailability of barbital was rather low because of the poor solubility in lipids of that drug. Furthermore, it was metabolized slowly so that the effects on drowsiness extended over 36 hours. Consequently, new drugs with short duration of action (amobarbital, pentobarbital, secobarbital) and later, in the 1930s, ultra-short duration of action (hexobarbital, thiopental, methohexital) were introduced into therapeutics. These drugs, termed barbiturates, are widely used as anaesthetics but they also have an excellent efficacy in alleviating anxiety. For example, pentobarbital (Figure XIX-3.1) is effective in most rodent models of anxiety. However, their development in the treatment of anxiety has been spurred by their high abuse potential. This has been shown in animal as well as human studies. Furthermore, it also elicited lethal effects (1500 deaths per year), principally due to accidental poisoning in drug abusers and to suicide.

### Carbamates

As in many other cases, the starting point of the use of carbamates in the treatment of anxiety had nothing to do with their action in the central nervous system. In the 1940s, the Wallace Laboratories were developing new antibacterial agents and therefore chemists attempted to improve the potency of phenoxetol, a compound used as disinfectant, by lengthening the carbon chain. When testing the toxicity of that newly synthesized compound, termed mephnesin, they observed that it produced muscle relaxation and a sleeping-like condition in animals. They described this action as 'quieting influence on the demeanor of the animal', an effect that was named in 1946 'tranquillization'. The drug was marketed in 1947 as a short-action muscle relaxant but in 1949

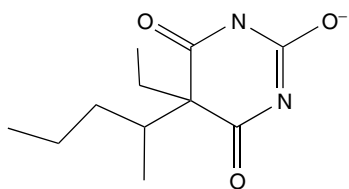


Figure XIX-3.1 The structure of pentobarbital

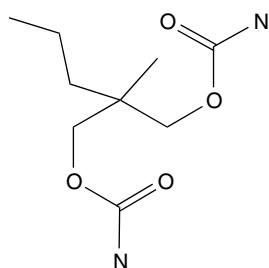


Figure XIX-3.2 The structure of meprobamate

several authors proposed that it may alleviate anxiety. However, mephnesin had several drawbacks, including a very short duration of action. Therefore, researchers attempted to alter the chemical structure of mephnesin to overcome these shortcomings: the result was meprobamate (Figure XIX-3.2), a compound whose duration of action was eight times that of mephnesin. In the early 1950s, Berger demonstrated that meprobamate possessed anxiolytic properties. This was shown in monkeys whose fear was reduced in threatening environmental situations, and in rats, in which meprobamate was effective in disinhibiting behaviour that was suppressed by punishment (Geller-Seifter test). Berger claimed that unlike alcohol or barbiturates, the anxiolytic effects of meprobamate were not associated with impairment of intellectual or physical performance. He explained these effects by an action on 'those specific areas in the brain as the thalamus and the limbic system, that represent the biological substrate of anxiety'. Consequently, between 1950 and 1960, meprobamate was one of the most commonly used drugs for the treatment of anxiety worldwide. Later, however, the image of meprobamate was tarnished by numerous reports of lethal overdoses. Moreover, the anxiolytic effect was accompanied by drowsiness and ataxia.

As will be discussed later, these three categories of compounds interfere with GABAergic neurotransmission, which is the principal inhibitory neurotransmitter within the central nervous system.

### The Discovery of Benzodiazepines

#### The Discovery of Chlordiazepoxide

At the end of the 1950s, pharmaceutical companies started to become interested in the field of psychopharmacology. This was related to the commercial success of meprobamate and other psychoactive agents such as chlorpromazine. In this context, a team at Hoffman-La Roche, led by Leo Sternbach, started to study some heptoxdiazines that Sternbach synthesized in the early 1920s when he was a postdoctoral student at the university of Cracow, looking for dyestuffs. In fact, these compounds had no interesting properties as dyes, and no evidence existed in favour of an action of these compounds on the central nervous system. Sternbach decided to study them only because they were unexplored and convenient to test because of their chemical versatility that allowed many transformations. First Sternbach discovered that these compounds were not heptoxdiazines but quinazolone 3-oxides. He then synthesized 40 derivatives and found that all of them, except one that was not tested, were biologically inactive. The last one, labelled RO 5-0690, was disregarded, mainly because of other research priorities. In May 1957, during a cleanup of the laboratory, one of the collaborators of Sternbach found this drug and suggested that it may be tested. Therefore RO 5-0690 was given to the team led by Randall for animal testing. Six tests were used for the screening: a test for sedation and muscle relaxation, a foot-shock test to measure 'taming' effects, another test for muscle relaxation and three tests for anticonvulsant activity. The drug was compared to phenobarbital, chlorpromazine and meprobamate. It was superior to the latter compounds in all tests and therefore Randall announced in 1960 that chlordiazepoxide (the new name of RO 5-0690) may have potent sedative, muscle relaxant, taming and anticonvulsant activity. It was introduced into pharmacotherapy under the tradename 'Librium' (from 'Equilibrium') in 1960, only two-and-a-half years after the first pre-clinical tests. It was then shown that this compound also elicited anti-anxiety effects.

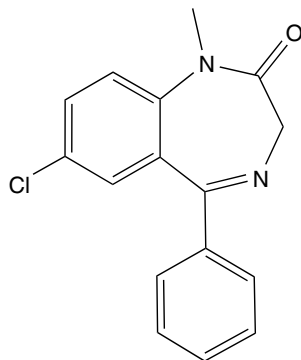
#### Synthesis of other BZs

A more potent analogue, diazepam, was synthesized in 1961 and, later, 50 other BZs were marketed throughout the world.

**Table XIX-3.1** Half-life of major BZs

BZ	Duration of action (h)*
Chlordiazepoxide	55 +/- 35
Diazepam	55 +/- 35
Oxazepam	17 +/- 11
Lorazepam	16 +/- 8
Alprazolam	11 +/- 5

\*Including active metabolite

**Figure XIX-3.3** Chemical structure of diazepam. The figure shows the core structure made up of the benzene ring (left ring) fused to a diazepine ring (right part, top). The third ring is an aryl substituent ring (bottom, right part)

These new compounds include oxazepam (1965), nitrazepam (1965), clorazepate (marketed in 1968), lorazepam (marketed in 1973), bromazepam (1974), clobazam (1975) and flunitrazepam (1978). The need for new compounds was mainly related to the necessity to develop compounds with a shorter half-life than chlordiazepoxide and diazepam (see Table XIX-3.1 for more details).

BZs are metabolized extensively, generating active metabolites. Some of these metabolites are biotransformed more slowly than the parent compound so that there is no clear relationship between the half-life of a BZ and its duration of action. It should also be noted that BZs display metabolic inter-relationship with common active metabolites. For example, chlordiazepoxide, clorazepate, prazepam, diazepam as well as demozepam are all metabolized in desmethyl diazepam. Some BZs (prazepam, flurazepam) reach the systemic circulation only in the form of active metabolites. All these compounds have a rather close chemical structure. In fact, the name BZ is derived from the benzene ring fused to the diazepine ring (Figure XIX-3.3). They all share common pharmacological properties as they are all sedative-hypnotic, muscle relaxant, anxiolytic and anticonvulsant. Unfortunately, they also display unwanted side effects: they induce anterograde amnesia, tolerance and dependence. However, when compared to the early anxiolytic agents (barbiturates and meprobamate), they displayed a high therapeutic index so that they became rapidly and widely prescribed and very popular, in particular in the treatment of anxiety. In 1972, chlordiazepoxide and diazepam accounted for half of all psychoactive prescriptions in the USA. This led to increasing concerns about their over-use, so that some countries such as the UK introduced a limited prescription list of these compounds in the mid 1980s. However, the prevalence of BZ use is still very high.

## Mechanisms of Action

### *Mechanisms of Action: First Discoveries*

The mechanism of action of BZs remained a mystery until a key observation was made in the unravelling of this knot. Indeed, in 1967, electrophysiological studies on the cat spinal cord revealed that diazepam could potentiate the dorsal root potential. The significance of this observation was, however, not realized until the discovery that the dorsal root potential was associated with the activation of local inhibitory neurotransmission using GABA. In fact, the effects of BZs on the dorsal root potential requires an intact GABA system within the spinal cord. It was later observed that the ability of BZs to potentiate GABAergic neurotransmission was a ubiquitous phenomenon, present in many brain areas. This evidence originates not only from electrophysiological studies but also from a biochemical study. The major type of GABA receptor in the brain, termed GABA<sub>A</sub> receptor, is associated with an ionophoric postsynaptic Cl<sup>-</sup> channel that mediates inhibitory neurotransmission in the brain regulating Cl<sup>-</sup> permeability. BZs do not bind to the GABA<sub>A</sub> receptor but they potentiate the action of GABA on the GABA<sub>A</sub> receptors. Therefore, they require the presence of GABA to express their pharmacological actions.

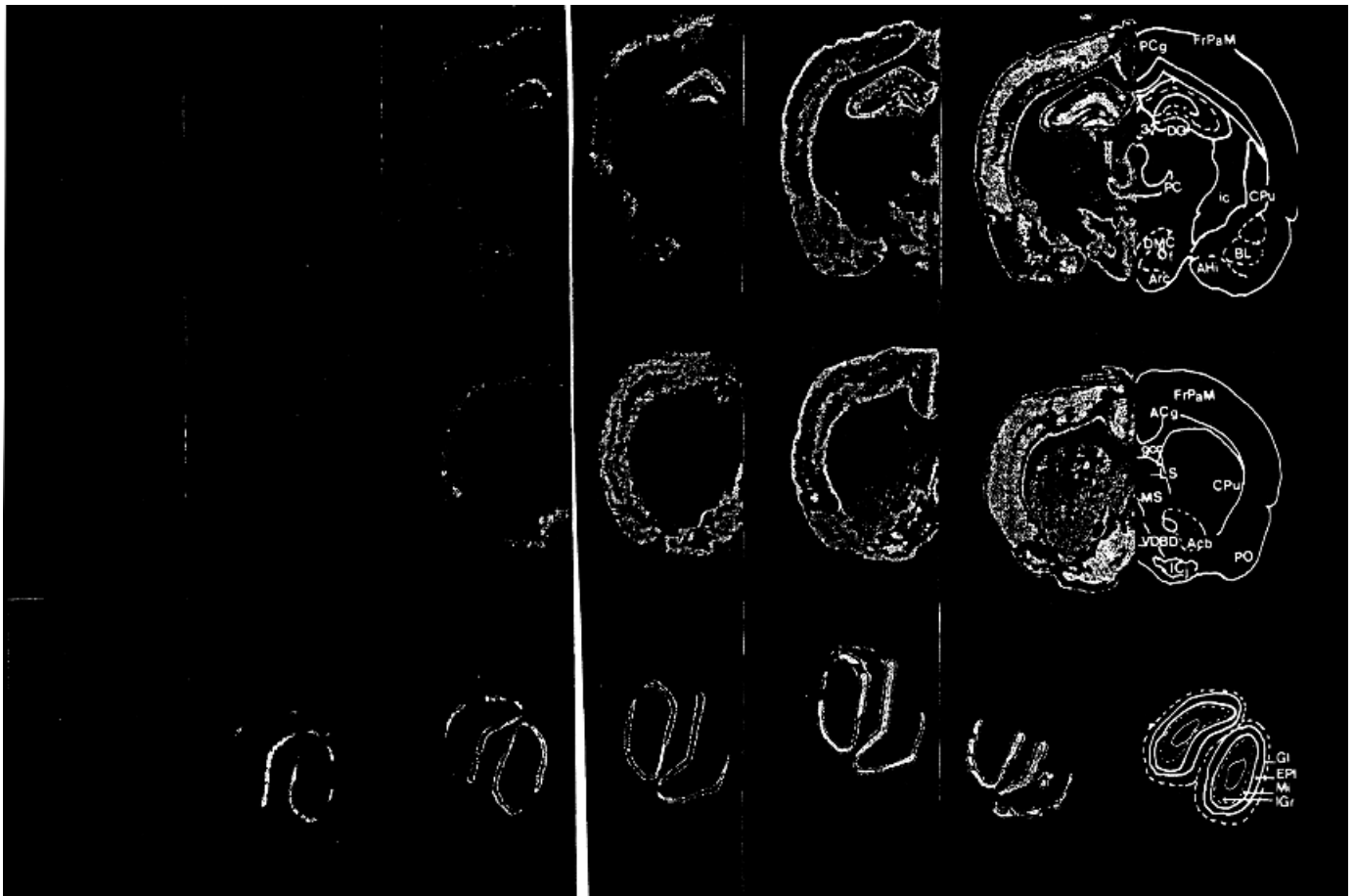
### *BZ Receptors*

The next milestone in the understanding of the mechanism of action of BZs was the discovery in membrane preparation of high affinity binding sites for [<sup>3</sup>H]-diazepam that were saturable and stereospecific. This was first reported in 1977 by two independent teams (Braestrup and Squires in Copenhagen, and Möhler and Okada in Basle). One year later, similar binding sites were identified in the human brain, using another ligand, [<sup>3</sup>H]-flunitrazepam. BZ receptors are located on dendrites, nerve cell bodies and nerve terminals. Autoradiographic studies showed that BZ binding sites are widely distributed within the central nervous system, with the highest concentrations in the cerebral cortex, intermediate concentrations in the limbic system and the cerebellum, and lowest in the pons-medulla and spinal cord (Figure XIX-3.4).

The clinical efficacy of BZs has been attributed to their ability to bind BZ receptors. Indeed, there is a positive correlation between the K<sub>i</sub> values for the inhibition of [<sup>3</sup>H]-diazepam binding by various BZs and their average therapeutic recommended doses (Figure XIX-3.5).

It is important to emphasize that BZs not only bind to BZ receptors. For example, they have also nanomolar affinity for adenosine receptors. However, this is often forgotten so that their psychoactive effects are always attributed to their binding to BZ receptors.

Binding sites are also found in certain peripheral tissues, and represent the so-called mitochondrial BZ binding site. They were first discovered in the kidney, and later they were found in other peripheral tissues such as the adrenal glands and the testes. Such sites also exist in the central nervous system, where they are mainly found on glial cells. These sites are not associated with the GABA<sub>A</sub> receptor. The binding site is different from the brain BZ receptor, since it does not recognize all psychoactive BZs (for example clonazepam) and binds some compounds such as the isoquinoline, PK 11195, with nanomolar affinity. They are located subcellularly on the outer membrane of the mitochondria, rather than on the cell membrane. Their function is not well known in all cell types, but it has been suggested that in steroid hormone-producing organs (adrenal glands, testes), they are involved in the transport of cholesterol from the outer to the inner mitochondrial membrane. In the brain, it has been proposed that these receptors may be involved in neurosteroidogenesis.



**Figure XIX-3.4** Autoradiography of  $^3\text{H}$ -Flunitrazepam binding sites in the rat brain. PCg: Posterior cingulate cortex; FrPaM: Frontoparietal cortex, motor area; 3v: third ventricle; DG: Dentate Gyrus; PC: Posterior Cingulate; iC: Internal capsule; Cpu: Caudate Putamen; BL: Basolateral amygdaloid nucleus; Ahi: Amygdalohippocampal area; Arc: Arcuate hypothalamus nucleus; f: fornix; ACg: anterior cingulate cortex; gcc: genu corpus callosum; LS: Lateral septum; MS: Medial septum; VDBD: Nucleus Vertical limb, diagonal band, dorsal part; Acb: Accumbens nucleus; PO: Primary olfactory cortex, Icj: Island of Calleja; EPI: external plexiform layer of the olfactory bulb. Reproduced from photostat by permission from Marcel, D., Weissmann-Nanopoulos, D., Mach, E. and Pujol, J.F., 1986. Benzodiazepine binding sites: localization and characterization in the limbic system of the rat brain. *Brain Research Bulletin*, **16**, 573–596

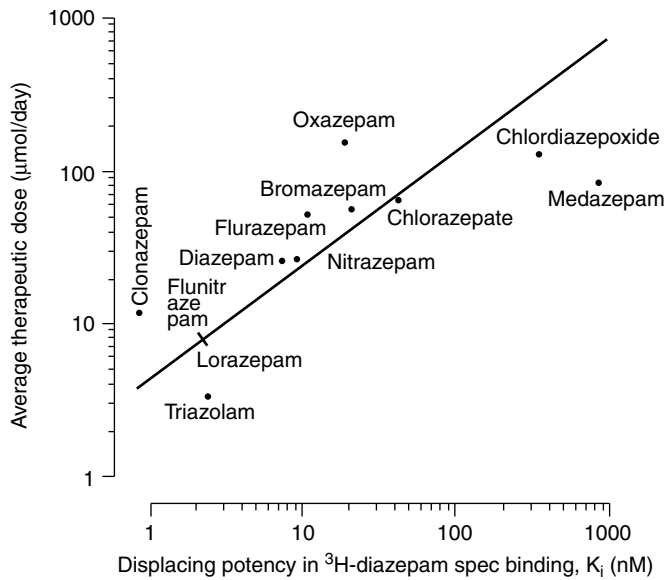
### Ligands of BZ Receptors

Up until the early 1980s, it was widely admitted that the chemical structure of BZs was a prerequisite for the binding to the BZ receptor. However, this view was challenged by the discovery that some chemically unrelated drugs (Figure XIX-3.6) such as cyclopyrrolones (zopiclone, suriclone), triazolopyridazines (CL 218,872), phenyl-imidazo-pyridine acetamides (alpidem, zolpidem), quinolines (PK 8165) and pyrazoloquinolines (CGS 8216, CGS 9896) bind to the same site as BZs, sometimes with dissociation constants in the low picomolar range, thus equalling the affinities of the most potent BZs. They act on the BZ receptor in a similar way to BZs.

However, not all compounds that bind with high affinity to the BZ receptor exhibit the same pharmacological profile as BZs. Indeed, compounds have been described that do not have any intrinsic activity when injected alone, but they block the pharmacological action of BZs. These compounds have therefore been termed antagonists of which the first to be identified was flumazenil, also called RO 15-1788 (Figure XIX-3.7). Indeed, this drug has antagonistic properties both *in vitro* and *in vivo*; it is

able to block the anxiolytic, anticonvulsant, amnesic, myorelaxant and sedative effects of BZs. Flumazenil is used therapeutically to control BZ anesthesia. Furthermore, other pharmacological agents that bind to the BZ receptor, such as ethyl- $\beta$ -carboline-3-carboxylate ( $\beta$ -CCE), have been described, but they induce a pharmacological profile opposite to the one induced by BZs. Indeed, they produce anxiogenic, proconvulsant and promnesic effects. They have therefore been termed inverse agonists. Not all inverse agonists have the same potency. For example, compounds such as DMCM and  $\beta$ -CCM (Figure XIX-3.7) are convulsant, while others such as  $\beta$ -CCE or FG 7142 cannot trigger seizures *per se*, but will sensitize animals to the convulsant effects of other pharmacological agents (proconvulsant effects): the first category has been termed full inverse agonists while the second is called partial inverse agonists.

In fact, subsequent studies have revealed compounds that span the complete efficacy spectrum from full agonists to full inverse agonists, including partial agonists such as bretazenil. Antagonists are able to block the effects of agonists and also the effects of inverse agonists or of partial agonists. See Table XIX-3.2



**Figure XIX-3.5** Correlation between BZ  $K_i$  and mean therapeutical dose recommended. Reproduced by permission from Möhler, H. and Okada, T., 1978. The benzodiazepine receptor in normal and pathological human brain. *British Journal of Psychiatry*, **133**, 261–268

**Table XIX-3.2** Properties of the various types of BZ receptor ligands

Different types of ligands	Example of compounds	Pharmacological action
Full agonist	Classical BZ (chlordiazepoxide, diazepam, oxazepam, flunitrazepam, etc.)	Sedative Anxiolytic Anticonvulsant Amnesic Myorelaxant
Partial agonist	Bretazenil	Anxiolytic
Antagonist	Flumazenil	No effect
Partial inverse agonist	$\beta$ -CCE, FG 7142, RO 15-4513	Anxiogenic Proconvulsant
Inverse agonist	DMCM, $\beta$ -CCM	Anxiogenic Convulsant Promnesic

A great amount of data exists showing that higher doses of a full agonist are required to produce sedative/myorelaxant effects than to produce anticonvulsant/anxiolytic effects. For example in mice, the dose to induce sedation is twice the minimal effective dose for anxiolytic activity. By combining this approach with *in vivo* binding studies, it is possible to assign a level of receptor occupancy to a given pharmacological effect. This type of analysis demonstrates that for full agonists such as diazepam, between 20 and 40 percent of receptor occupancy is required to produce anxiolytic or anticonvulsant effects. For the same compound, the receptor occupancy must be higher than 60% to elicit sedation or myorelaxant effects (Figure XIX-3.8). This idea has led to the development of partial agonists as anxiolytic agents devoid of sedative side effects. Indeed, partial agonists are not able to activate all the receptors they occupy, so that the dose eliciting sedation may be considerably larger than that inducing anxiolysis (Doble and Martin, 1996).

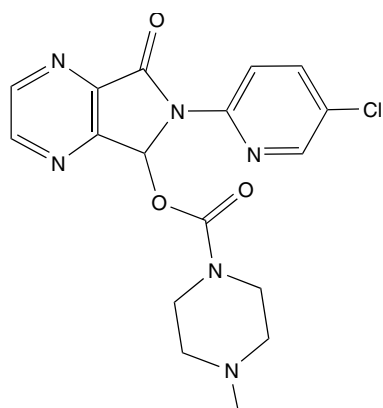
### Endogenous Ligand of BZ Receptors

The presence of BZ receptors has provided some support for the notion that some natural BZ receptor ligands may exist in the central nervous system. Therefore, some research began in the early 1980s that aimed at finding an endogenous ligand for BZ receptors. Such a compound must be present in the organism, bind to the BZ receptors with high affinity and elicit behavioural effects. Diazepam Binding Inhibitor (DBI) is an 86 amino acid peptide that was initially isolated from rat brain on the basis of its ability to displace diazepam from BZ receptors. Splicing of DBI generates several biologically active fragments including the triakontatetrapeptide DBI<sub>17-50</sub> (TTN) and the octadecaneuropeptide DBI<sub>33-50</sub> (ODN) which are designated by the generic term endozeptines. Intracerebroventricular injections of endozeptines in rodents elicit anxiogenic effects (Garcia de Mateos-Verchere *et al.*, 1998) and block the anxiolytic action of diazepam. Evidence from *in vitro* and *in vivo* studies indicates that these compounds may act as inverse agonists at the BZ receptor, thus negatively modulating the GABA<sub>A</sub> receptor function. Subsequently, it was also observed that endozeptines interact with peripheral BZ receptors and stimulate cholesterol transport in the mitochondria, thus participating in the biosynthesis of neurosteroids by brain tissues. It is to be noted here that neurosteroids also modulate the GABA<sub>A</sub> receptor function. *In situ* hybridization experiments showed strong DBI mRNA expression in the vicinity of the third ventricle, the hypothalamus and the cerebellum. Long-term isolation in mice, which is a rather stressful procedure in this species, has been shown to induce a decrease in mRNA expression for DBI in the hypothalamus, further suggesting that these peptides may have a biological function related to anxiety and/or stress (Dong *et al.*, 1999).

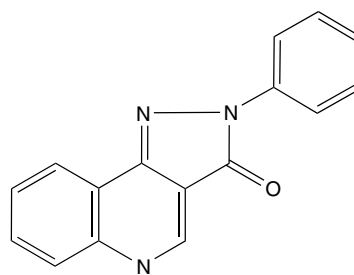
### Interaction with GABA

As mentioned above, BZs potentiate the action of GABA on the GABA<sub>A</sub> receptors. Interestingly, the ability of anxiolytic compounds to act on GABAergic neurotransmission is shared by other anti-anxiety agents, such as barbiturates, meprobamate and ethanol. For example, barbiturates such as pentobarbital increase the affinity of GABA<sub>A</sub> receptors to GABA and increase the duration of the opening of GABA-activated Cl<sup>-</sup> ionophoric channels. Moreover, at high doses, this compound is able to directly open Cl<sup>-</sup> channels, even in the absence of GABA. This action is exerted via a specific binding site, termed the barbiturate binding site. As to the mechanism of action of carbamates, recent data suggest that meprobamate may also act at the barbiturate binding site of the GABA<sub>A</sub> receptor (Rho, Donevan and Rogawski, 1997). However, the enigma of the mechanism of action of meprobamate is not completely resolved, because meprobamate does not always have the same effects as barbiturates (Haefely *et al.*, 1981). Finally, ethanol is also able to interact with the GABAergic neurotransmission. Indeed, ethanol activates the GABA<sub>A</sub> receptor-coupled Cl<sup>-</sup> channel, thereby increasing Cl<sup>-</sup> conductance and mimicking the action of GABA. Although some effects of ethanol are also mediated via other molecular targets such as the NMDA and the 5-HT<sub>3</sub> receptors, one may suggest that its anxiolytic effects are mediated via GABA<sub>A</sub> receptors. Indeed, BZ antagonists such as flumazenil or BZ inverse agonists such as RO 15-4513 are able to block the anxiolytic effects of ethanol in rodents at doses where they do not have any intrinsic activity.

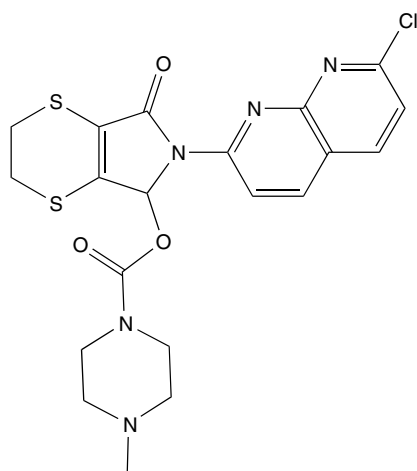
Present knowledge proposes a model in which the GABA<sub>A</sub> receptor may in fact be allosterically modulated by compounds binding to at least six different sites (for a review, see Hevers and Lüddens (1998)): the BZ receptors; a binding site for barbiturates; a site for neurosteroids; a site for the convulsant drugs picrotoxin and TBPS; one for furosemide and one for loreclezole. Binding



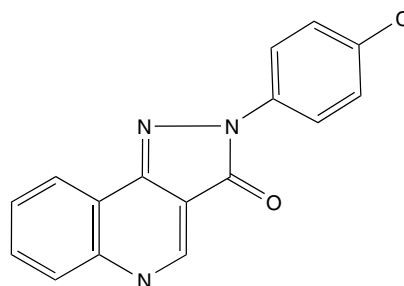
Zopiclone



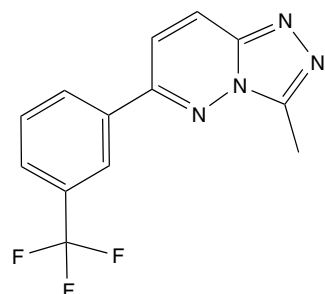
CGS 8216



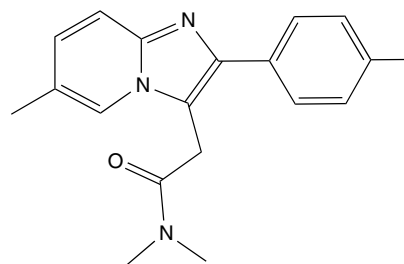
Suriclone



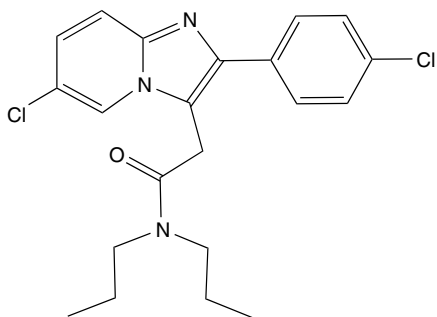
CGS 9896



CL218,872

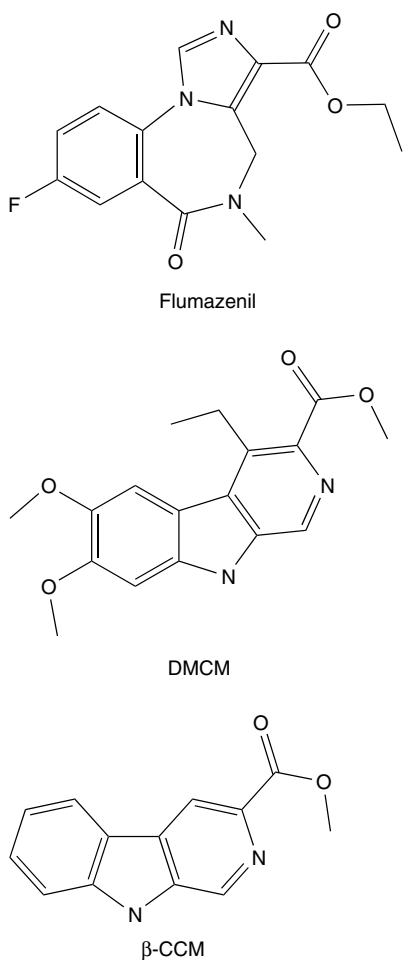


Zolpidem



Alpidem

Figure XIX-3.6 Chemical structure of zopiclone, suriclone, CL 218,872, alpidem, zolpidem, CGS 9896 and CGS 8216



**Figure XIX-3.7** Chemical structure of the BZ antagonist flumazenil and of the BZ inverse agonists DMCM and  $\beta$ -CCM

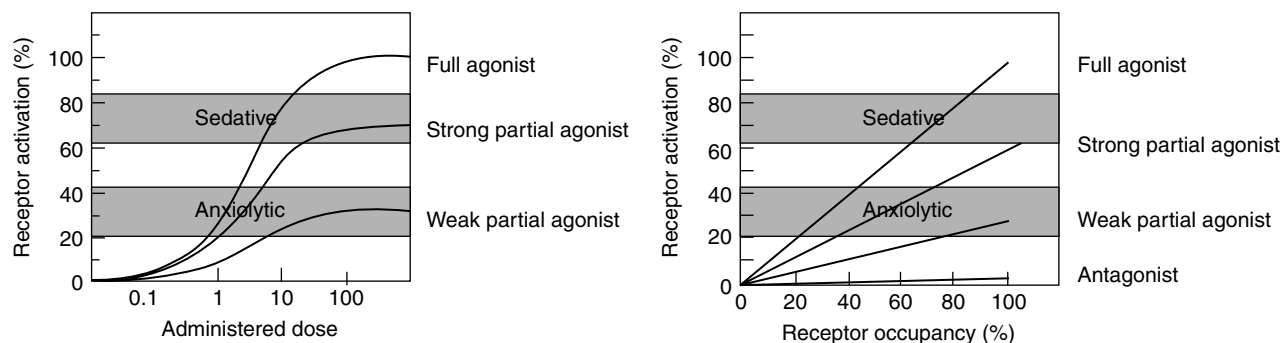
of BZs to the BZ receptors, of barbiturates to the barbiturate binding site, of steroids such as metabolites of progesterone to the neurosteroid site, or of loreclezole to the loreclezole site, are all associated with an anticonvulsant effect underlined by a positive modulation of GABAergic neurotransmission, that is an increase in

GABA function. By contrast, binding of picrotoxin to the picrotoxin site induces convulsions, an effect related to the ability of this compound to block GABA-evoked  $\text{Cl}^-$  conductance. Furosemide, a loop diuretic, inhibits GABA function in some (for example in the cerebellum) but not all neuronal population (for example not in hippocampal neurons) via a mechanism independent of the other allosteric sites.

None of these sites are associated with anxiolytic or anxiogenic effects. As mentioned above, BZs and barbiturates are potent anxiolytics. Antagonists of the picrotoxin site such as etifoxin also induce anxiolysis: this effect is not blocked by flumazenil, thereby indicating that it is not linked to an action at BZ receptors. Some endogenous steroids such as progesterone and its  $3\alpha$ -reduced metabolite produce a dose-dependent anxiolytic response in animal models of anxiety. However, negative modulators of the neurosteroid site, such as pregnenolone sulphate have not been shown to induce the opposite effect in a consistent manner. Finally, some data suggested that loreclezole may induce anxiolytic effects in the rat: these effects are not blocked by flumazenil, suggesting that they are not related to the BZ site. To our knowledge, no data have shown any effect of ligands at the furosemide binding site on anxiety. Moreover, facilitation of GABAergic neurotransmission by the GABA<sub>A</sub> receptor agonist THIP or the GABA transaminase inhibitor  $\gamma$ -acetylene GABA (a compound that increases GABA function by inhibiting the degradation enzyme of GABA) does not elicit anxiolytic effects in the rat while drugs that have the opposite action, such as GABA synthesis inhibitors or GABA<sub>A</sub> receptor antagonists fail to induce anxiogenic effects (Ågmo *et al.*, 1991).

#### Molecular Biology of GABAergic Pentamer

The findings mentioned in the precedent chapter can appear contradictory as GABA mimetic drugs are not able to elicit an anxiolytic effect, while the positive modulation of allosteric sites linked to this receptor induce anxiolysis. An explanation of this apparent discrepancy is related to the molecular structure of the GABA<sub>A</sub> receptor complex. Indeed, molecular biology has revealed that the GABA receptor is composed of five subunits that co-assemble. Six classes of subunits have been described:  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$  and  $\rho$  (for a review, see Hevers and Lüddens, 1998). Within each type of subunit, several isoforms are possible: in mammals, these are  $\alpha 1-6$ ,  $\beta 1-3$ ,  $\gamma 1-3$ ,  $\delta$ ,  $\epsilon$  and  $\rho 1-3$ . The  $\rho 1-3$  subunits do not seem to co-assemble with  $\alpha$  or  $\beta$  subunits within GABA<sub>A</sub> receptors and are mainly described in the retina. As to the  $\epsilon$  subunit, little information is available because it has only been recently described. The most frequent stoichiometric combination



**Figure XIX-3.8** Receptor occupancy–activity relationships for full and partial agonists. Left: receptor activation as a function of receptor occupation. Right: receptor activation as a function of dose administered. Reproduced by permission from Doble, A. and Martin, I.L., 1996. The GABA<sub>A</sub>/Benzodiazepine Receptor as a Target for Psychoactive Drugs. Springer

includes 2  $\alpha i$ , 1  $\beta j$  and 2  $\gamma k$  as well as 2  $\alpha i$ , 2  $\beta j$  and 1  $\gamma k$  (with  $i = 1-6$ ,  $j = 1-3$  and  $k = 1-3$ ). However, other stoichiometries have been described, such as 3  $\alpha i$ , 1  $\beta j$  and 1  $\gamma k$ . Thus, the number of possible isoforms of the GABA<sub>A</sub> receptor may exceed 100 000. However, not all of the possible isoforms have been described within the central nervous system and approximately 20 isoforms seem to represent the most abundant ones.

Among the  $\alpha 1-6$  subunits, the  $\alpha 1$  is the most frequent and it has been described in almost all brain areas. It is often co-localized with  $\beta 2$  and  $\gamma 2$ . Clustering is characteristic of GABA-A receptor genes on human chromosomes. In humans, the  $\alpha 1$  subunit maps on Chr5q34-q35, in a cluster including the gene for the  $\gamma 2$  and the  $\alpha 6$  subunit. The human gene for  $\alpha 1$  does not appear to have any mutations associated with disease entities. The  $\alpha 2$  subunit is mainly localized in the cortex, in limbic areas and in the striatum (where it represents the only  $\alpha$  subunit reported). None are found in the cerebellum. It often co-localizes with  $\beta 3$  subunits.  $\alpha 2$  (with  $\alpha 4$  and  $\gamma 2$ ) is expressed at low levels during early development, with a significant increase about one week after birth. However, the biological importance of that is not well understood for the moment. The  $\alpha 2$  and the  $\beta 1$  subunits map close together Ch4 p13-p12. The human gene for this subunit does not appear to have any mutations associated with anxiety disorders or other diseases. The  $\alpha 3$  subunit is mainly localized in the monoaminergic nuclei, particularly on serotonergic cell bodies in the raphe nuclei, as well as on cholinergic neurons of the basal forebrain. The gene coding for the  $\alpha 3$  subunit is located in the central region of chromosome X and has been suggested as a candidate for X-linked manic depression, a psychiatric disease that has some comorbidity with anxiety disorders. The  $\alpha 4$  subunit is localized in the hippocampus and the thalamus and the gene encoding for this subunit is located on chromosome 4 in humans. The  $\alpha 5$  subunit is located quasi exclusively in the hippocampus and the gene encoding for this subunit is located on human chromosome 15, in the region of the Angelman and Prader-Willi syndromes. In fact, a single paternal allele of this gene is found in cases of Angelman syndrome, and a single maternal allele in cases of Prader-Willi syndrome. However, evidence of abnormal anxiety in these disorders is scarce. Finally, the  $\alpha 6$  subunit is located mainly in the cerebellar granule cells and the cochlear nuclei. The gene encoding for this subunit forms a cluster on chromosome 5 with the genes encoding for the  $\gamma 2$  and  $\alpha 1$  subunits. All these data are summarized in Table XIX-3.3.

Interestingly, the  $\alpha$  subunits seem to determine the ability of the BZ receptors to respond to BZ. Indeed, receptors containing  $\alpha 4$  or  $\alpha 6$  subunits lack the modulation by classical BZs. Classical BZs such as chlordiazepoxide interact indiscriminately with receptors containing  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$  or  $\alpha 5$  subunits, that are termed benzodiazepine-sensitive. These receptors have a histidine at a conserved position ( $\alpha 1$ -H101,  $\alpha 2$ -H101,  $\alpha 3$ -H126 and  $\alpha 5$ -H105) while the benzodiazepine insensitive receptors containing  $\alpha 4$  or  $\alpha 6$  subunit have an arginine in the corresponding position. Therefore,

diazepam-sensitive receptors can be rendered insensitive by replacing this histidine by an arginine while the regulation of this receptor by GABA is preserved. Recent data show that replacement of histidine by arginine at the 101 position of the gene encoding for the murine  $\alpha 1$  subunit gene has rendered the  $\alpha 1$ -type GABA<sub>A</sub> receptors insensitive to diazepam ( $\alpha 1$ -knock-in mice). These  $\alpha 1$ -knock-in mice display no overt change in spontaneous behaviour and bred normally but they failed to show the sedative, amnesic and anti-convulsant action of diazepam. By contrast, the anti-anxiety and myorelaxant properties of the BZ are fully retained (Rudolph *et al.*, 1999). A similar point-mutation technique has been used to render  $\alpha 2$  and  $\alpha 3$  insensitive to BZs. As for the  $\alpha 1$ -knock-in mice, no obvious modification of basal behaviour was observed. However, pharmacological challenge with diazepam failed to induce anxiolysis in the  $\alpha 2$ -knock-in mice (Low *et al.*, 2000). No similar modification could be found with the  $\alpha 3$ -knock-in mice. These observations point to new strategies for drug design, as one can imagine drugs of the future acting specifically on some  $\alpha$ -subunit subtypes, that is on BZ receptors within a particular brain area. For example, drug acting in an agonistic way specifically on  $\alpha 2$ -type GABA<sub>A</sub> may elicit an anxiolysis not accompanied by sedation or amnesic effects (see below).

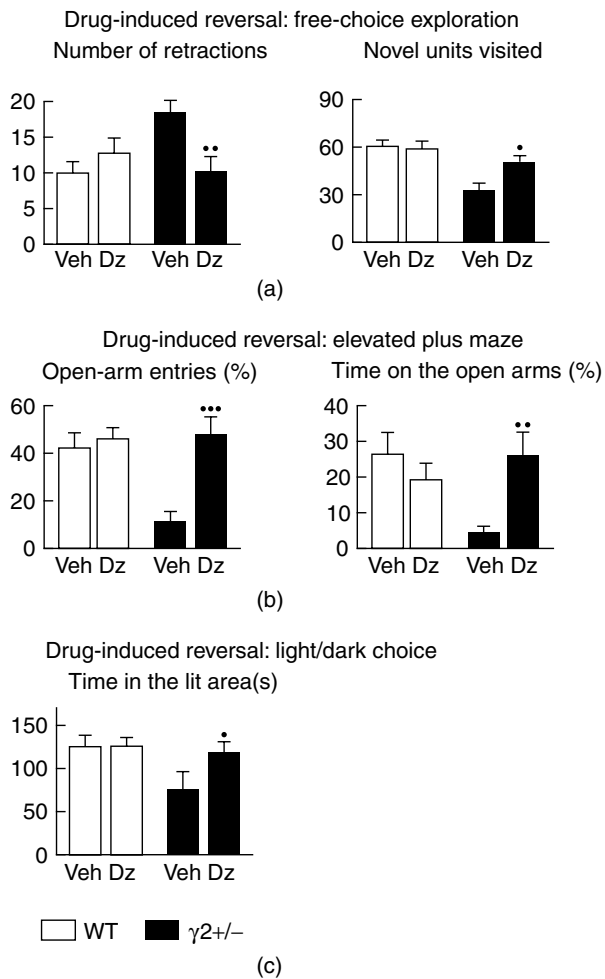
As to the  $\beta$  subunits, they seem to have little influence on the action of BZ receptor ligands. Indeed, *in vitro* studies showed that the type of  $\beta$  isoform did not modify the ability of diazepam to increase GABA-activated Cl<sup>-</sup> currents. In fact, they seem to be involved in brain development as they are all expressed in the developing mouse cerebellum, the  $\beta 2$  and  $\beta 3$  subunits being present at birth, and displaying spatial correspondence with areas of GABAergic synapses. The  $\beta 1$  subunit mRNA does not appear until the second week after birth, and may be associated with Bergmann glia or basket cells.

More data suggest that the  $\gamma$  subunits may be involved in the action of BZ. When compared to the  $\gamma 2$  isoform, the  $\gamma 3$  isoforms displays a marked decrease in affinity for the BZ antagonist flumazenil or the inverse agonist DMCM while replacement of the  $\gamma 2$  isoform with a  $\gamma 1$  isoform results in an agonistic affinity of DMCM. By using targeted mutation,  $\gamma 2$  knock-out mice have been generated ( $\gamma 2$  -/-). These mice display severe growth retardation, sensorimotor abnormalities and a reduced life span as survival was never superior to 17 days of postnatal life. They are insensitive to BZs, indicating that this subunit is critical for BZ sensitivity. As no behavioural studies could be undertaken in such young mice, features of anxiety-related disorders have been investigated in  $\gamma 2$  +/- mice. These mice exhibited a region-specific reduction of BZ receptors. This decrease was more pronounced in some areas of the hippocampus (reduction of 35% in CA1 and 28% in CA3), in the cingulate cortex (-25%), in the frontal cortex (-23%), in the piriform cortex (-25%) and in the lateral septum (-30%) than in other brain areas such as the striatum (-6%), the globus pallidus (-13%) or the amygdala. These mice exhibited enhanced state and trait anxiety that was reversed by diazepam. Furthermore, they displayed a bias for threat cues, resulting in an increased sensitivity

**Table XIX-3.3** The properties of different types of  $\alpha$  subunits

Type of $\alpha$ subunit	Ability to bind BZ	CNS localization	Human chromosome	Mouse chromosome	Cluster
$\alpha 1$	Yes	All brain areas	Chr 5q34-q35	Chr 11	$\gamma 2$ and $\alpha 6$
$\alpha 2$	Yes	Cortex, limbic areas, striatum	Chr 4p13-p12	Chr 5	$\beta 1$
$\alpha 3$	Yes	Raphe nuclei Basal forebrain	X q28	Central part of X	No
$\alpha 4$	No	Hippocampus, thalamus	Chr 15 p14-q12	Chr 5	No
$\alpha 5$	Yes	Hippocampus	Chr 15q11-q15	Chr 7	$\beta 3$ and $\gamma 3$
$\alpha 6$	No	Cerebellar granule cells, cochlear nuclei	Chr 5q31.1-q35	Chr 11	$\gamma 2$ and $\alpha 1$





**Figure XIX-3.9** Behaviour of wildtype (WT) and  $\gamma 2^{+/-}$  mice subjected to three tests of anxiety (a) the free exploratory test; (b) the elevated plus maze; (c) the light/dark choice test and treated either with vehicle or diazepam ( $0.3 \text{ mg kg}^{-1}$ , i.p.). Results show that  $\gamma 2^{+/-}$  mice display increased anxiety when compared to wildtype mice as they exhibit an increased number of retraction, a decrease in novel units visited, a decrease of entries and time spent on the open arms and a decreased time spent in the lit box. At the dose used, diazepam did not elicit anxiolysis in vehicle-treated WT while it reversed the anxiogenic pattern observed in  $\gamma 2^{+/-}$  mice. Reproduced by permission from Crestani, F., Lorez, M., Baer, K., Essrich, C., Benke, D., Laurent, J.P., Belzung, C., Fritschy, J.M., Luscher, B. and Mohler, H., 1999. Decreased GABA<sub>A</sub>-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nature Neuroscience*, **2**, 833–839

to negative associations (Figure XIX-3.9) (Crestani *et al.*, 1999). Alternate RNA splicing produces two alternative forms of the  $\gamma 2$  subunit ( $\gamma 2L$  and  $\gamma 2S$ ) that are expressed in different brain regions, more  $\gamma 2L$  being found in the cerebellum, while  $\gamma 2S$  is in the ascendant in the cerebral cortex. Moreover,  $\gamma 2S$  is expressed at a more or less constant level throughout brain development, while  $\gamma 2L$  production increases with maturation. Mice lacking  $\gamma 2L$  ( $\gamma 2L^{-/-}$ ) display increased sensitivity to BZ agonists associated with a decreased sensitivity to BZ inverse agonists, indicating a possible shift of the BZ receptor from an inverse agonist, leaning towards an agonist preferring configuration (Quinlan, Firestone and Homanics, 2000). However, in that case, one might expect a decrease of anxiety levels in those mice, while the contrary was observed. Surprisingly,

$\gamma 2$  over-expression did not elicit any changes in several animal models of anxiety (Wick *et al.*, 2000).

Little information is available concerning the relevance of the  $\delta$  subunit in the GABA<sub>A</sub> receptor in relation to anxiety. A GABA<sub>A</sub> $\delta$  null mutant ( $\delta^{-/-}$ ) mouse has been described that did not exhibit any modification of anxiety in animal models. However, the neuroactive steroid ganaxolone was unable to elicit anxiolytic action in the knock-out mice, as it does in wildtype mice suggesting a possible involvement of this subunit in the anti-anxiety-like effect modulators of the neurosteroid site of the GABA<sub>A</sub> receptor.

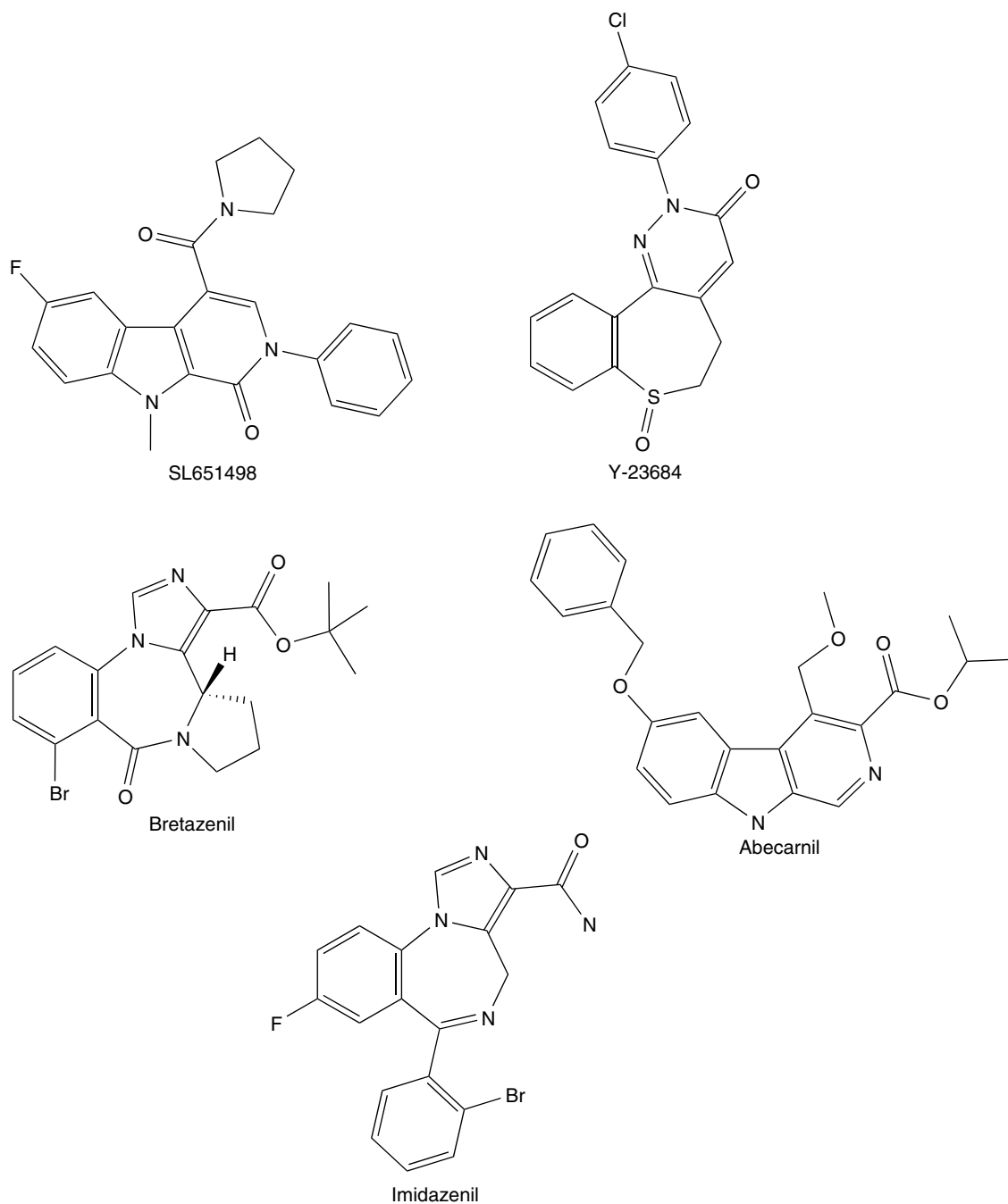
### Drugs of the Future Acting at the GABA<sub>A</sub> Receptor Subtypes

The search for compounds chemically unrelated to the BZs with more specific therapeutic actions and without their concomitant unwanted effects has led to the development of drugs that selectively bind to a specific GABA<sub>A</sub> receptor subtype, display low efficacies at each GABA<sub>A</sub> receptor subtype, or combine selective affinity and differential intrinsic activity at these receptors (Griebel, Perrault and Sanger, 2000).

While there are GABA<sub>A</sub> receptor ligands claimed in patents or shown to bind selectively for all BZ-sensitive GABA<sub>A</sub> receptor subtypes, only compounds selective for the  $\alpha 1$  subtype have been studied extensively. These latter include compounds with greatly varying chemical structures. The most widely studied selective  $\alpha 1$  subtype ligands include the imidazopyridine zolpidem (see Figure XIX-3.6), the  $\beta$ -carboline abecarnil (Figure XIX-3.10) and the pyrazolopyrimidine zaleplon. These compounds are either marketed (zolpidem, zaleplon) or pre-registered (abecarnil). In animal studies, zolpidem and abecarnil were found to produce sedative activity at much lower doses than those producing ataxia and myorelaxation, and after repeated treatment, did not produce tolerance and physical dependence as was observed with most BZs. However, selective GABA<sub>A</sub>  $\alpha 1$  subtype agonists are generally found to display weaker (if any) anxiolytic-like activity in animals than non-selective agents, thereby confirming the findings with  $\alpha 1$  knock-in mice which showed that this subtype is not primarily involved in the anxiolytic effects of GABA<sub>A</sub> receptor agonists (Rudolph *et al.*, 1999).

Unlike selective GABA<sub>A</sub>  $\alpha 1$  subtype agonists, non-selective GABA<sub>A</sub> receptor partial agonists such as bretazenil, imidazenil and Y-23684 (Figure XIX-3.10) were found to display comparable or even greater efficacy in anxiety models than BZs. In addition, they had lower liabilities for sedation and muscle relaxation compared to conventional BZs.

Based on the findings from experiments using mice with point-mutated diazepam-insensitive GABA<sub>A</sub> receptor subtypes, that the anxiolytic effects of GABA<sub>A</sub> receptor agonists are mediated by the  $\alpha 2$  GABA<sub>A</sub> receptor, research for anxiolytic compounds acting at the GABA<sub>A</sub> receptor subtypes has focused on the development of ligands that display functionally selective agonist activity at the  $\alpha 2$  GABA<sub>A</sub> receptor subtype. The recently discovered pyridoindole derivative, SL651498 fulfils this criterion. Although the drug has also high affinities for the  $\alpha 1$  and  $\alpha 3$  subtypes, it displays higher intrinsic efficacy at the  $\alpha 2$  subtype as compared to the other GABA<sub>A</sub> receptor subtypes. In animal experiments, SL651498 elicited anxiolytic-like activity qualitatively and quantitatively similar to that of BZs, but unlike these latter, it induced central depressant effects at doses much higher than those producing anxiolytic-like activity. Moreover, in contrast to BZs, SL651498 did not produce tolerance to its anticonvulsant activity or physical dependence, and was much less active than BZs in potentiating the depressant effects of ethanol. The 'anxiolytic' profile of SL651498 is in agreement with the idea that GABA<sub>A</sub>  $\alpha 2$  subtype plays a major role in regulating anxiety, and suggests that targeting selectively GABA<sub>A</sub> receptor subtypes can lead to drugs with increased clinical specificity (Low *et al.*, 2000).



**Figure XIX-3.10** Drugs of the future. Chemical structure of the partial agonists bretazenil, imidazenil and Y-23684, of the  $\alpha_1$  selective agent abecarnil and of the  $\alpha_2$  selective compound SL 651498

## AMINO ACID NEUROTRANSMITTER SYSTEM IN ANXIETY DISORDERS

### Anxiety Disorders

Anxiety can be considered as an everyday life emotion that ones experiences when subjected to threatening or stressful situations. However, in certain cases anxiety can become excessive, as in anxiety disorders. Anxiety disorders have a lifetime incidence of 16% (Walley, Beebe and Clark, 1994) and it is considered that in any six-month period, 9% of Americans are affected by such an

affliction. Anxiety disorders include generalized anxiety disorder (GAD), panic disorder, obsessive-compulsive disorders, phobias and post-traumatic stress disorder (PTSD). In this chapter, we shall not consider dissociative and somatoform disorders as they are studied in further chapters of this book.

### Generalized Anxiety Disorder (GAD)

GAD is considered to be a constant state of anxiety, worries occurring for almost any ordinary event. It frequently has other associated disorders accompanied by apprehension, increased tension

and hyperalertness. Even if it is the most common anxiety disorder, (14.80% worldwide incidence), little research has been carried out on investigating the underlying psychobiological features of that affliction. It has been shown that platelet and lymphocyte BZ receptors have low binding in GAD. As for cerebral BZ receptor binding in subjects with GAD, the findings are contradictory. Some have found a decrease (Tiihonen *et al.*, 1997), while others did not see any difference (Abadie *et al.*, 1999).

BZs are largely prescribed in the treatment of GAD (Hoehn-Saric and McLeod, 1991). Patients are generally treated with BZ having long elimination half-life, so that they do not have to take the treatment several times a day to prevent rebound anxiety. The main risk is linked to the ability of BZs to elicit tolerance and to have abuse potential. As patients need long-term medication, there is a high risk to elicit withdrawal symptoms when the treatment is discontinued. It should be noted that these compounds do not only relieve anxiety, but they are also effective in reducing hyperalertness, insomnia, tension and some somatic symptoms. However, one has to keep in mind the fact that some symptoms of GAD, such as tension, somatic modifications or hyperalertness may in fact contribute to the increase in anxiety, as suggested by some emotion theoreticians. In that case, neither anxiety nor other symptom of GAD can be suppressed by compounds that are not acting on the somato-visceral perception.

### Panic Disorder and Conditioned Fears

Though the delineation of panic disorder as a specific category had heuristic value, it has also left other fruitful conceptions in the dark (Marks, 1987), which are now implicitly coming back when categorizing panic disorder as a fear-conditioned disorder (Gorman *et al.*, 2000). Indeed, in order to understand the onset of panic disorder (characterized by episodic paroxysmic anxiety states, phobic fears, and several autonomic- and endocrine-related symptoms) beside heritable aetiological factors, environmental disruption is needed. Several studies have shown an association between disruptions of early attachment to parents and the development of panic disorder (Tweed *et al.*, 1989; Stein *et al.*, 1996).

When the term 'panic attacks' was introduced in the DSM-III in 1980, the prevalent view was that the anxiety disorder characterized by severe spontaneous panic attacks was mainly alleviated by tricyclic antidepressants, while the anxiety disorder corresponding to the absence of such acute crisis (GAD) was treated by BZs. This was the rationale for splitting anxiety neurosis in two different anxiety disorders in the DSM-III. This historical background led to the prejudice that BZs may be ineffective in panic attacks. This view was reinforced by the observation that imipramine was more effective than chlordiazepoxide in panic attacks (McNair and Kahn, 1981). However, the dose of chlordiazepoxide used in that study was rather low. More recently, the contribution of amino acids in panic disorders has been evoked by the demonstration of the efficacy of higher doses of BZs in blocking panic attacks. The prototypical BZs used in the treatment of panic are alprazolam and clonazepam, even if other BZs are also effective agents. Clonazepam has a longer half-life than alprazolam, which allows a decrease in the number of daily administrations and thus avoids interdose rebound anxiety. However, many clinicians continued to claim that BZs had weak antipanic efficacy when compared with antidepressants. This prompted some researchers to compare the potency of alprazolam with imipramine and placebo in a double-blind study (Sheehan and Raj, 1990). Results showed that alprazolam was as effective as imipramine (except for the depressive symptoms that are often co-morbid with panic disorder, which were only treated by imipramine), both compounds being superior to placebo. Alprazolam was also effective in attenuating lactate-induced panic attacks. The common problem is related to

the abuse potential observed with BZs. Therefore, these compounds are often prescribed for their short-term effects and then dosage is rapidly tapered and antidepressant treatment initiated. BZ are not the sole treatment of panic attack involving increase in GABA function. Indeed, when patients do not respond to BZ or to antidepressants, a medication with valproic acid can be prescribed. Valproic acid increases GABA function by stimulating the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis, and by inhibiting GABA degradation enzymes such as GABA transaminase. Three studies show that valproic acid was superior to placebo on various components of panic attacks and was able to block lactate-induced panic attacks.

Moreover, psychobiological data have also revealed some abnormalities in GABA neurotransmission in patients with panic disorder, suggesting that pharmacological treatments with BZs or valproic acid may in fact act on these abnormalities. For example, patients with panic disorder have been shown less sensitive to BZs on several psychophysiological measures (Roy-Byrne *et al.*, 1996). Furthermore, pre-clinical findings suggested that a decrease of GABA transmission in a network centred in the amygdala — and involving hippocampus and prefrontal cortex — with projections to midbrain central grey and hypothalamus, leads to paroxysmic anxiety responses (LeDoux *et al.*, 1988; Davis, 1992). Finally, intravenous lactate, which induces panic attacks, produces a decrease in circulating plasma GABA levels.

However, most studies of binding at the BZ-GABA<sub>A</sub> receptor are contradictory. Indeed, some findings suggest global reduction of binding (Malizia *et al.*, 1998); other findings affirm only local or state-related panic anxiety reduction (Bremner *et al.*, 2000); and further studies found no peculiarity or increase in BZ receptor density in association with up-regulation hypotheses (Brandt *et al.*, 1998; Abadie *et al.*, 1999). The idea that in this disorder the subject has difficulty in protecting himself in a state of stress could bring forward new hypotheses (Kellner and Yehuda, 1999; Strohle *et al.*, 1999).

### Obsessive Compulsive Disorder (OCD)

OCD is characterized by repetitive thoughts or behaviour that are felt by the subject and are difficult to prevent. Most of the time the behaviour or thoughts are absurd, and consume so much time that they alter social functioning of the subject.

The psychobiological approach accumulates arguments — including those from lesional models (Laplante *et al.*, 1989) — for abnormalities associated with obsessive and compulsive manifestations in frontocortico-striatal-thalamic networks. Indeed, a hyperactivity of this axis has been shown within orbito-frontal and anterior cingulate cortex, as well as caudate nucleus and thalamus, and a decrease of N-Acetyl-Aspartate (NAA), a putative marker of neuron viability, within striatal areas — which could be sites of primary pathology — and thalamus, a site of integration and relay, especially involved in compulsions (Fitzgerald *et al.*, 2000).

Functional neuroimaging studies in OCD patients during symptom provocation suggests increased glutamatergic activity. Current psychobiological research on OCD puts forward that *basal ganglia* dysfunctions could contribute to the aetiology of the symptoms. Indeed, it has been suggested through functional imaging techniques on subjects with OCD, before and after treatment by Selective Serotonin Reuptake Inhibitors (SSRIs), (substances known for their positive effect on OCD symptomatology) that *caudate nucleus* seems primarily involved in the medication's efficacy on OCD (Baxter, Schwartz and Bergman, 1992). The interrelations of amino acids such as  $\gamma$ -aminobutyric acid (GABA) or *glutamate* with serotonin (5-HT) pathways could be enlightened by some findings. First, the 5-HT<sub>2a</sub> stimulation on GABA neurons opposes the glutamate action on the striatum, an area known for being rich in neurons

with serotonin synthesis (Chugani *et al.*, 1998). Second, the prefrontal cortex highly innervates the caudate nucleus (Modell *et al.*, 1989) by glutamatergic projections. Third, glutamate can decrease the release of 5-HT in the caudate nucleus in humans (Becquet, Faudon and Hery, 1990) and be at the same time affected in its action by serotonin neurons (Edwards *et al.*, 1996).

As BZs are effective in the treatment of anxiety, their efficacy in OCD has also been evaluated. In fact, BZs are devoid of anti-obsessive–compulsive effect *per se* but they can alleviate the high anxiety or insomnia that are in some case associated with OCD. In this way they can be considered as a symptomatic treatment of OCD. They can also provide a short-term relief for the distress of the patients, before the anti-obsessive–compulsive effects of serotonin reuptake inhibitors can be observed, or before a cognitive therapy can be undertaken. It should be noted that this observation is not true for all BZs. In fact, there is an exception as the BZ clonazepam has been shown significantly more effective as a monotherapy than some antidepressant after 3-week treatment (Hewlett, Vinogradov and Agras, 1992). A proposed explanation as to the superiority of clonazepam over other BZs in the treatment of OCD is related to the fact that this drug may have specific effects on the serotonergic system.

### Post-Traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is a condition specified by repetition in thoughts, nightmares, physical and mental re-experiencing of the traumatic experience. It is associated with a negative semiology, such as numbing or amnesia, related to the dissociative field. Preclinical data put forward that stress induces cortico-limbic release of glutamate. Clinical observations suggest that *N*-methyl-*D*-aspartate antagonists may also induce glutamate release while provoking dissociative-like symptoms. Some authors then hypothesized that hyperglutamatergic states could contribute to acute and chronic consequences of trauma (Chambers *et al.*, 1999).

Among the psychobiological alterations observed in PTSD, the neuroendocrinological peculiarities in the hypothalamic-pituitary-adrenal axis, including cortisol decrease and reactivity alterations are documented the most. Specificities of GABA function have been studied in subjects with PTSD. The failure of the benzodiazepine antagonist flumazenil to produce flashbacks in PTSD suggest a weak role of attention deficit in the chronic disorder. The background of this topic includes the fact that GABA<sub>A</sub> receptors inhibit meso-prefrontal dopamine neurons, that BZs and neurosteroids enhance GABA<sub>A</sub> inhibition, that altered BZ receptors or a diminution of an endogenous ligand may decrease GABA inhibition and lead to excessive anxiety, and that PTSD may deplete an endogenous ligand and produce receptor alteration.

Morris and his colleagues (Morris *et al.*, 2000) presented an interesting positron emission tomography (PET) study to estimate the binding potential (BP) of GABA<sub>A</sub> benzodiazepine receptors *in vivo* using the BZ receptor antagonist radioligand C<sup>11</sup>-flumazenil, comparing 13 subjects with PTSD and 13 without disorder.  $B_{max}$  was the same for subjects both with and without PTSD;  $K_d$  was lower in PTSD cases, and the  $B_p$  constant of the BZ receptor was higher in PTSD cases, especially in cerebellar, latero-temporal, occipital and prefrontal areas.

BZ medication in PTSD is still controversial. A study has shown that alprazolam induces a slight improvement in anxiety on the Hamilton scale in PTSD patients but there is no superiority of alprazolam over placebo on the PTSD scale. Moreover, alprazolam and clonazepam have positive effects on hyperarousal, as does valproic acid. The GABA transaminase vigabatrin has also been shown to ameliorate the exaggerated startle response that is found in PTSD. These observations suggest that increasing the GABA function may be a treatment of some symptoms of PTSD such as

anxiety, increased startle or hypervigilance, rather than a treatment of PTSD *per se*. Furthermore, PTSD is co-morbid with other anxiety disorders such as GAD so that BZ may in fact alleviate some symptoms related to GAD, rather than treat PTSD.

### Phobias

The main characteristic of phobias is marked and persistent fear of some specific situations (for example enclosed spaces) or objects/animals (small animals such as mice). Here we may emphasize on a particular type of phobia—social phobia—which is characterized by fear of social situations in which embarrassment may occur, as some arguments suggesting an involvement of the GABAergic system have been proposed. Indeed, compounds potentiating the action of GABA such as benzodiazepines and conventional anticonvulsants have been evaluated as treatments for social phobia. Among the benzodiazepines, clonazepam is the best studied, and showed efficacy in several studies. Among the anticonvulsants, gabapentin and pregabalin, which are analogues of GABA, have been shown to be more effective than placebo in double-blind studies. Furthermore, subjects with social phobia showed abnormalities in peripheral benzodiazepine receptors, which suggests that these receptors may play a role in the pathophysiology of this disorder.

### BZ and Anxiety Disorders

With the exception of GAD and, to a lesser extent panic attack, it is noteworthy that BZs or drugs that increase GABA transmission may alleviate some symptoms associated with anxiety disorders (including anxiety, insomnia, tension, startle), rather than treat anxiety disorders *per se*. In these disorders, anxiety is often a symptom subsequent to a core semiology resulting mainly from cognitive activations, for instance ‘re-experiencing’ in PTSD or ‘obsession’ in OCD, rather than a primary autonomic dysregulation and first rank therapeutic target. This is the reason why the ICD-10 classification of the World Health Organization (WHO) preferred to call the chapter from the DSM-IV ‘Somatoform, stress-related and neurotic disorders’ rather than ‘Anxiety disorders’.

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