Neurotransmitters, Drugs and Brain Function.
Edited by Roy Webster

ISBN: Hardback 0-471-97819-1 Paperback 0-471-98586-4 Electronic 0-470-84657-7

19 Anxiety

S. C. STANFORD

INTRODUCTION

Emotional states that would now be classified as anxiety were recognised as long ago as the classical Greek period but have undergone many phases of medical classification since then. Nowadays, 'anxiety' is a term used loosely to cover the clusters of physiological and emotional changes shared by several disorders in which anxiety is a major component (Table 19.1). The current diagnostic criteria for these disorders are defined in the *Diagnostic and Statistical Manual* of the American Psychiatric Association (4th edition: 'DSM–IV' (1994)). The extent to which they share a common neurobiological basis is far from clear but it is evident that different anxiety disorders do not all respond to the same drug treatments. In particular, whereas generalised anxiety disorder (GAD) is treated preferentially with so-called 'anti-anxiety' agents (e.g. the benzodiazepines), these compounds are regarded as relatively ineffective in relieving panic disorder (except, possibly, at high doses) and they are of no benefit at all in treating phobias. In fact, it is antidepressant drugs, especially the selective serotonin reuptake inhibitors (SSRIs), that are turning out to be the most effective treatments for some anxiety disorders and their use has undoubtedly been encouraged by fears that prolonged treatment with benzodiazepines might induce a dependence syndrome. The pharmacology of antidepressants is described in Chapter 20.

The beneficial effects of antidepressants in anxiety are often interpreted as support for a neurobiological link between anxiety and depression. Also, because anxiety often progresses to depression and because these disorders can co-exist in the same patients, it has even been suggested that they might be different manifestations of a single problem (Tyrer 1989). Whether or not this is the case, it is clear that key features of all anxiety disorders, especially the sympathetic arousal, resemble the response to aversive stimuli ('stress') and this overlap has strongly influenced research into the neurobiology of anxiety. However, whereas anxiety drives people to seek medical help, the response to stress is a normal physiological event. A distinctive feature of anxiety, therefore, is that it can be regarded as an inappropriate stress response that is chronic or intermittent, for which the stimulus is either not obvious (as in GAD) or irrational (as in the phobias), or provokes a prolonged emotional disturbance (as in post-traumatic stress disorder).

There are two main approaches to research into anxiety. The first is to establish experimental models of anxiety in animals and humans in order to discover its neurobiological basis. The second is to investigate the actions of anti-anxiety drugs in the brain in the hope that this will give some clues to the cause(s) of anxiety. This chapter will discuss evidence from both these lines of research.

Table 19.1 Anxiety disorders

The diagnostic criteria for, and general features of, disorders in which anxiety is a prominent component are described in detail in the *Diagnostic and Statistical Manual* of the American Psychiatric Association (DSM–IV, 4th edition (1994)) and are regarded as either 'phobias' or 'anxiety states':

PHOBIC DISORDERS: profound fear of, and avoidance of, a dreaded object or situation.

Agoraphobia:	Fear of places or situations from which escape is difficult — can occur with or without a history of panic disorder
Social phobia:	Fear of social or performance situations
Specific phobia:	Fear of a specific object or situation

ANXIETY STATES

Panic disorder (with or without agoraphobia):
Recurrent unexpected panic attacks

Generalised anxiety disorder:
At least 6 months of persistent and excessive anxiety and worry

Obsessive compulsive disorder:
Obsessions (which cause anxiety) and or compulsions (which serve to neutralise anxiety)

Post-traumatic stress disorder (PTSD):
Re-experiencing of traumatic event outside normal human experience with increased arousal and avoidance of stimuli associated with the trauma

Acute stress disorder:
Symptoms similar to PTSD but occur within 1 month of the traumatic event

Anxiety disorder due to a general medical condition:
e.g. Disorders of thyroid function, cardiovascular system, respiratory system, head injury, etc.

Substance-induced anxiety disorder:
e.g. Caffeine, cocaine, alcohol

Anxiety disorder not otherwise specified:
Prominent symptoms of anxiety that do not fit any of the above categories

SYMPTOMS AND SIGNS OF ANXIETY

(Modified from Nutt 1990)

Mood:	Apprehension, worry, difficulty in concentration, irritability, insomnia
Cognitions:	Fear of (for example): death, ineffectiveness, failure, humiliation, mental illness
Somatic:	Cardiovascular (tachycardia, palpitations), sweating, respiration, GIT, muscle tension, tremor, muscle aches or soreness, nausea, exaggerated startle reflex, increased urinary frequency
Behaviour:	Hypervigilance, nail-biting, scratching

ANIMAL MODELS OF ANXIETY

All preclinical animal models of anxiety involve exposing animals (usually rats or mice) to environmental stimuli that disrupt their normal pattern of behaviour (Table 19.2). Obviously, it can never be confirmed that animals are actually experiencing the equivalent of human anxiety and so the validity of all preclinical models rests largely on confirming that the change in behaviour is prevented by drugs that have established anti-anxiety effects in humans.

An influential theory proposed by Gray (1987) suggests that environmental stimuli that induce anxiety in both rats and humans fall into three major groups, all of which present 'threats' to the individual. One of these is 'novelty' in which the subject's innate

Table 19.2 Environmental stimuli that induce changes in behaviour which are prevented by anti-anxiety drugs

'*Ethological models*'
Elevated plus-maze
Social interaction test
Light/dark shuttle-box
Isolation-induced ultrasonic vocalisation (rodent pups)
Models dependent on conditioned cues
Fear-potentiated startle reflex (conditioned fear)
Four-plate test (conditioned fear)
Geller–Seifter test ('signals' of conflict)
Vogel conflict test ('signals' of conflict)
Frustrative non-reward ('signals' of non-reward)

tendency to explore ('approach') novel stimuli is opposed by a tendency to avoid them; it is the conflict between approach and avoidance that gives rise to anxiety. The two other forms of anxiety-inducing ('anxiogenic') stimuli are those that are normally regarded as neutral but, as a result of innate factors (genetic programming) or the subject's previous experiences (associative learning), are interpreted as a signal for a stimulus that the subject would normally avoid. The signal can either warn that behaviour which is reinforced by reward will also be punished (e.g. by a footshock to rats; 'conflict') or warn that the reward will not materialise ('non-reward').

Testing the effects of drugs on animals' behavioural response to novel environmental stimuli offers the major advantage that it relies on evaluating changes in their innate behaviour (so-called 'ethological' models). Contrasting with this, testing the effects of drugs on animals' behavioural response to an environmental 'signal' requires extensive prior training (see below). The important point about this approach is that it is the 'signal' or 'threat' of the aversive event that triggers anxiety, rather than the aversive event itself. By analogy, environmental conditions that are associated with a threat of attack will provoke anxiety in humans whereas an actual attack triggers an (appropriate) acute stress response that recruits the 'fight or flight' (stress) reaction. In the following sections, specific behavioural models used to study anxiety and the effects of anti-anxiety drugs are described.

EVALUATING DRUG EFFECTS ON INNATE BEHAVIOUR (ETHOLOGICAL MODELS)

Most of these models evaluate the effects of drugs on the behaviour of animals when they are exposed to a novel environment. Novelty normally reduces animals' exploratory activity but established anti-anxiety drugs consistently increase exploration of, and approaches to, the novel stimulus and reduce the neophobic ('avoidance') reaction. There are several examples of tests based on this principle (Table 19.2) but two that are widely used are the 'plus-maze' and the 'social interaction' tests.

The plus-maze

This consists of a raised platform with four narrow arms, two of which have walls ('closed arms') and two which do not ('open arms') (Fig. 19.1). When placed on the

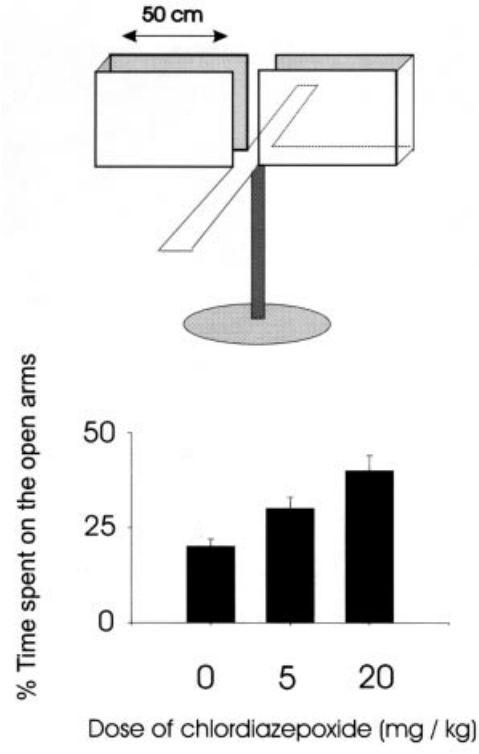


Figure 19.1 The elevated plus-maze. (*Top*) The apparatus is arranged with two open arms, two closed arms and a central zone, raised above the ground. Animals are placed in the central zone (usually facing an open arm) and their movements scored for: number of entries to the open and closed arms and the percentage time spent in the open arms. (*Bottom*) Chronic administration (5 days) of the anti-anxiety drug, chlordiazepoxide, increases the percentage time spent on the open arms to approximately 50% of the total. (Figure kindly provided by S. E. File)

apparatus for the first time, animals explore all zones of the maze but spend most time (approximately 75%) in, and make most entries to, the closed arms. Pretreatment with an anti-anxiety drug increases exploration of the open arms so that approximately equal times are spent on the open and closed arms of the maze. When interpreting results from this test, it is important to establish that any drug effects are independent of non-specific effects on the animals' overall locomotor activity (i.e. their ability to make appropriate movements), particularly since many anti-anxiety drugs are highly sedative when given acutely. Detailed insight into some of the many assumptions and refinements of the use of the plus-maze is to be found in Rodgers and Dalvi (1997).

Social interaction test

In this test, it is the interaction (sniffing, grooming, etc.) between two rats in a test arena that is scored. Social interaction is dependent on the familiarity of the animals with the test arena (social interaction is reduced in an unfamiliar arena) and the intensity of illumination (social interaction is reduced in bright light). The reduction in social

interaction under aversive conditions (unfamiliar arena and bright light) is prevented by pretreatment with anti-anxiety drugs (File and Hyde 1979). However, it is again important to establish that any drug effects are directed specifically at the behavioural response to the test environment, rather than overall locomotor activity.

MODELS THAT REQUIRE CONDITIONING

There are several models that depend on monitoring changes in animals' behaviour when they are exposed to conditioned threatening cues. One of these, the fear-potentiated startle reflex, rests on the development of an exaggerated startle on presentation of the conditioned cue. Although this response is prevented by anti-anxiety drugs, there is considerable debate over whether 'fear' is the same as 'anxiety'.

A model that fits better with Gray's criteria is the Geller–Seifter ('conflict') test. This is named after the two scientists who developed it and is still often used to screen putative anti-anxiety drugs (Geller, Kulak and Seifter 1962). Briefly, animals are trained to associate the pressing of a lever with a food reward ('operant' or 'instrumental conditioning'). After reaching a stable response on the lever, the rats are then trained to realise that when a (normally) neutral stimulus is presented, such as a buzzer or a light, they will experience a mild footshock, *as well as* receive the reward, when they press on the lever. This invokes a classic approach/avoidance conflict and animals invariably respond on the lever less frequently when the conditioned cue is presented (the 'punished phase'). An important distinction is that their response on the lever in the absence of the signal (the 'unpunished' phase) is unaffected. Anti-anxiety drugs abolish the inhibition of responding during the punished phase but do not affect unpunished responding (Fig. 19.2). Obviously, a drug that increases punished responding could be increasing animals' overall activity (as with amphetamine) but this can be excluded if it has no effect on lever responses during the unpunished phase. A drug-induced reduction in the discomfort caused by the footshock (as is achieved with analgesics) or amnesia (i.e. under the influence of the test drug the animal forgets that the cue warns of a footshock) must be ruled out also.

There are many variations of this model, a commonly used example being the Vogel licking (conflict) test. This evaluates the effects of drugs on the punished phase of drinking from a water spout (Vogel *et al.* 1980) which has the advantage that the animals do not have to be trained to initiate the behavioural response (drinking). However, the increase in baseline fluid intake induced by some anti-anxiety drugs, in the absence of any anxiogenic stimuli, can be a confounding factor.

INDUCING ANXIETY IN HUMANS

One advantage of studying humans is that it is possible to confirm that a given experimental intervention does actually induce anxiety in the subject. A disadvantage is that any research into the neurobiological changes that underlie the subjects' psychological status is limited to the analysis of accessible tissue samples, such as plasma or urine. Such measurements will, at best, be indirect indications of what is happening in the brain. As a result, research of anxiety in humans has concentrated on drugs with a known pharmacological target (usually a neurotransmitter receptor) and has compared their effects in anxious patients and normal subjects. Some treatments that induce or

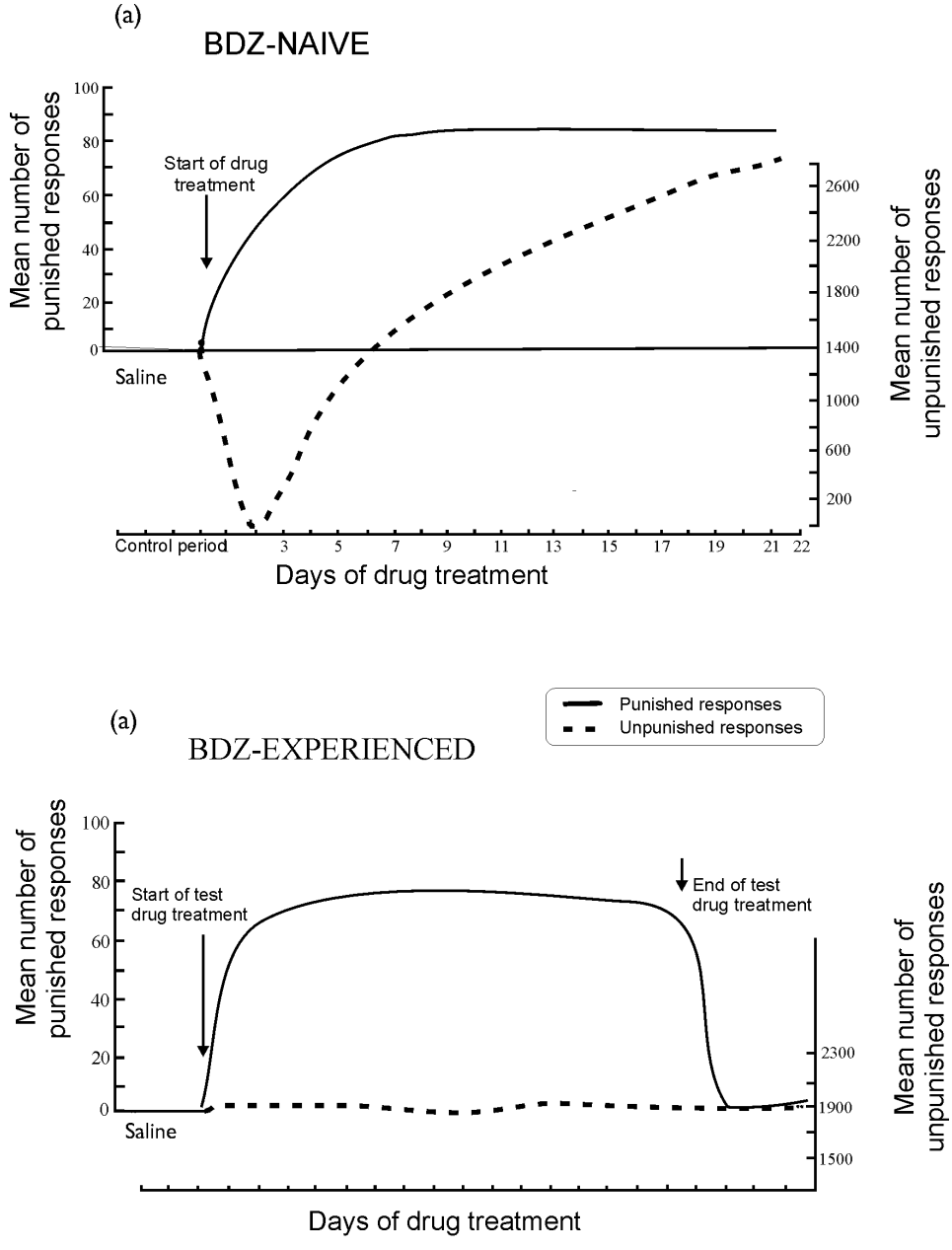


Figure 19.2 (a) Average curves of punished and unpunished responses of rats, never previously treated with a benzodiazepine (BDZ), during several days of administration of a test BDZ. The apparent delay in the increase in punished responses is due to the reduction in all responses (including unpunished ones) at the start of drug administration. The progressive recovery of unpunished responses reflects the development of tolerance to the sedative effects of the test compound. (b) Average curves of punished and unpunished responses of rats, previously treated with a BDZ, during administration of a test BDZ. Note the immediate increase in punished responses and the lack of a decline in unpunished responses, indicating pre-existing tolerance to the sedative effects of the test compound. (Based on Margules and Stein 1968)

Table 19.3 Substances that induce panic attacks in humans

Sodium lactate (mechanism unresolved)
CO_2 (inhalation) (mechanism unresolved)
Caffeine (adenosine, A_2 receptor antagonism?)
Yohimbine (α_2-adrenoceptor antagonism?)
m-Chlorophenylpiperazine (mCPP, 5-$HT_{2A/2C}$ receptor agonism?)
CCK-4 (CCK_B receptor agonism?)
FG7142 (benzodiazepine receptor inverse agonism?)

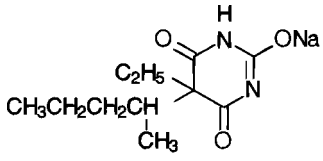
exacerbate anxiety in humans are listed in Table 19.3 and their presumed neurobiological targets have formed the bases of theories to explain the cause(s) of this disorder. A full appraisal of this topic is beyond the scope of this chapter but the links between drugs that affect central monoamine transmission and anxiety are discussed in later sections. Details of findings from research in humans can be found in Ballenger (1990) and Coupland, Glue and Nutt (1992).

DRUG TREATMENTS FOR ANXIETY

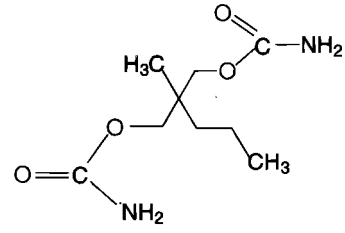
The oldest anti-anxiety agent is undoubtedly alcohol and it is certain that this drug is still routinely self-administered for this purpose. Towards the end of the eighteenth century, bromide salts were used to relieve conditions akin to anxiety despite the risk of a characteristic toxic delirium, known as 'bromism'. Alternative treatments, such as paraldehyde and chloral hydrate, were also widely used but these too had adverse effects; the former can cause psychosis but the latter is still used as a sedative and anaesthetic agent.

By the turn of the century, barbiturates (e.g. pentobarbitone, Fig. 19.3) were gradually replacing these treatments. Early reports (one as early as 1903) described a 'toxic' behavioural reaction to barbiturates that was attributed to a form of poisoning. It was not until the 1930s that it was recognised that this adverse behavioural effect of barbiturates in fact represented a drug-withdrawal syndrome (Seevers and Tatum 1931). This, together with the overt sedation caused by barbiturates, their narrow therapeutic index and their lethal toxicity in overdose, motivated the search for non-sedative anti-anxiety agents. One compound to carry such claims was meprobamate ('Miltown'; Fig. 19.3), synthesised in the 1950s. However, the initial enthusiasm over the use of this compound as a treatment for anxiety rapidly abated because it too proved to be a potent sedative and, of even more concern, it induced dependence and was widely abused.

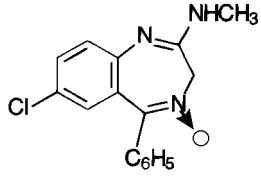
This background set the scene for the arrival of the benzodiazepines. The first of these was chlordiazepoxide ('Librium') launched in 1960, followed by diazepam ('Valium'; Fig. 19.3). Like their predecessors, but with greater justification, these drugs were claimed to relieve anxiety at non-sedative doses (see Sternbach, Randall and Gustafson 1964). However, the benzodiazepines are members of the sedative/hypnotic group of anti-anxiety drugs, which also include alcohol, meprobamate and the barbiturates. This means that the liability of all these compounds to induce sedation, or even hypnosis (sleep), is largely a question of dose (Fig. 19.4) although it is offset by the rapid development of tolerance to their sedative effects. Like their predecessors, the



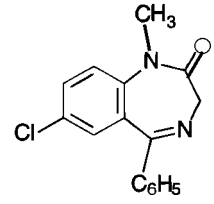
Pentobarbitone



Meprobamate



Chlordiazepoxide



Diazepam

Figure 19.3 The chemical structure of some leading sedative/hypnotic anti-anxiety agents

actions of benzodiazepines are not restricted to relief of anxiety: they also induce ataxia, muscle relaxation (used in relief of muscle spasm), anterograde amnesia (used to relieve dental phobia) and increase seizure threshold (used to treat some forms of epilepsy).

BENZODIAZEPINES AND BENZODIAZEPINE RECEPTORS

MOLECULAR TARGETS FOR THE $GABA_A$ RECEPTOR

The first clues to the mechanism of action of benzodiazepines came from landmark experiments (Squires and Braestrup 1977; Moehler and Okada 1977) which showed that

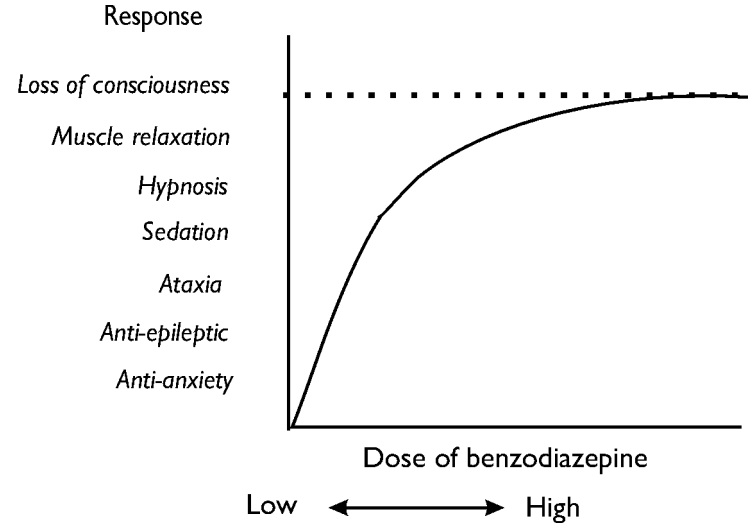


Figure 19.4 The activity spectrum of the benzodiazepines. Motor impairment and CNS depression increases with drug dose. (Based on data for chlordiazepoxide (Sternbach, Randall and Gustafson 1964))

[^{3}H]diazepam binds to a specific site in the brain. Studies of solubilised receptors confirmed that this binding site was a component of the $GABA_A$ receptor which incorporates a Cl^- channel. GABA did not compete with [^{3}H]benzodiazepine for binding to this receptor and so it was clear that their binding domains were not the same. It was soon realised that there is an allosteric interaction between them such that binding of [^{3}H]benzodiazepines is increased by GABA (Fig. 19.5). This is thought to be due to an interaction between the GABA recognition site on a β-subunit of the $GABA_A$ receptor and the benzodiazepine recognition site on an α-subunit (see Chapter 11 and Doble and Martin 1996). The overall effect of benzodiazepines is to augment the increase in Cl^- conductance caused by GABA and thereby potentiate its inhibitory actions; they achieve this by increasing the probability (and as a consequence, the frequency) of Cl^- channel opening. This action is thought to play a crucial role in the anti-anxiety effects of these drugs. The progressive increase in CNS depression, as drug dose is increased, is attributed to an increase in receptor occupancy, although the extent to which binding in different brain regions contributes to these different actions is not known.

Barbiturates bind non-competitively to yet another, functionally distinct, domain on the receptor which is thought to be directly associated with the Cl^- channel itself. Although there is an allosteric interaction with GABA, as with the benzodiazepines, barbiturates also directly increase Cl^- conductance by increasing the duration of channel opening. Thus, by contrast with the benzodiazepines, the barbiturates activate this receptor even in the absence of GABA. This explains why antagonists of the GABA binding site, such as bicuculline, block the actions of benzodiazepines, but not those of the barbiturates, and probably accounts for the greater toxicity of the barbiturates in overdose. In contrast, so-called 'cage convulsants' (e.g. picrotoxinin, pentylenetetrazol and *t*-butylbicycloorthobenzoate ('TBOB')) are thought to bind directly to another site on the Cl^- channel and to *reduce* Cl^- conductance. In recent years, the range of

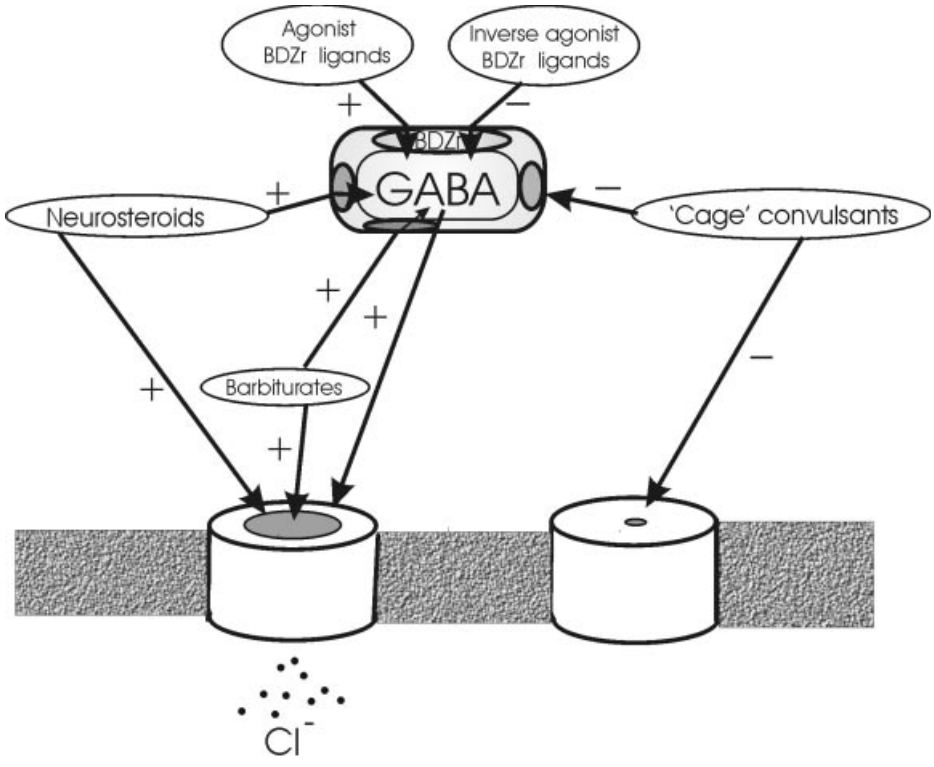


Figure 19.5 A schematic diagram of the $GABA_A$ receptor. Binding of GABA to its domain on the receptor opens a Cl^- channel. This action of GABA is augmented (+) by agonist benzodiazepines that bind to their own domain (BDZr) on the $GABA_A$ receptor and trigger an allosteric interaction with the GABA binding site. Other compounds that act in this way include the neurosteroids (e.g. allopregnanolone) and barbiturates but these compounds also bind to the Cl^- channel directly and, at high concentrations, increase Cl^- conductance in the absence of GABA. Some compounds, such as BDZr inverse agonists, have the opposite effect: i.e. they bind to the BDZr site but reduce Cl^- channel opening through a negative allosteric interaction (−) with the GABA binding site. The so-called 'cage convulsants' (e.g. picrotoxinin, pentylenetetrazol and *t*-butylbicycloorthobenzoate ('TBOB'), which also reduce Cl^- conductance, are thought to bind directly to a site on the Cl^- channel

compounds shown to have binding domains on the $GABA_A$ receptor has steadily increased and it is thought that there could be even more.

BENZODIAZEPINE RECEPTOR SUBTYPES

The discovery of the benzodiazepine receptor was quickly followed by their subdivision as evidenced by (see also Chapter 11):

(1) The biphasic dissociation of [^{3}H]flunitrazepam binding (Chiu, Dryden and Rosenberg 1982).
(2) Competition binding studies showing that when using compounds like β-CCE (ethyl-β-carboline-3-carboxylate), which bind to the benzodiazepine receptor, the displacement curve for [^{3}H]flunitrazepam was shallow in the hippocampus and

cortex. In contrast, in the cerebellum, the curve was steep with a Hill coefficient of 1 (Duggan and Stephenson 1988).

(3) Photoaffinity labelling confirming that there was more than one type of benzodiazepine receptor, rather than multiple sites on the same receptor. In this technique [^{3}H]flunitrazepam binding is carried out under ultra-violet light which renders most of the ligand binding irreversible. Purification of the radioligand/receptor complex revealed a 51 kDa protein in the cerebellum (now known to be the α1 subunit of the 'BZ_1 receptor') but quite different protein(s) in the hippocampus (now known to be the α2 and α3 subunits of the 'BZ_2 receptor') (Sieghart and Karobath 1980).

The discovery of these receptor subtypes kindled the hope that it would be possible to develop subtype-selective drugs with specific clinical actions: i.e. that it would at last be feasible to produce an anti-anxiety agent that was genuinely non-sedative. It is now known that the $GABA_A$ receptor comprises different combinations of subunits, probably in a pentameric complex (see Chapter 11), and so it might even be possible to develop drugs that target different subunits, thereby increasing their functional specificity. One such compound has already been developed, the imidazopyridine zolpidem (Fig. 19.6), which was initially regarded as a BZ_1 receptor ligand but is now classified as a high-affinity ligand for the α1 subunit of $GABA_A$ receptors (in contrast to α2 and α3 subunits which produce the so-called BZ_2 receptor). Notwithstanding this selectivity, zolpidem turns out to be a potent hypnotic agent. Zopiclone is another hypnotic, available in the clinic; this drug displaces benzodiazepines from the $GABA_A$ receptor but lacks subunit selectivity and does not seem to target precisely the same binding domain as the benzodiazepines.

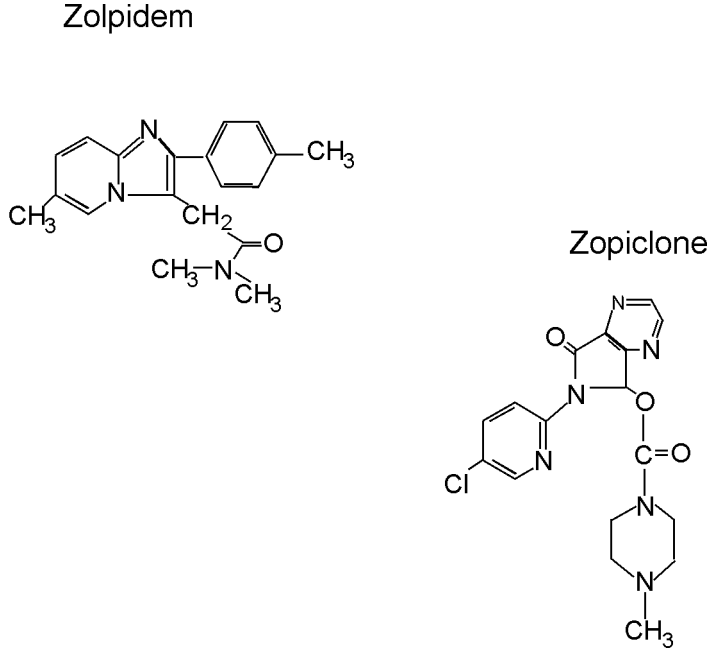


Figure 19.6 The chemical structure of the imidazopyridine and benzodiazepine (BZ_1) receptor ligand, zolpidem, and the cyclopyrrolone, zopiclone

Whether simple augmentation of $GABA_A$ receptor function accounts for the anti-anxiety effects of these compounds remains equivocal. If this was the case then other agents that augment GABAergic transmission such as inhibitors of GABA uptake (e.g. vigabatrin) or metabolism (e.g. tiagabine) should also have anti-anxiety effects. Indeed, there are reports of their anti-anxiety effects in patients receiving these treatments for relief of epilepsy. There is also some supporting evidence from preclinical studies but the behavioural effects of these drugs in animal models are less robust than are those of the benzodiazepines. It remains to be seen whether this is because they are just less effective anti-anxiety agents than the benzodiazepines or whether existing preclinical models show a bias that detects preferentially the anti-anxiety effects of benzodiazepines.

'PERIPHERAL' BENZODIAZEPINE RECEPTORS

In the 1980s, a further binding site for benzodiazepines was identified and, because it was first discovered in the rat adrenal gland, the term 'peripheral benzodiazepine receptor' was coined. This is regrettably confusing because this receptor has now been found in the brain also (Awad and Gavish 1987). These benzodiazepine receptors differ from those described above in a number of important respects, not least because they do not affect, nor are they affected by, GABA binding. They also have their own specific ligands: the isoquinolone, PK 11195, and the benzodiazepine, Ro 4864, neither of which binds to the $GABA_A$ receptor. Moreover, the benzodiazepine, clonazepam, which is a high-affinity, partial agonist ligand for the benzodiazepine domain on the $GABA_A$ receptor, does not bind to the 'peripheral' receptor.

Another difference is that these peripheral benzodiazepine receptors are located mainly intraneuronally, on mitochondrial outer membranes, rather than on the plasma membrane. In the brain, they are associated with glial cells but in the periphery they are found in a range of tissues, including mast cells and platelets. Their function is still a matter of intense debate but one possibility is that they regulate cholesterol uptake and, secondary to this, the synthesis of neurosteroids (Do Rego *et al.* 1998). Since neurosteroids also have a binding domain on the $GABA_A$ receptor, there might be some indirect functional coupling between these two types of receptors after all. To some extent, the possibility of such an interaction is supported by evidence that the density of peripheral benzodiazepine receptors differs in inbred strains of rats which are distinguished by their behavioural reactivity ('fearfulness') to novel stimuli (Drugan *et al.* 1987). Other possible, albeit controversial, functions of these receptors are reviewed in Doble and Martin (1996).

OTHER LIGANDS FOR THE BENZODIAZEPINE RECEPTOR

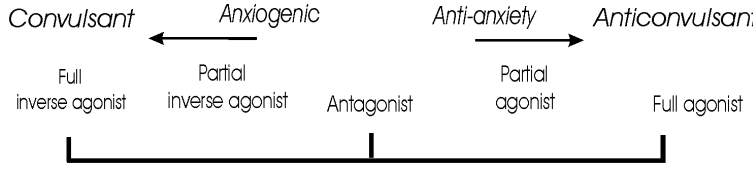
Among the early indications that the benzodiazepines were not the only compounds to bind to the benzodiazepine receptor were findings that emerged from a search for an endogenous ligand for this receptor site. This effort produced a non-benzodiazepine ligand, ethyl-β-carboline-3-carboxylate (β-CCE), and this pointed the way to a whole family of compounds that are high-affinity ligands for this receptor. However, not all turned out to share the properties of the protypical benzodiazepines (anti-anxiety, anticonvulsant, etc.). Some, including β-CCE itself, had the opposite effects in animals: i.e. they *induced* anxiety and *reduced* seizure threshold and some, such as 3-carbomethoxy-4-ethyl-6,7-dimethoxy-β-carboline (DMCM), caused overt seizures.

These new recruits to the activity spectrum were named 'inverse agonists' and subsequent studies confirmed that they reduce the affinity of GABA for its binding site on the $GABA_A$ receptor and attenuate the $GABA_A$ receptor-mediated increase in Cl^- conductance (Fig. 19.5).

THE BENZODIAZEPINE RECEPTOR AGONIST/INVERSE AGONIST SPECTRUM

The rich portfolio of compounds that bind to the benzodiazepine receptor includes many compounds which, despite not being benzodiazepines, share the properties of the prototypical benzodiazepines, chlordiazepoxide and diazepam. However, all these groups of compounds, including the benzodiazepines themselves, span the activity spectrum: from full inverse agonist to full agonist. In between these extremes are compounds which have either partial agonist or partial inverse agonist activity and some are antagonists (Fig. 19.7). This spectrum of actions reflects the overall effects of these drugs on native receptors and is usually assessed in whole animals. However, the synthesis of receptors comprising different combinations of subunits has shown that the activity of these drugs depends greatly on subunit composition. For instance, $GABA_A$ receptors have been characterised to which diazepam does not bind at all (see Chapter 11).

The first antagonist to be developed was the (imidazo)benzodiazepine, flumazenil. This compound blocks the actions of both agonists and inverse agonists *in vitro*. It will



Benzodiazepines	Ro19-4603	Sarmazenil	Flumazenil	Bretazenil	Diazepam
β-*carbolines*	DMCM	FG7142	ZK93426	Abecarnil	ZK94323
Cyclopyrrolones				Rp60503	Zopiclone
Imidazopyrimidines	Ru34000			Ru32698	
Imidazopyridines		S-8510		Alpidem	Zolpidem
Phenylquinolines				Pk8165	
Pyrazoloquinolinones			CGS8216	CGS9895	
Triazolopyridazines				CI218872	

Figure 19.7 The activity spectrum for different generic groups of compounds that bind to the 'benzodiazepine' domain on the $GABA_A$ receptor

also block the effects of these agents *in vivo*, but there is some argument over whether it is a true antagonist: i.e. whether it really lacks any intrinsic activity (as would be required of an antagonist) or whether it is merely a weak partial agonist. The subunit composition of the $GABA_A$ receptor could be one confounding factor in resolving this question. For instance, flumazenil has been reported to *augment* the action of GABA at cloned receptors comprising α_4 β_2 γ_2 subunits. Apparent effects of the antagonist *in vivo* could also depend on whether there is any tonic activation of the benzodiazepine receptor by an endogenous ligand. Flumazenil is available in the clinic for intravenous infusion to reverse benzodiazepine-induced sedation (e.g. in the post-anaesthetic context) or coma (after overdose). However, because it has a half-life of only 1 h in humans, it is only of realistic benefit in reversing the actions of agonist benzodiazepines with a short half-life, such as midazolam.

The potential benefits of benzodiazepine partial agonists are as non-sedative, anti-anxiety agents. Because of their low efficacy, it was predicted that a partial agonist should not induce sedation even if their receptor occupancy exceeds that normally required for an anti-anxiety effect when using a full agonist. One such compound, bretazenil, has been developed but failed to reach the clinic because it displayed some sedative activity and, more problematic, there were end-of-dose rebound effects that were undoubtedly exacerbated by its short half-life. Currently, the partial agonist, abecarnil (a β-carboline), is undergoing clinical trials. For the current status of the development of partial agonists and other promising benzodiazepine receptor ligands see Cheetham and Heal (2000).

Even benzodiazepine inverse agonists might yet find some useful applications such as in the relief of cognitive deficits (which are increased by benzodiazepine full agonists) (Abe, Takeyama and Yoshimura 1998). With the rapidly expanding understanding of different combinations of subunits that comprise the $GABA_A$ receptor, it is hoped to develop compounds that target specific subunit combinations and improve cognitive function in dementia but which lack any proconvulsant or anxiogenic actions.

THE QUEST FOR THE ENDOGENOUS BENZODIAZEPINE RECEPTOR LIGAND ('ENDOZEPINES')

The discovery of the opioid receptor, followed by isolation of endogenous opioids, provided the impetus for a search for an endogenous ligand for the established benzodiazepine receptor. Although many candidates have emerged (De Robertis *et al.* 1988; Table 19.4), most are present in the CNS at concentrations far too low for them to be feasible endogenous modulators of $GABA_A$ receptor function. However, three candidates have been given prominent attention, albeit for different reasons, and are worthy of mention.

Table 19.4 Putative endogenous ligands for the benzodiazepine binding domain on the $GABA_A$ receptor

β-carbolines (β-CCB)
Desmethyldiazepam
'Endozepines' (unknown chemical structure)
Peptides (nepenthin, octodecaneuropeptide)
Purines (inosine, hypoxanthine, guanosine, nicotinamide)
Thromboxane A_2

The first, β-CCE, was the product of an arduous attempt to isolate an endogenous ligand from human urine. Although subsequently found to be an artefact of the extraction process, this compound turned out to be a ligand for the benzodiazepine receptor, nonetheless, and was the first inverse agonist to be identified. The anxiogenic effects in humans of its more stable congener, FG 7142, are described graphically in a report by Dorow *et al.* (1983). β-Carbolines are realistic candidates for an endogenous ligand because they can be synthesised in the brain (Han and Dryhurst 1996) but, although other members of this group of compounds have at various times been suggested to fulfil the role of an endogenous ligand, none has been confirmed as such.

Another, more recent, candidate is an endogenous propeptide, 'diazepam binding inhibitor' (also known as Acyl-CoA Binding Protein (DBI/ACBP)), which yields 'octodecaneuropeptide' ('ODN') and 'triakontatetraneuropeptide' ('TTN') (Costa and Guidotti 1991). Both these peptides are neuroactive and ODN turns out to have inverse agonist activity at $GABA_A$ receptors both *in vivo* and *in vitro* and to have marked effects on behaviour (e.g. Reddy and Kulkarni 1998). However, there is scepticism as to whether the brain can manufacture sufficient peptide to regulate the ubiquitous $GABA_A$ receptor on a moment-to-moment basis. Currently, the binding of TTN to the peripheral benzodiazepine site, and its effect on neurosteroid synthesis, is attracting greater interest (Do Rego *et al.* 1998).

Finally, the presence in human post-mortem brain tissue of the active metabolite of diazepam, desmethyldiazepam, raised some curiosity and frank alarm (Sangameswaran *et al.* 1986). At the time of its discovery in the brain it was thought that there was no enzyme system capable of producing such halogenated compounds and that its presence in the brain reflected dietary intake from an environment contaminated by overuse of its parent compound. However, its discovery in stored brain tissue which had been obtained before the synthesis of the benzodiazepines allayed these fears. It is now thought possible that some benzodiazepines, including desmethyldiazepam, occur naturally and that they are taken in as part of a normal diet (Table 19.5).

Although, by analogy with the opioids, one would expect there to be an endogenous ligand for the widely distributed benzodiazepine receptor, its existence remains uncertain and we must be alert to the possibility that any such ligand(s) could have either agonist or inverse agonist activity.

Table 19.5 Examples of plants containing ligands for benzodiazepine receptors

Plant source	Active agent(s)
Valeriana officinalis	Hydroxypinoresinol (a lignan)
Hypericum perforatum L. Hypericaceae (St John's Wort)	Unknown
Matricaria recutita L.	5,7,4′-trihydroxyflavone (apigenin)
Passiflor coeruleus L.	Chrysin
Wheat grain	Diazepam, desmethyldiazepam, lormetazepam
Potato	Diazepam, desmethyldiazepam, lormetazepam
Karmelitter Geist	Amentoflavon

ENDOGENOUS LIGANDS AND BENZODIAZEPINE RECEPTORS: AN EXPLANATION FOR THE CAUSE OF ANXIETY

The undisputed efficacy of benzodiazepines in relief of anxiety led to the question of whether this disorder could arise from abnormal concentrations in the brain of an endogenous ligand or a malfunction of the benzodiazepine/GABA receptor system. An important study, aimed at distinguishing between these possibilities, has been carried out in humans (Nutt *et al.* 1990) and was based on the premise that anxiety could be caused by either:

(1) Inadequate activity of an endogenous ligand which is a benzodiazepine receptor agonist and suppresses anxiety. In this case, the administration of the antagonist, flumazenil, should induce anxiety in normal subjects and exacerbate anxiety in anxious patients.
(2) Excessive activity of an endogenous ligand which is a benzodiazepine receptor inverse agonist and induces anxiety. In this case, the administration of flumazenil should relieve anxiety in anxious patients and have no, or sedative, effects in healthy subjects.
(3) Dysfunction of the $GABA_A$ receptor complex such that the effects of all benzodiazepine receptor ligands are shifted in the direction of inverse agonism. In this case, flumazenil (which normally has zero efficacy) should induce anxiety in anxious patients but have no effects in healthy subjects because they have normal receptors.

To distinguish between these possibilities, flumazenil was administered to panic patients and control subjects. The results of the experiment were consistent with the third possibility: flumazenil induced panic attacks in 8 of 10 patients but not in control subjects (Fig. 19.8). Unfortunately, the change(s) in the benzodiazepine receptor or its coupling to the rest of the $GABA_A$ receptor are unknown, as are the stimuli that could explain this functional change. Recent studies suggest that the binding of [^{11}C]flumazenil is abnormally low in panic patients (Malizia *et al.* 1998), although this finding does not relate in any obvious way to the '$GABA_A$ receptor shift' hypothesis. However, this is the only tested theory so far to connect panic anxiety directly with a disorder of the $GABA_A$ receptor. The receptor shift theory could also explain why benzodiazepines are ineffective in treating panic disorder but, because these drugs do effectively relieve generalised anxiety, it seems that the theory might explain the origin of the former, but not the latter disorder, and that they have different causes.

MONOAMINES IN ANXIETY

NORADRENALINE

The first suggestion that abnormal noradrenergic transmission was linked with anxiety came from Redmond's laboratory in the 1970s when he drew attention to the similarities in the symptoms and signs of anxiety with those of the acute stress response (Redmond and Huang 1979). He went on to stimulate the locus coeruleus of (chair-restrained) monkeys and showed that this caused behavioural changes, some of which resembled a cluster of behaviours displayed by the animals when under threat. This work led to the proposal that anxiety was due to (or exacerbated by) excessive

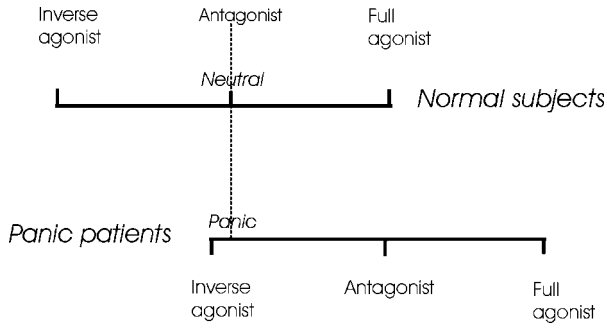


Figure 19.8 A schematic representation of the $GABA_A$ receptor shift hypothesis. This proposes that patients with panic disorder have dysfunctional $GABA_A$ receptors such that the actions of drugs that behave as antagonists in normal subjects are expressed as inverse agonism in panic patients. It is unlikely that this theory extends to generalised anxiety disorder (GAD), for which benzodiazepine agonists are highly effective treatments, but it could explain why these drugs are relatively ineffective at treating panic disorder. (Based on Nutt *et al.* 1990)

noradrenergic transmission in the brain. This hypothesis has survived, largely unchallenged, for over 20 years.

Redmond's explanation of anxiety has been underpinned by investigations of changes in noradrenaline release in humans given the anxiogenic drug, yohimbine. This is an α_2-adrenoceptor antagonist that increases the firing rate of, and release of noradrenaline from, noradrenergic neurons by blockade of presynaptic α_2-adrenoceptors on the neuronal cell bodies and terminals, respectively. Increases in noradrenaline release, inferred from measurement of the noradrenaline metabolite, 3-methoxy, 4-hydroxyphenylglycol (MHPG), in plasma, have shown that the noradrenergic response in panic patients who experience a panic attack with yohimbine is greater than that in either panic patients who do not express this response or in normal patients (Bremner *et al.* 1996). Unfortunately, the noradrenergic response to yohimbine is not exaggerated in patients with GAD, suggesting that the aetiology of this form of anxiety could differ from that of panic disorder. Nevertheless, the α_2-adrenoceptor agonist, clonidine, which has the opposite effect to yohimbine on noradrenergic neurons, is sometimes used to relieve anxiety, especially that associated with alcohol and opiate withdrawal. However, it is not a viable long-term treatment for anxiety because of its effects on the cardiovascular system.

One complication with the above concept is that, in some brain regions, the majority of α_2-adrenoceptors are postsynaptic and so a *reduction* in α_2-adrenoceptor-mediated noradrenergic transmission, after treatment with yohimbine, cannot be ruled out as a causal factor for the anxiety induced by this drug. Another problem is that yohimbine is also a 5-HT_{1A} receptor agonist, a 5-HT_{1D} partial agonist and a 5-HT_{2B} antagonist; all these receptors could be involved in anxiety (see below). Furthermore, a finding that argues against excessive noradrenergic transmission as a cause of anxiety is that stimulating the locus coeruleus in humans induces a pleasant sensation, rather than anxiety (Libet and Gleason 1994) and that not all anxiogenic challenges increase plasma MHPG (Silverstone *et al.* 1994).

Measurements of noradrenaline release in animals have not helped to resolve this confusion. Microdialysis studies *in vivo* have confirmed that anxiogenic doses of

yohimbine do increase the extracellular concentration of noradrenaline in the frontal cortex of rats but an anxiogenic dose of the benzodiazepine inverse agonist, FG 7142, does not. Moreover, anxiogenic doses of these two drugs have opposite effects on the rats' response to a novel environment: the increase in noradrenaline efflux caused by this aversive stimulus is *reduced* by yohimbine but *increased* by FG 7142 (Mason, Heal and Stanford 1998). A further complication is that, despite its anxiogenic effects, yohimbine actually *augments* the anti-anxiety effects of benzodiazepines in the 'conflict' test (Söderpalm, Blomqvist and Söderpalm 1995) while a more selective α_2-adrenoceptor antagonist, idazoxan, has anti-anxiety, rather than anxiogenic, effects in animal models of conflict (La Marca and Dunn 1994).

These observations question the role of noradrenaline as an initiator of anxiety as does the finding that the anti-anxiety drug, buspirone (see Chapter 9), increases the concentration of noradrenaline in the extracellular fluid in the frontal cortex of freely-moving rats (Done and Sharp 1994). Whether this is because buspirone is metabolised to 1-(2-pyrimidinyl)-piperazine (1-PP), which is an α_2-adrenoceptor antagonist, is uncertain. Unfortunately, no studies have investigated the effects of chronic administration of this drug on noradrenergic transmission; this could be important because, unlike benzodiazepines, buspirone is effective therapeutically only after several weeks of treatment.

The finding that infusion of the β-adrenoceptor agonist, isoprenaline, has an anxiogenic effect in humans implicates this receptor subytype also but little (if any) isoprenaline crosses the blood–brain barrier and so any anxiogenic effects are likely to be an indirect consequence of the autonomic arousal it will cause (i.e. increased heart rate, reduced salivation, etc.). Of course, this alone does not rule out a role for these receptors in the psychological component of anxiety. It has long been claimed that peripheral responses can serve as 'interoceptive cues' and cause secondary (anxiogenic) changes in the brain. This is the 'James–Lange hypothesis' which broadly suggests that we experience anxiety because of an increase in heart rate and a dry mouth, rather than the other way round. Blocking such β-adrenoceptor-mediated effects in anxiety would also explain the 'anti-anxiety' effects of their antagonists (e.g. propranolol) because β-adrenoceptors are postsynaptic in the CNS and so their antagonists would blunt noradrenergic transmission. However, one complication is that propranolol, like many other β-adrenoceptor antagonists, is also a 5-HT_{1A} receptor antagonist which could contribute to its anti-anxiety effects (see below). Another is that β-adrenoceptor antagonists are only of clear benefit in prevention of 'situational' anxiety ('competition nerves') which should really be regarded as a normal stress response rather than an anxiety disorder. Indeed, subjects claim that, whereas these drugs relieve the peripheral manifestations of anxiety, they have no appreciable effects on its psychological component. This is supported by evidence that these drugs are of little, if any, long-term benefit in GAD (Nutt 1990) and that they can even exacerbate this condition.

If excessive noradrenergic transmission is a causal factor in anxiety, then it would be predicted that a lesion of central noradrenergic neurons would have an anti-anxiety effect in behavioural models of this condition. Unfortunately, the behavioural effects of such lesions are notoriously inconsistent and there are many reports of negative findings (e.g. Salmon, Tsaltas and Gray 1989). One study has even shown that a lesion of central noradrenergic neurons, induced by the selective neurotoxin, DSP-4, abolishes the anti-anxiety effects of tricyclic antidepressants and MAO inhibitors, but not those of the benzodiazepine, alprazolam, or the barbiturate, phenobarbitone (Fontana,

McMiller and Commissaris 1999). This suggests that the central noradrenergic system is actually needed to express the anti-anxiety effects of some drugs, but not others.

Finally, many early studies suggested that benzodiazepines attenuate the increase in turnover of noradrenaline in the brain caused by stressful stimuli such as footshock and restraint (Taylor and Laverty 1969; reviewed by Stanford 1995). It has also been reported that they prevent the phasic increase in firing rate of neurons in the locus coeruleus caused by such stimuli (Rasmussen and Jacobs 1986). These actions would certainly support Redmond's theory. However, recent microdialysis studies suggest that the actions of these drugs might not be so straightforward. Anti-anxiety doses of the benzodiazepine, diazepam, reduced spontaneous efflux of noradrenaline but had no effect on the noradrenergic stress response on exposure to a novel environment (Dalley, Mason and Stanford 1996). This points to an important limitation of many studies in this area, namely that stimuli used to investigate the neurochemical effects of test anti-anxiety drugs are usually crude and involve somatosensory stress, often involving physical discomfort. This approach disregards the criteria, defined by Gray (1987; discussed above) for the types of environmental stimuli that trigger anxiety in rodent models or humans. In fact, surprisingly few studies have investigated the effects of *anxiogenic* stimuli on neurochemical changes in the brain. This is probably because techniques of sufficient sensitivity to detect the neurochemical changes provoked by these procedures have been developed only recently.

In one such study, using *in vivo* microdialysis, exposure to an aversive novel environment (a brightly lit, novel arena) increased the concentration of extracellular noradrenaline (suggestive of increased noradrenaline release) in both the rat frontal cortex and the hypothalamus. However, if animals were trained to associate the sound of a tone (which becomes a conditioned cue) with imminent transfer to the aversive environment, a different pattern of noradrenaline responses ensued. After a series of such conditioning trials, the sound of the tone alone increased the concentration of extracellular noradrenaline in the rat frontal cortex, but not the hypothalamus (McQuade and Stanford 2000). This suggests that the noradrenergic innervation of these two brain areas might have different roles in the response to conditioned and unconditioned aversive environmental stimuli. They also suggest that noradrenergic neurons innervating the frontal cortex are recruited in the response to anxiogenic environmental signals (of the type described by Gray) whereas those projecting to both brain regions could have a role in coordinating or triggering the flight/fight response to unconditioned stimuli. Clearly, different components of the central noradrenergic system could have different roles in anxiety, a possibility that is considered in more detail later.

5-HYDROXYTRYPTAMINE

It has been known for many years that aversive stimuli increase serotonergic transmission (reviewed by Chaouloff 1993) and so it was inevitable that exaggerated serotonergic transmission in a hypothetical brain 'punishment system' became linked with anxiety. Unfortunately, much of the evidence for this idea was gleaned from unreliable measures of changes in 5-HT concentration in rodent brain tissue post-mortem after experience of moderately severe forms of stress *in vivo*. Nevertheless, this concept was encouraged by reports that a reduction in 5-HT transmission, following administration of either the 5-HT synthesis inhibitor, *p*-chloroamphetamine (*p*CPA),

or the selective neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), usually increased responding in the punished (anxiety) phase of a 'conflict' test.

Equally inevitably, many confounding factors have come to light which undermine this simplistic explanation of anxiety. For instance, 5-HT release in the brain suppresses food and fluid intake and so its depletion will distort results from any procedure that relies on changes in ingestive behaviour (such as conflict tests). In contrast, benzodiazepines increase food intake. Ethological models avoid this problem but offer others instead: e.g. both *p*CPA and 5,7-DHT neurotoxic lesions of 5-HT neurons in the dorsal Raphé nucleus increase social interaction and yet lesions of neurons in the median Raphé nucleus decrease it. Moreover, *p*CPA exaggerates the flight/fight response induced by stimulation of the periaquaductal grey (PAG), an action that is hard to reconcile with the anti-anxiety effect of this drug in the social interaction test. Studies using *in vivo* microdialysis have complicated the picture even more: e.g. the concentration of extracellular 5-HT is increased in the forebrain of rats tested in the plus-maze and yet isolation rearing, which increases animals' behavioural reactivity to the plus-maze, abolishes the increase in extracellular 5-HT (Marsden *et al.* 1993). Another complication is that different serotonergic nuclei project to different brain areas (reviewed by Jacobs and Azmitia 1992) and it is now thought that different components of the brain 5-HT system have different roles in behaviour (see below). Finally, the wide variety of responses to 5-HT mediated by its many receptor subtypes is problematic (see Barnes and Sharp 1999), especially when coupled with the dubious selectivity of most test drugs.

In recent years, attention has been directed to the azapirones such as gepirone, ipsapirone and, in particular, buspirone since this is the only one which is available clinically as an anti-anxiety agent. It was developed as a neuroleptic, because it is a dopaminergic D_2 receptor antagonist, but turned out to be more effective as a treatment for anxiety. In preclinical models, buspirone generally produces behavioural changes that are consistent with an 'anti-anxiety' effect but this has been much harder to demonstrate than for the benzodiazepines. These anti-anxiety effects of buspirone were thought to rest on its activation of inhibitory 5-HT_{1A} autoreceptors on serotonergic cell bodies in the Raphé nuclei (Dourish, Hutson and Curzon 1986). The ensuing reduction in the firing rate of serotonergic neurons and 5-HT release in the terminal field fitted well with the idea that a hyperresponsive serotonergic system was a causal factor in anxiety. Evidence that both *p*CPA and 5,7-DHT lesions abolish the anti-anxiety effects of this drug in conflict tests (Eison *et al.* 1986) was deemed to support this scheme, although it is hard to understand why such a lesion should abolish the effects of a drug that is supposed to work through inhibition of 5-HT release.

One problem with the idea that the anti-anxiety effects of buspirone are mediated by its activation of 5-HT_{1A} autoreceptors is that it disregards the postsynaptic 5-HT_{1A} receptors which are found in many key areas of the limbic system (e.g. frontal cortex, hippocampus and hypothalamus). Buspirone would be expected to activate these receptors, regardless of any inhibition of neuronal firing rate and 5-HT release. A solution to this problem was offered by the suggestion that buspirone is a full agonist at presynaptic receptors but only a partial agonist at postsynaptic sites. An alternative suggestion was that there is a greater receptor reserve on cell bodies than postsynaptically. In either case, a reduction of neuronal firing mediated by buspirone's activation of inhibitory autoreceptors would be accompanied by minimal postsynaptic effects.

More recently, evidence favouring an anti-anxiety effect mediated by activation of postsynaptic 5-HT_{1A} receptors has emerged. However, it must be remembered that, since buspirone is a partial agonist, its postsynaptic effects will depend on the degree of tonic activation of the target receptor(s). Indeed, this could account for the many conflicting reports in the literature. It would certainly explain why a neurotoxic lesion of serotonergic neurons has no effect on the anti-anxiety effects of ipsapirone in the conflict test (Przegalinski, Chojnacka-Wojcik and Filip 1992) whereas buspirone prevents the anxiogenic effects of 5-hydroxytryptophan, which increases 5-HT transmission, in rats placed in a novel environment. Antagonists of 5-HT_{1A} receptors (e.g. WAY100635) have now been developed as well and, over a limited range of doses, they too have anti-anxiety effects in preclinical models (Cao and Rodgers 1997). Unfortunately, it is still unclear whether this is due to a presynaptic action (i.e. an increase in 5-HT release and transmission) or postsynaptic action (i.e. a reduction in 5-HT_{1A}-mediated transmission) (see Beckett and Marsden 1997).

Overall, the extent to which postsynaptic receptors contribute to the actions of 5-HT_{1A} receptor agonists seems to depend on the behavioural test used, whether the drugs are administered systemically or locally and, if the latter, into which brain region (see Handley 1995). Moreover, an important limitation of much of this work is that buspirone is effective in humans only after prolonged administration and yet most experimental studies have investigated its behavioural sequelae only after acute drug administration. The outcome of the few chronic studies that have been attempted seems to differ across different models, with buspirone being ineffective in the plus-maze but effective in conflict tests (see Handley 1995). An understanding of the effects of chronic treatment with buspirone is a notable gap in the field because it is possible that its anti-anxiety effects rest on long-latency changes in receptor populations that culminate in a shift in the balance of 5-HT responses from one set of receptors to another.

Unfortunately, research in humans has not helped to resolve these difficulties. There is some evidence which implicates increased 5-HT transmission as a causal factor in anxiety: For instance, some patients experienced anxiety when given the anti-obesity agent, fenfluramine (now withdrawn), which increases release of 5-HT in the brain. Also, *m*-1-(3-chlorophenyl) piperazine (*m*CPP), which is a 5-HT_{2C} partial agonist (and has some, albeit even lowers efficacy, at 5-HT_{2A} and 5-HT_{1B} receptors) reliably induces panic in patients. Finally, unlike other antidepressants, trazodone is ineffective in treatment of panic disorder, probably because it is metabolised to *m*CPP which is anxiogenic. On the other hand, there is no consistent evidence that administration of the 5-HT precursor, 5-hydroxytryptophan, causes anxiety in humans and selective serotonin reuptake inhibitors (SSRIs), which are thought to increase 5-HT transmission after chronic administration, do not cause anxiety disorders. Indeed they are now used routinely to treat them. In short, evidence for either an excess or a deficit in serotonergic transmission as a causal factor in anxiety in humans is equally (un)convincing (Bell and Nutt 1998).

Currently, hopes for compounds with greater clinical efficacy and faster onset of action than buspirone rest on the development of selective ligands for 5-HT receptors. So far, antagonists of 5-$HT_{2A/C}$ (e.g. ritanserin), 5-HT_3 (e.g. ondansetron) and 5-HT_4 (e.g. zacopride) receptors have all been explored but their anti-anxiety effects are, at best, equivocal. Full appraisals of the role of 5-HT systems in anxiety and the actions of anti-anxiety drugs are to be found in Handley (1995), Barnes and Sharp (1999) and Olivier, van Wijngaarden and Soudijn (2000).

INTEGRATED THEORIES OF ANXIETY

The evidence outlined so far does little to explain how monoamines or anti-anxiety drugs might influence anxiety states. To achieve this, an integrated view of the relevant brain systems is required, together with an appreciation of how their function is regulated.

One scheme focuses on the roles of the septum and hippocampus. Detailed justification of this theory is beyond the scope of this chapter but can be found in Gray (1987). Briefly, the 'septohippocampal system' is thought to form part of a neuronal network that functions as a 'comparator', i.e. it compares anticipated and actual stimuli. It is envisaged that, when the comparator detects a mismatch between events that are suggested by 'signals' and prevailing stimuli (as in novelty, conflict or frustrative non-reward), a 'behavioural inhibition system' is activated by the septohippocampal system (Fig. 19.9). This system arrests ongoing behaviour and increases vigilance, as is evident in animal models of anxiety (e.g. suppression of rewarded responses or exploration). Ascending noradrenergic and serotonergic inputs are thought to activate this behavioural inhibition system, with these two monoamines playing complementary roles. Moreover, there is extensive evidence that anti-anxiety drugs prevent activation of the behavioural inhibition system by blunting monoaminergic transmission in the hippocampus.

An additional ('defence') system was proposed as early as the 1960s which mediates the flight and fight response. This comprises the amygdala, hypothalamus and central grey in the midbrain. It is generally agreed that the periaquaductal grey area (PAG) of the central grey is responsible for eliciting the flight/fight response which incorporates autonomic changes and analgesia as well as the locomotor response. Gray (1987) proposes that the central grey is normally inhibited by the (ventromedial) hypothalamus and that the influence of the hypothalamus is governed in opposing ways by the behavioural inhibition system and the amygdala. Whereas the former augments hypothalamic inhibition of the flight/fight response, the latter inhibits it, thereby releasing the flight/fight response (Fig. 19.9).

A more recent hypothesis, which incorporates many features of Gray's hypothesis, has concentrated on the central serotonergic system and proposes that different serotonergic pathways underlie GAD and panic (Fig. 19.10). This theory, like that described above, focuses on the amygdala as part of the neuronal 'defence' system and highlights evidence for its key role in the response to conditioned fear. The amygdala is thought to be a major target for conditioned sensory inputs and to organise the conditioned fear response (LeDoux and Muller 1997); this is effected by its connections to the hypothalamus and PAG. Different zones of the PAG seem to evoke different components of this response: whereas stimulation of the dorsal PAG (dPAG) evokes 'explosive running', the ventral PAG (vPAG) is responsible for 'freezing', both of which are common features of a panic attack.

Serotonergic neurons, originating in the dorsal Raphé nucleus (DRN), innervate both the amygdala and the PAG. In the former region, they are thought to augment active avoidance of aversive signals by exaggerating the amygdalar response to conditioned aversive stimuli (Deakin and Graeff 1991; Graeff *et al.* 1996). Excessive serotonergic activity of neurons originating in the DRN is proposed to underlie anticipatory (or 'learned') anxiety which is regarded as akin to GAD. This response could be modulated, at the level of both the DRN and the amygdala, by neuronal inputs from both the frontal

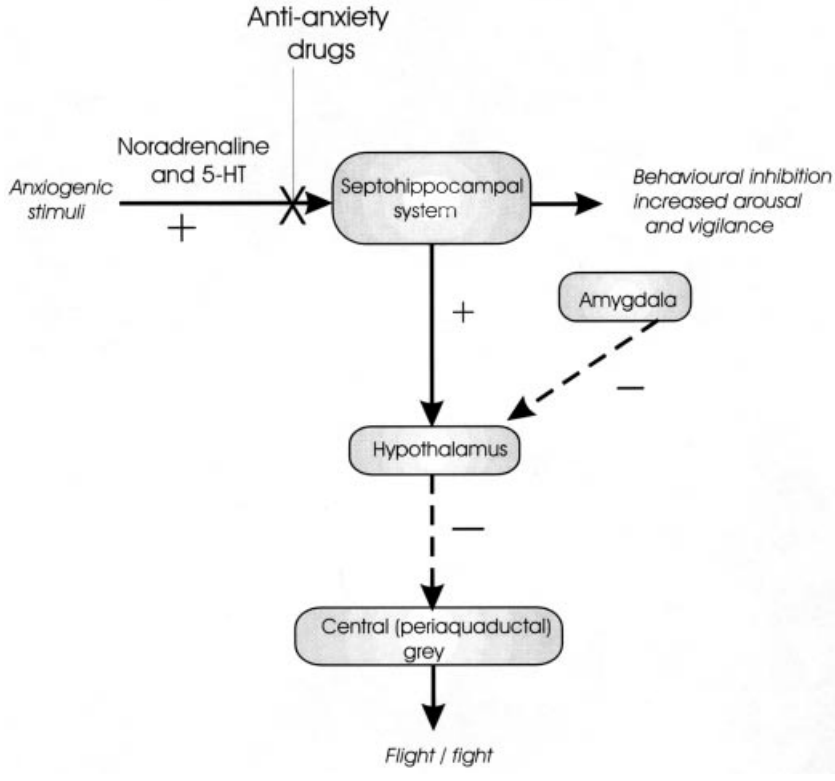


Figure 19.9 A schematic representation of key elements of Gray's explanation for anxiety and the flight/fight response. Noradrenergic and serotonergic inputs to the septohippocampal component of a neuronal 'comparator' activate a behavioural inhibition system which suppresses ongoing behaviour and increases vigilance ('anxiety'). Inputs from the behavioural inhibition system also augment the activity of the (ventromedial) hypothalamus which suppresses the flight/fight response generated in the periaquaductal grey. In contrast, the amygdala inhibits hypothalamic activity and releases the flight/fight response. Anti-anxiety drugs are thought to inhibit monoaminergic activation of the behavioural inhibition system

cortex, which is thought to process the perception of sensory information, and the hippocampus, which processes contextual (environmental) cues.

Deakin and Graeff (1991) further propose the existence of a pathway, again arising in the DRN, which inhibits activation of the PAG. It is suggested that a reduction of serotonergic transmission in this area releases the flight/fight response. Under normal conditions, activity in this system is governed by higher centres in the forebrain (the cortex and hippocampus) so that, when interpretation of prevailing stimuli deems it appropriate, the flight/fight response is suppressed. A deficit in serotonergic inhibition of the PAG is thought to be the origin of panic. There are several ramifications of this interesting theory. For instance, during low arousal states, a decline in the activity of forebrain serotonergic systems would diminish the inhibition of the PAG. This would ensure that threatening stimuli would evoke a protective escape response by default until cortical systems switch off the PAG response, if appropriate, as arousal increases (Handley 1995). It could also explain why patients often report that they are woken up during the night by their panic attacks.

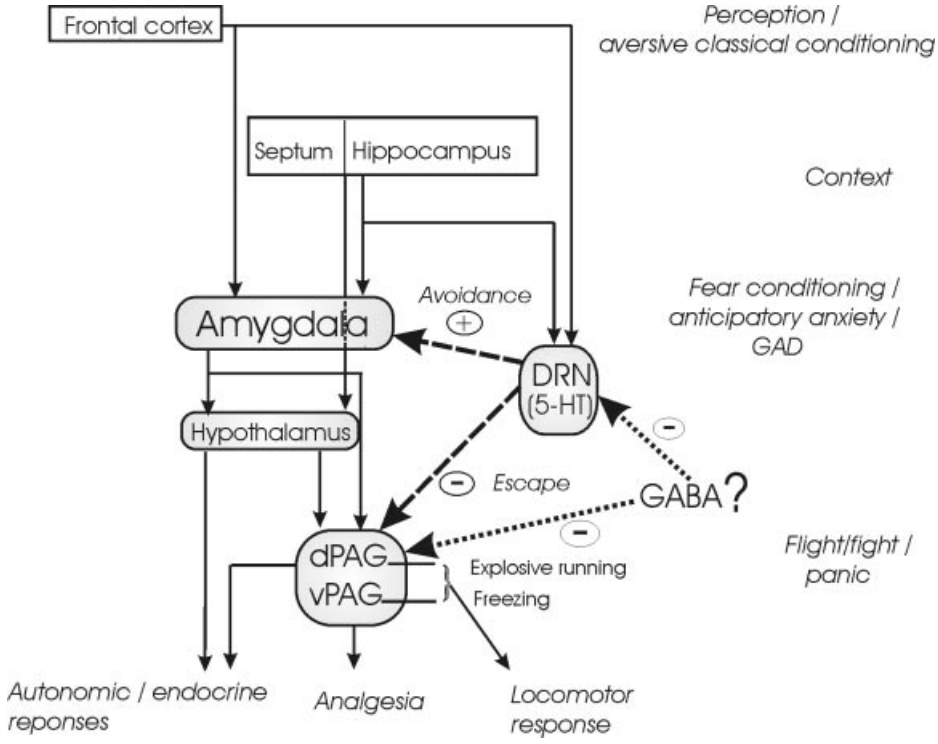


Figure 19.10 The influence of 5-HT pathways (represented by the dashed lines) projecting from the dorsal Raphé nucleus (DRN) on brain regions comprising the 'brain defence system'. This system comprises the amygdala, hypothalamus and periaquaductal grey (PAG) and coordinates behavioural and neuroendocrine responses to conditioned and unconditioned aversive stimuli. Activity within the defence system is governed by higher centres, such as the frontal cortex and hippocampus. Serotonergic neurons projecting from the dorsal Raphé nucleus are proposed to activate the amygdala (+) thereby promoting the response to conditioned aversive stimuli (anxiety). Projections from this nucleus to the dorsal and ventral periaquaductal grey (dPAG and vPAG) are thought to suppress (−) the flight/fight response to aversive stimuli. A deficit in serotonergic transmission to this brain region is thought to underlie panic. Possible targets for anti-anxiety drugs, acting via the $GABA_A$ receptor, are indicated by the dotted arrows. See text for further details

There is a good deal of evidence that postsynaptic $5\text{-}HT_{2A/2C}$ receptors mediate the actions of 5-HT in both the amygdala and the PAG (Deakin, Graeff and Guimaraes 1992). Thus local infusion of $5\text{-}HT_{2A/2C}$ antagonists into the amygdala has an anti-conflict effect in animals while their systemic administration might have (albeit controversially) anti-anxiety effects in humans. In contrast, these drugs promote the flight/fight response to aversive stimuli. This leads to the prediction that drugs that relieve anxiety, through inhibition of 5-HT transmission in the amygdala, will exacerbate panic by inhibiting the restraining influence of 5-HT in the PAG. In fact, this has been offered as an explanation for the panic attacks experienced by some patients given buspirone. It could also explain the increase in panic attacks in the early stages of treatment with antidepressants. These drugs first decrease the firing rate of serotonergic neurons and the terminal release of 5-HT; recovery of neuronal firing and

increased release of 5-HT, like the relief of panic by these drugs, requires prolonged treatment (see Chapter 20).

Obviously, any explanation of anxiety must account for the actions of benzodiazepines. Gray's theory suggests that they inhibit monoaminergic inputs to the septohippocampal system and switch off behavioural inhibition. A related suggestion is that, whereas the behavioural inhibition system is located in the medial septum/dorsal hippocampus, there is also a 'safety system' in the lateral septum/ventral hippocampus. According to this scheme, benzodiazepines might activate this latter system and generate spurious safety signals (see Handley 1995). Alternatively, Deakin and Graeff's theory suggests that benzodiazepines could directly inhibit activity generated in the PAG. However, they could also inhibit the activity of serotonergic neurons in the DRN and suppress the amygdala response to conditioned fear stimuli. In this case, suppression of the serotonergic inhibitory inputs to the PAG might also be anticipated, an action that could explain why benzodiazepines are ineffective in treating panic disorder.

The finding that noradrenergic neurons innervating the frontal cortex, but not those projecting to the hypothalamus, respond to conditioned environmental cues (McQuade and Stanford 1999) suggests that there could be a similar subdivision of function in this monoamine system as well. However, activation of 5-HT receptors modulates release of noradrenaline (Stanford 1999) and vice versa (Gobert *et al.* 1997). The function of both these neuronal systems is influenced by GABAergic systems. Clearly, any theory for anxiety must eventually take account of evidence that serotonergic and noradrenergic systems do not operate independently.

PEPTIDES

Many peptidergic and amino acid receptors have been suggested as potential targets for anti-anxiety drugs. The outcome of preclinical and clinical investigations (together with further references) are detailed in Jackson and Nutt (1996). So far, no compounds have emerged as clear candidates for the clinic, not least because pharmacokinetic considerations and adverse effects in the periphery are common confounding factors. How any of the peptides could actually influence the neuronal networks considered to be involved in anxiety, as outlined above, is uncertain but the targets which hold greatest promise for the future are:

(1) *The angiotensin system*: The possibility that drugs targeting this system might be efficacious in anxiety first came to light from self-reports of patients who were being treated with inhibitors of angiotensin-converting enzyme ('ACE' inhibitors) for their hypertension. Since then, these agents (e.g. captopril) have been found to mimic the effects of benzodiazepines in a range of preclinical models. Experiments with selective angiotensin receptor antagonists, e.g. the AT_1 receptor antagonist, losartin, have so far confirmed that these agents are effective in many preclinical models whereas AT_2 receptor antagonists are not.

(2) *Cholecystokinin receptor ligands*: The actions of receptor-selective ligands have been explored following reports that peptides targeting CCK receptors can induce anxiety in humans. CCK_B receptor agonists have anxiogenic effects in animal models whereas antagonists have an anti-anxiety effect, including the prevention of behavioural changes resulting from ethanol withdrawal (Wilson, Watson and Little

1998). In humans, CCK_B receptor ligands can induce a panic attack, especially in panic patients, whereas antagonists (e.g. CL988) have the opposite effect. Nevertheless, the results from clinical trials of this compound are not promising, mainly due to low bioavailability and unacceptable side-effects.

(3) *Neurokinin receptors*: NK_2 receptor agonists (e.g. GR64349) have an anxiogenic profile in animal models while the antagonists (GR100679) have an anti-anxiety effect. However, NK_1 receptor antagonists have also been reported to have anti-anxiety activity in the social interaction test (File 1997).

(4) *Neuropeptide Y receptors*: The NPY_1 receptor agonist, (leu^{31}, Pro^{34})neuropeptide Y has anticonflict activity. Although NPY_2 receptor ligands are generally thought to lack anti-anxiety effects, there is some evidence that they are active in preclinical models (Kask, Rago and Harro 1998). The picture is complicated by findings that NPY itself can have anti-anxiety or anxiogenic effects, depending on dose (Nakajima *et al.* 1998).

(5) *Corticotropin-releasing factor (CRF) receptors*: CRF is well known for its role in the stress response and for its interactions with monoamine systems in the brain. As might be expected, this hormone has anxiogenic activity in preclinical models. The CRF receptor antagonist, α-helical CRF^{9-14}, prevents the actions of anxiogenic drug treatments (such as ethanol withdrawal) but seems to be inactive in preclinical tests when given alone. Interestingly, it also seems to prevent the anxiogenic effects of NPY_1 antagonists suggesting some functional interactions between these two peptide systems (Kask, Rago and Harro 1997).

(6) *NMDA receptors*: The non-competitive NMDA receptor antagonist, dizocilpine, and competitive antagonists such as AP-5, have anti-anxiety activity in preclinical models. However, the psychotropic effects of other NMDA receptor antagonists, such as the potent hallucinogen, phencyclidine, warn against these compounds being realistic targets for future drug development. Antagonists of the glycine site on the NMDA receptor (e.g. HA-966) also have anti-anxiety effects in preclinical models and are more promising.

(7) *Adenosine receptors*: The possibility that adenosine receptors will be a useful target is suggested by the marked anxiogenic effects of the adenosine receptor antagonist, caffeine. Whether it is this action of caffeine (which has many molecular targets in the brain) that explains its anxiogenic actions is not at all certain and, so far, selective adenosine receptor agonists have not yielded promising results.

CONCLUSIONS

Without doubt, the benzodiazepines are the most successful of the anti-anxiety agents in respect of their safety and tolerability and so one might ask why there is a need to search for better agents at all. One problem is that, while they are highly efficacious in treating GAD, the benzodiazepines are not without their drawbacks, particularly in respect of concern about their potential for dependency and their clear liability for abuse. Another is the need to develop better treatments for other manifestations of anxiety. Novel agents, targeting peptidergic systems, might provide solutions to both these problems. It is only through the combined efforts of all the approaches outlined in this chapter that we are likely to identify the cause(s) of anxiety and develop the ideal treatment.

REFERENCES

Abe, K, Takeyama, C and Yoshimura, K (1998) Effects of S-8510, a novel benzodiazepine receptor partial inverse agonist, on basal forebrain lesioning-induced dysfunction in rats. *Eur. J. Pharmacol.* **347**: 145–152.

Awad, M and Gavish, M (1987) Binding of [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 to cerebral cortex and peripheral tissues of various species: species differences and heterogeneity in peripheral benzodiazepine binding sites. *J. Neurochem.* **49**: 1407–1414.

Ballenger, JC (1990) *Neurobiology of Panic Disorder*, Wiley-Liss, New York.

Barnes, NM and Sharp, T (1999) A review of central 5-HT receptors and their function. *Neuropharmacology* **38**: 1083–1152.

Beckett, S and Marsden, CA (1997) The effect of central and systemic injection of the 5-HT_{1A} receptor agonist 8-OHDPAT and the 5-HT_{1A} receptor antagonist WAY100635 on peri-aquaductal grey-induced defence behaviour. *J. Psychopharmacol.* **11**: 35–40.

Bell, CJ and Nutt, DJ (1998) Serotonin and panic. *Brit. J. Psychiatry* **172**: 465–471.

Bremner, JD, Krystal, JH, Southwick, SM and Charney, DS (1996) Noradrenergic mechanisms in stress and anxiety. II. Clinical studies. *Synapse* **23**: 39–51.

Cao, BJ and Rodgers, RJ (1997) Influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour in mice. II WAY 100635, SDZ 216-525 and NAN-190. *Pharmacol. Biochem. Behav.* **58**: 593–603.

Chaouloff, F (1993) Physiopharmacological interactions between stress hormones and central serotonergic systems. *Brain Res.* **18**: 1–32.

Cheetham, SC and Heal, DJ (2000) Antidepressant and anxiolytic drugs. In *Biological Psychiatry* (Eds Bittar, EE and Bittar, N), JAI Press, Stanford, CT, pp. 551–567.

Chiu, TH, Dryden, DM and Rosenberg, HC (1982) Kinetics of [^{3}H]-labelled flunitrazepam binding to membrane-bound benzodiazepine receptors. *Mol. Pharmacol.* **21**: 57–65.

Costa, E and Guidotti, A (1991) Diazepam binding inhibitor (DBI): a peptide with multiple biological actions. *Life Sci.* **49**: 325–344.

Coupland, N, Glue, P and Nutt, DJ (1992) Challenge tests: assessment of the noradrenergic and GABA systems in depression and anxiety disorders. *Molec. Aspects Med.* **13**: 221–247.

Dalley, JW, Mason, K and Stanford, SC (1996) Increased levels of extracellular noradrenaline in the frontal cortex of rats exposed to naturalistic environmental stimuli: modulation by acute systemic administration of diazepam or buspirone. *Psychopharmacology* **127**: 47–54.

Deakin, JFW and Graeff, FG (1991) 5-HT and mechanisms of defence. *J. Psychopharmacol.* **5**: 305–315.

Deakin, JFW, Graeff, FG and Guimaraes, FS (1992) 5-HT receptor subtypes and the modulation of aversion. In *Central Serotonin Receptors and Psychotropic Drugs* (Eds Marsden, CA and Heal, DJ), Blackwell Scientific Publications, Oxford, pp. 147–174.

De Robertis, E, Peña, C, Paladini, AC and Medina, JH (1988) New developments on the search for endogenous ligand(s) of central benzodiazepine receptors. *Neurochem. Int.* **13**: 1–11.

Doble, A and Martin, IL (1996) *The $GABA_A$/Benzodiazepine Receptor as a Target for Psychoactive Drugs*, RG Landes Co., Austin, TX.

Done, CJ and Sharp, T (1994) Biochemical evidence for the regulation of central noradrenergic activity by 5-HT_{1A} and 5-HT_2 receptors: Microdialysis studies in the awake and anaesthetised rat. *Neuropharmacology* **33**: 411–421.

Do Rego, JL, Mensah-Nyagan, AG, Feuilloley, M, Ferrara, P, Pelletier, G and Vaudry, H (1998) The endozepine triakontatetraneuropeptide diazepam-binding inhibitor [17–50] stimulates neurosteroid biosynthesis in the frog hypothalamus. *Neuroscience* **83**: 555–570.

Dorow, R, Horowski, R, Paschelke, G, Amin, M and Braestrup, C (1983) Severe anxiety induced by FG7142, a β-carboline ligand for benzodiazepine receptors. *Lancet* **ii**: 98–99.

Dourish, CT, Hutson, PH and Curzon, G (1986) Putative anxiolytics 8-OHDPAT buspirone and TVXQ 7821 are agonists at 5-HT_{1A} autoreceptors in the raphé nuclei. *Trends Pharmacol. Sci.* **7**: 212–214.

Drugan, RC, Basile, AS, Crawley, JN, Paul, SM and Skolnick, P (1987) 'Peripheral' benzodiazepine binding sites in the Maudsley Reactive rat: selective decrease confined to peripheral tissues. *Brain Res. Bull.* **18**: 143–145.

DSM–IV: *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (1994), American Psychiatric Association, Washington, DC, pp. 393–444.

Duggan, MJ and Stephenson, FA (1988) Benzodiazepine binding site heterogeneity in the purified $GABA_A$ receptor. *Eur. J. Pharmacol.* **154**: 293–298.

Eison, AS, Eison, MS, Stanley, M and Riblet, LA (1986) Serotonergic mechanisms in the behavioural effects of buspirone and gepirone. *Pharmac. Biochem. Behav.* **24**: 701–707.

File, SE (1997) Anxiolytic action of a neurokinin1 receptor antagonist in the social interaction test. *Pharmacol. Biochem. Behav.* **58**: 747–752.

File, SE and Hyde, JR (1979) A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants. *Pharmacol. Biochem. Behav.* **11**: 65–69.

Fontana, DJ, McMiller, LV and Commissaris, RL (1999) Depletion of brain norepinephrine: differential influence on anxiolytic treatment effects. *Psychopharmacology* **143**: 197–208.

Geller, I, Kulak, JT and Seifter, J (1962) The effects of chlordiazepoxide and chlorpromazine on a punished discrimination. *Psychopharmacologia* **3**: 374–385.

Gobert, A, Rivet, JM, Cistarelli, L, Melon, C and Millan, MJ (1997) Alpha2-adrenergic receptor blockade markedly potentiates duloxetine- and fluoxetine-induced increases in noradrenaline, dopamine and serotonin levels in the frontal cortex of freely moving rats. *J. Neurochem.* **69**: 2616–2619.

Graeff, FG, Guimarães, FS, De Andrade, TGC and Deakin, JFW (1996) Role of 5-HT in stress, anxiety and depression. *Pharmacol. Biochem. Behav.* **54**: 129–141.

Gray, JA (1987) *The Psychology of Fear and Stress*, 2nd edition, Cambridge University Press, Cambridge.

Han, QP and Dryhurst, G (1996) Influence of glutathione on the oxidation of 1-methyl-6-hydroxy-1,2,3,4-tetrahydro-beta-carboline; chemistry of potential relevance to the addictive and neurodegenerative consequences of ethanol use. *J. Med. Chem.* **39**: 1494–1508.

Handley, SL (1995) 5-Hydroxytryptamine pathways in anxiety and its treatment. *Pharmac. Ther.* **66**: 103–148.

Jackson, HC and Nutt, DJ (1996) Anxiety and panic disorders. In *Neurotherapeutics, Emerging Strategies* (Eds Pullan, LM and Patel, J), Humana Press Inc., Totowawa, NJ, pp. 85–131.

Jacobs, BL and Azmitia, EC (1992) Structure and function of the brain serotonin system. *Pharmacol. Rev.* **72**: 165–229.

Kask, A, Rago, L and Harro, J (1997) Alpha-helical CRF(9-41) prevents the anxiogenic-like effect of NPY Y1 receptor antagonist BIBP3226 in rats. *Neuroreport* **8**: 3645–3647.

Kask, A, Rago, L and Harro, J (1998) Anxiolytic-like effect of neuropeptide Y (NPY) and NPY13-36 microinjected into vicinity of locus coeruleus in rats. *Brain Res.* **788**: 345–348.

La Marca, S and Dunn, RW (1994) The α_2-antagonists idazoxan and rauwolscine but not yohimbine or piperoxan are anxiolytic in the Vogel lick-shock conflict paradigm following intravenous administration. *Life Sci.* **54**: 179–184.

LeDoux, JE and Muller, J (1997) Emotional memory and psychopathology. *Phil. Trans. Roy. Soc. Lond. B* **352**: 1719–1726.

Libet, B and Gleason, CA (1994) The human locus coeruleus and anxiogenesis. *Brain Res.* **634**: 178–180.

Malizia, AL, Cunningham, VJ, Bell, CJ, Liddle, PF, Jones, T and Nutt, DJ (1998) Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. *Arch. Gen. Psychiatry* **55**: 715–720.

Margules, DL and Stein, L (1968) Increase of 'antianxiety' activity and tolerance of behavioural depression during chronic administration of oxazepam. *Psychopharmacologia* **13**: 74–80.

Marsden, CA, Bickerdike, M, Cadogen, A-K, Wright, I, Rex, A and Fink, H (1993) Serotonergic mechanisms and animal models of anxiety. In *Anxiety, Neurobiology, Clinic and Therapeutic Perspectives* (Eds Hamon, M, Oilat, H and Thiebot, M-H), John Libbey Eurotext Ltd, 232, pp. 75–82.

Mason, K, Heal, DJ and Stanford, SC (1998) The anxiogenic agents, yohimbine and FG 7142 disrupt the noradrenergic response to novelty. *Pharmacol. Biochem. Behav.* **60**: 321–327.

McQuade, R and Stanford, SC (2000) A microdialysis study of the noradrenergic response in rat frontal cortex and hypothalamus to a conditioned cue for aversive, naturalistic environmental stimuli. *Psychopharmacol.* **148**: 201–208.

Moehler, H and Okada, T (1977) Properties of [3H]diazepam binding to benzodiazepine receptors in rat cerebral cortex. *Life Sci.* **20**: 2101–2110.

Nakajima, M, Unui, A, Asakawa, A, Momose, K, Ueno, N, Teranishi, A, Baba, S and Kasuga, M (1998) Neuropeptide Y produces anxiety via Y2-type receptors. *Peptides* **19**: 359–363.

Nutt, DJ (1990) The pharmacology of human anxiety. *Pharmacol. Ther.* **47**: 233–266.

Nutt, DJ, Glue, P, Lawson, C and Wilson, S (1990) Flumazenil provocation of panic attacks. Evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Arch. Gen. Psychiatry* **47**: 917–925.

Olivier, B, van Wijngaarden, I and Soudijn, W (2000) 5-HT_3-receptor antagonists and anxiety: a preclinical and clinical view. *Eur. Neuropsychopharmacol.* **10**: 77–95.

Przegalinski, E, Chojnacka-Wojcik, E and Filip, M (1992) Stimulation of postsynaptic 5-HT_{1A} receptors is responsible for the anticonflict effect of ipsapirone in rats. *J. Pharm. Pharmac.* **44**: 780–782.

Rasmussen, K and Jacobs, BL (1986) Single unit activity of locus coeruleus neurons in the freely moving cat II. Conditioning and pharmacological studies. *Brain Res.* **371**: 335–344.

Reddy, DS and Kulkarni, SK (1998) The role of GABA-A and mitochondrial diazepam-binding inhibitor receptors on the effects of neurosteroids on food intake in mice. *Psychopharmacology* **137**: 391–400.

Redmond, DE and Huang, YH (1979) New evidence for a locus coeruleus-norepinephrine connection with anxiety. *Life Sci.* **25**: 2149–2162.

Rodgers, RJ and Dalvi, A (1997) Anxiety, defence and the elevated plus-maze. *Neurosci. Biobehav. Rev.* **21**: 801–810.

Salmon, P, Tsaltas, E and Gray, JE (1989) Disinhibition by propranolol and chlordiazepoxide of nonrewarded lever-pressing in the rat is unaffected by dorsal noradrenergic bundle lesion. *Neuropharmacology* **28**: 207–210.

Sangameswaran, L, Fales, HM, Friedrich, P and De-Blas, AL (1986) Purification of a benzodiazepine from bovine brain and detection of benzodiazepine-like immunoreactivity in human brain. *Proc. Natl. Acad. Sci. USA* **83**: 9236–9240.

Seevers, MH and Tatum, AL (1931) Chronic experimental barbital poisoning. *J. Pharmacol. Exp. Ther.* **42**: 217–231.

Sieghart, W and Karobath, M (1980) Molecular heterogenity of benzodiazepine receptors. *Nature* **286**: 285–287.

Silverstone, PH, Rue, JE, Franklin, M, Hallis, K, Camplin, G, Laver, D and Cowen, PJ (1994) The effects of administration of *m*CPP on psychological, cognitive, cardiovascular, hormonal and MHPG measurements in human volunteers. *Int. Clin. Psychopharmacol.* **9**: 173–178,

Söderpalm, B, Blomqvist, O and Söderpalm, B (1995) The yohimbine-induced anticonflict effect in the rat, part I. Involvement of noradrenergic, serotonergic and endozepinergic(?) mechanisms. *J. Neural Trans.* **100**: 175–189.

Squires, RF and Braestrup, C (1977) Benzodiazepine receptors in rat brain. *Nature* **266**: 732–734.

Stanford, SC (1995) Central noradrenergic neurones and stress. *Pharmac. Ther.* **68**: 297–342.

Stanford, SC (1999) SSRI-induced changes in catecholaminergic transmission. In Selective Serotonin Reuptake Inhibitors (SSRIs): Past, Present and Future, Ron Landes Bioscience, Austin, TX, pp. 148–170.

Sternbach, LH, Randall, LO and Gustafson, SR (1964) 1,4-Benzodiazepines (chlordiazepoxide and related compounds). In Medicinal Chemistry, Volume 1, Psychopharmacological Agents (Ed. Gordon, M), Academic Press, New York & London, pp. 137–224.

Taylor, KM and Laverty, R (1969) The effect of chlordiazepoxide, diazepam and nitrazepam on catecholamine metabolism in regions of the rat brain. *Eur. J. Pharmacol.* **8**: 296–301.

Tyrer, P (1989) *The Psychopharmacology of Anxiety*, Oxford Medical Publications, Oxford.

Vogel, RA, Frye, GD, Wilson, JH, Kuhn, CM, Koepke, KM, Mailman, RB, Mueller, RA and Breese, GR (1980) Attenuation of the effects of punishment by ethanol: comparisons with chlordiazepoxide. *Psychopharmacology* **71**: 123–129.

Wilson, J, Watson, WP and Little, HJ (1998) CCK(B) antagonists protect against anxiety-related behaviour produce by ethanol withdrawal, measured using the elevated plus-maze. *Psychopharmacology* **137**: 121–131.