New insights into the role of the $GABA_A$–benzodiazepine receptor in psychiatric disorder

DAVID J. NUTT and ANDREA L. MALIZIA

Background In the 40 years since the first benzodiazepine was brought into clinical use there has been a substantial growth in understanding the molecular basis of action of these drugs and the role of their receptors in disease states.

Aims To present current knowledge about the role of the $GABA_A$–benzodiazepine receptor in anxiety disorders, new insights into the molecular biology of the receptor complex and neuroimaging studies suggesting involvement of these receptors in disease states.

Method An overview of published literature, including some recent data.

Results The molecular biology of this receptor is detailed. Molecular genetic studies suggesting involvement of the $GABA_A$–benzodiazepine receptor in animal behaviour and learning are outlined; possible parallels with human psychopathology are discussed.

Conclusions Current insights into the role of the $GABA_A$–benzodiazepine receptor in the action of benzodiazepines and as a factor in disease states, in both animals and humans, may lead to new, more sophisticated interventions at this receptor complex and potentially significant therapeutic gains.

Declaration of interest D.J.N. has received grants from various pharmaceutical companies with an interest in drugs acting at the GABA–benzodiazepine receptor.

Since their introduction into clinical practice 40 years ago, the use of benzodiazepines has become widespread, because of their efficacy, safety and tolerability. However, their mechanism of action was unknown until 1977, when was it discovered that they interacted with specific receptors in the central nervous system (CNS). In 1987 this receptor, the $GABA_A$–benzodiazepine receptor, was cloned. Now sophisticated techniques in molecular biology and in neuroimaging are giving us precise information not only into how – and where – benzodiazepines and other anxiolytics/hypnotics act, but also further insights into the pathophysiology of anxiety and related disorders including benzodiazepine dependence and addiction.

THE $GABA_A$–BENZODIAZEPINE RECEPTOR

Following the clinical impact of the first benzodiazepines in the mid-1950s considerable effort went into investigating their mechanism of action. It was not until 1974 that researchers at Roche and in the USA independently showed that there was a highly specific potentiation of gamma-aminobutyric acid (GABA) by benzodiazepines. In 1977 it was discovered that the benzodiazepines interacted with a specific binding site in the CNS, which turned out to be an integral part of the $GABA_A$ receptor complex (reviewed in Haefely, 1978). The receptor complex was isolated and sequenced in 1987 (Schofield *et al*, 1987) and was visualised by electron microscopy in 1994 (Nayeem *et al*, 1994) (see Fig. 1a). The $GABA_A$–benzodiazepine receptor comprises five protein sub-units (Fig. 1b), arranged like a rosette around a central pore, crossing the cell membrane, which is permeable to chloride and other anions. In addition to GABA and the benzodiazepines, other psychoactive compounds, such as barbiturates and anaesthetic steroids, can also bind to the receptor and open the chloride channel (see Fig. 1c). Benzodiazepine site ligands, however, do not act directly to open the channel, but rather modulate the capacity of GABA to do so, resulting in augmentation or diminuition of its inhibitory effects (Barnard *et al*, 1998).

GABA: REINING IN THE NERVOUS SYSTEM

Gamma-aminobutyric acid is quantitatively the most important inhibitory transmitter in the CNS. GABAergic neurons are distributed widely in the CNS, but are virtually absent

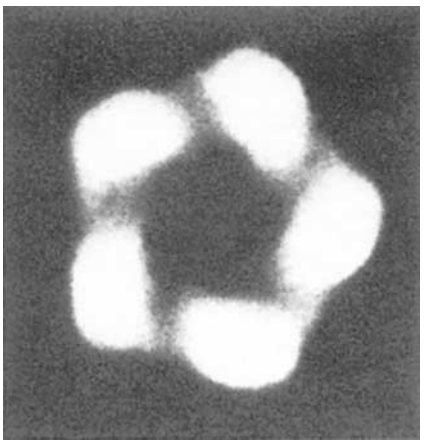

Fig. Ia The $GABA_A$–benzodiazepine receptor complex, visualised by electron microscopy, showing five protein sub-units arranged around a central core (from Nayeem *et al* (1994), with permission).

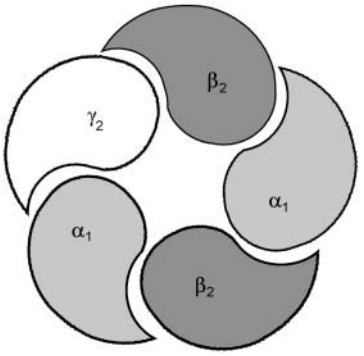

Fig. Ib The most important and most prevalent $GABA_A$–benzodiazepine receptor in the brain is made up from α_1, β_2 and γ_2 sub-units, encoded by the same cluster of genes on chromosome 5. The composition of the receptor sub-units, particularly α and γ sub-units, seems to determine the benzodiazepine pharmacology of the receptor, with different subtypes having different sensitivities to benzodiazepine receptor ligands.

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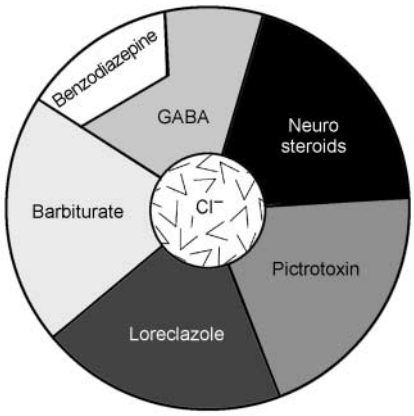

Fig. Ic Schematic representation of the binding sites on the GABA$_A$–benzodiazepine receptor complex. Note that agonists binding to the benzodiazepine receptor site do not open the chloride channel directly, but rather augment the capacity of GABA to do so. (This is a schematic diagram and does not correspond directly to the protein sub-units seen in Fig. Ib.)

outside the brain and the spinal cord. GABA controls the state of excitability in all brain areas and the ongoing level of neuronal activity is regulated by the balance between excitatory inputs (mostly glutamatergic) and inhibitory GABAergic activity. If the balance swings in favour of GABA, then sedation, amnesia and ataxia appear. On the other hand, the mildest attenuation of the GABAergic system results in arousal, anxiety, restlessness, insomnia and exaggerated reactivity. It has been estimated that, depending on the brain region, 20–50% of all central synapses use fast-signalling GABA$_A$–benzodiazepine receptors.

AGONISTS, ANTAGONISTS, INVERSE AGONISTS

When GABA binds with the GABA$_A$–benzodiazepine receptor complex, it acts as an agonist: inducing conformational changes, which increase the permeability of the central pore to chloride ions. The resulting chloride flux hyperpolarises the neuron, reducing its excitability and producing a general inhibitory effect on neuronal activity. Classical benzodiazepines in clinical use act to enhance the effectiveness of GABA in a unique way, by lowering the concentration of GABA required for opening the channel. Benzodiazepine binding allosterically changes the receptor complex to increase the efficiency of GABA, so enabling the GABAergic circuits to produce a larger inhibitory effect. The action of benzodiazepines is thus markedly different from drugs such as barbiturates, chloral hydrate, chlormethiazole and ethanol, which, as well as enhancing GABA, can also directly open the chloride channel. The propensity of these latter drugs to be fatal in overdose probably reflects this direct action on the chloride channel. Benzodiazepines are safer, perhaps because the vital brain circuits cannot be inhibited over and above the level that would be achieved by natural GABAergic effects.

Further insights into the sophistication of the benzodiazepine site came 15 years ago with the discovery of drugs which bind to it, yet have the opposite effects to the classical benzodiazepine receptor agonists. These drugs decrease the probability of the chloride channel opening in response to GABA and have stimulant, anxiogenic and proconvulsant properties. They are now termed 'inverse agonists'. Subsequently, benzodiazepine receptor antagonists, notably flumazenil, have been discovered which block the activities of both agonists and inverse agonists. Moreover, the recent discovery of both partial agonists and partial inverse agonists shows that the benzodiazepine receptor mediates a spectrum of different actions (see Fig. 2).

WHY DOES THE BENZODIAZEPINE SITE EXIST?

The benzodiazepine site is the most evolutionary recent part of the GABA$_A$ complex discovered (Nielsen *et al*, 1978). So what evolutionary pressures have led to the emergence of benzodiazepine receptors and their widespread presence in neurons? One theory is that they are necessary to regulate anxiety, and that the brain itself produces an anxiety-reducing compound (an 'endogenous agonist'). Anxiety states and insomnia could be the result of a deficiency in the production of this compound and, by analogy with other deficiency diseases (such as insulin in Type 1 diabetes), might require continual, long-term replacement therapy. Attempts to isolate an endogenous agonist have led to some interesting findings. Benzodiazepines are found in the (paraffin-preserved) brains of individuals who died long before the first laboratory synthesis of benzodiazepines (Sangameswaran *et al*, 1986). Endogenous benzodiazepine agonists (endozapines) are found in the rare familial condition, idiopathic recurrent stupor (Tinuper *et al*, 1994) and possibly in hepatic encephalopathy (Cossar *et al*, 1997). Plants, notably *Aspergillus* fungi, can make a range of benzodiazepines, and these naturally occurring benzodiazepines can also be stored in human brains after being eaten – so it is possible that the receptors evolved to take advantage of these naturally occurring anxiolytics.

It is also possible that benzodiazepine receptors exist for exactly the opposite purpose – to mediate the activities of endogenous inverse agonists. Such compounds could keep brain arousal optimal and if levels fell, sleep could result. In the search for an endogenous benzodiazepine receptor ligand, several compounds with inverse agonist activity have emerged. One early candidate, ethyl-β-carboline-3-carboxylate (β-CCE), was the first compound shown

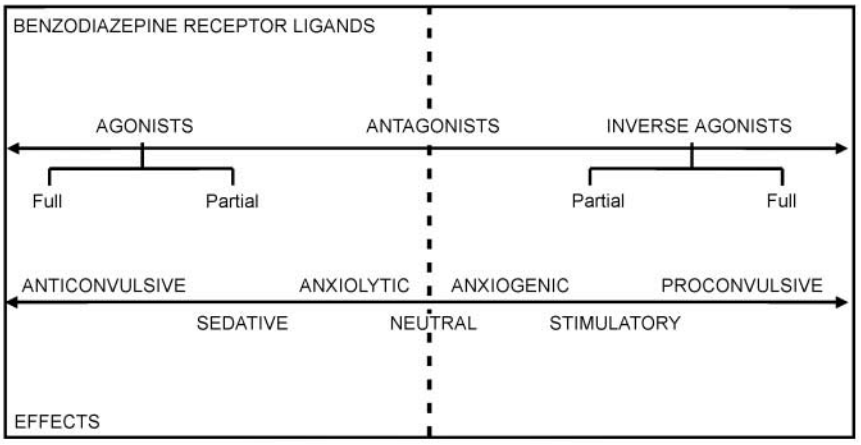

Fig. 2 The benzodiazepine receptor mediates a spectrum of actions, via a range of ligands, including full and partial agonists and inverse agonists.

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to promote anxiety by a direct action at a receptor in the brain (Braestrup *et al*, 1980). But it was later shown that β-CCE was not endogenous, being formed in the extraction process. Another, called tribulin (Hucklebridge *et al*, 1998), is found in urine and its levels are elevated in many conditions where there is increased anxiety (post-traumatic stress disorder and alcohol withdrawal, for example; reviewed by Glover, 1998). However, its structure has not yet been determined and its status remains uncertain.

A third theory suggests that there is no endogenous benzodiazepine receptor ligand and that the site may simply be a particular protein conformation that 'fine tunes' GABA function, possibly altering maximal efficacy, or the rate of desensitisation. Recent evidence suggests that the benzodiazepine receptor spectrum is not fixed and that the 'set-point' – where drugs bind, but have no effect – can be moved, perhaps as a result of differential sub-unit expression (see below). Among the effects of a set-point shift could be tolerance of, and/or dependence on, benzodiazepine receptor agonists, differential sensitivity to alcohol, anxiety predisposition, panic disorder and stress responsiveness (see Fig. 3). Genetically determined factors may also lead to different positions of the set-point (see also benzodiazepine dependence).

ANXIETY: A BENZODIAZEPINE RECEPTOR ABNORMALITY?

Evidence that anxiety disorders may be caused by abnormalities in benzodiazepine receptors has come from a series of studies using the benzodiazepine receptor antagonist, flumazenil, both as a challenge test and as an imaging ligand.

When patients with panic disorder were given an intravenous 2 mg dose of flumazenil, enough to occupy more than half of the receptors in the brain, it provoked panic in most of the patients but was quite innocuous in the control subjects (Nutt *et al*, 1990). This finding, which has been replicated in a number of studies (though not in all) (Woods *et al*, 1991; Maddock, 1998; Strohle *et al*, 1999) clearly demonstrates that panic disorder is not due to the actions of an inverse agonist, as in this case the antagonisit would reduce anxiety. The anxiogenic effect of flumazenil could reflect displacement of an endogenous agonist, but this would only be present in patients (since controls did not experience an increase in anxiety). Another, and more likely, hypothesis is that the 'set-point' of the benzodiazepine receptor has moved in the inverse agonist direction, making flumazenil a weak inverse agonist, thus generating anxiety. Further, we could expect the effects of full agonists to be reduced in patients with panic disorder and this is indeed the case. It has been shown that patients with panic disorder are subsensitive to the central effects of diazepam (Roy-Byrne *et al*, 1990) and we also know, from clinical experience, that treatment of these patients requires either a high-dose or a high-potency benzodiazepine.

Abnormality at the GABA–benzodiazepine receptor may be specific to, or more pronounced in, severe episodic anxiety – as patients with generalised anxiety disorder, post-traumatic stress disorder and depression do not panic when given flumazenil. Since there is a significant hereditary factor in panic disorder, the receptor abnormality could be due to the transmission of a defective receptor gene (see 'Identifying the role of receptor sub-units', below).

IMAGING STUDIES IN ANXIETY

Modern neuroimaging techniques, notably positron emission tomography (PET) and single photon emission tomography (SPET), allow us to measure the $GABA_A$–benzodiazepine receptor complex in the living human brain. In a recent study, PET scans were used to measure brain $GABA_A$–benzodiazepine receptor binding using flumazenil, radio-labelled with ^{11}C. The major finding of the study was that, compared with controls, there is a significant global reduction in flumazenil binding to benzodiazepine sites throughout the brain in patients with panic disorder (Malizia *et al*, 1998); PET scans showing flumazenil binding in controls and patients with panic disorder are shown in Fig. 4. SPET studies using the related benzodiazepine receptor ligand ^{123}I-iomazenil, have shown similar decreases in binding (reviewed in Malizia, 1999). A localised reduction in benzodiazepine binding in the temporal lobe has also been reported in generalised anxiety disorder (Tiihonen *et al*, 1997).

These findings are consistent with the idea that some anxiety disorders may be due to defective neuroinhibitory processes. The greatest decreases observed in benzodiazepine binding occurred in areas thought to be involved in the experience of anxiety in man, such as the orbitofrontal and temporal cortex and insula. Interestingly, in a recent PET study of anxiety generation, we have shown that the anxiolytic effects of the benzodiazepine most closely relate to modulation in brain metabolism in insula and orbitofrontal cortex, among others (Malizia, 2000) (see Fig. 5). Care should be

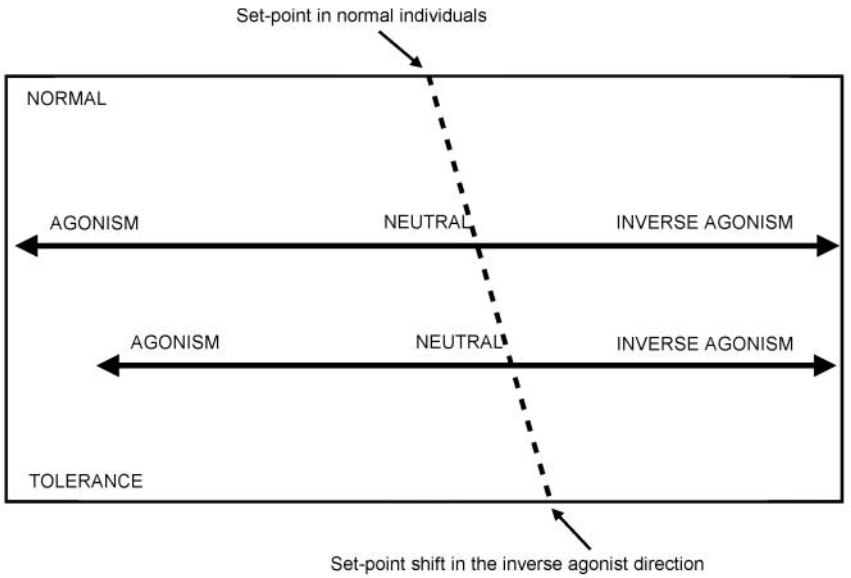

Fig. 3 Recent evidence suggests that the benzodiazepine receptor spectrum is not fixed and that the 'set-point' – where drugs bind, but have no effect – can be moved. Anxiety states, particularly panic disorder, may be due to receptor abnormalities which lead to a movement of the set-point in the inverse agonist direction.

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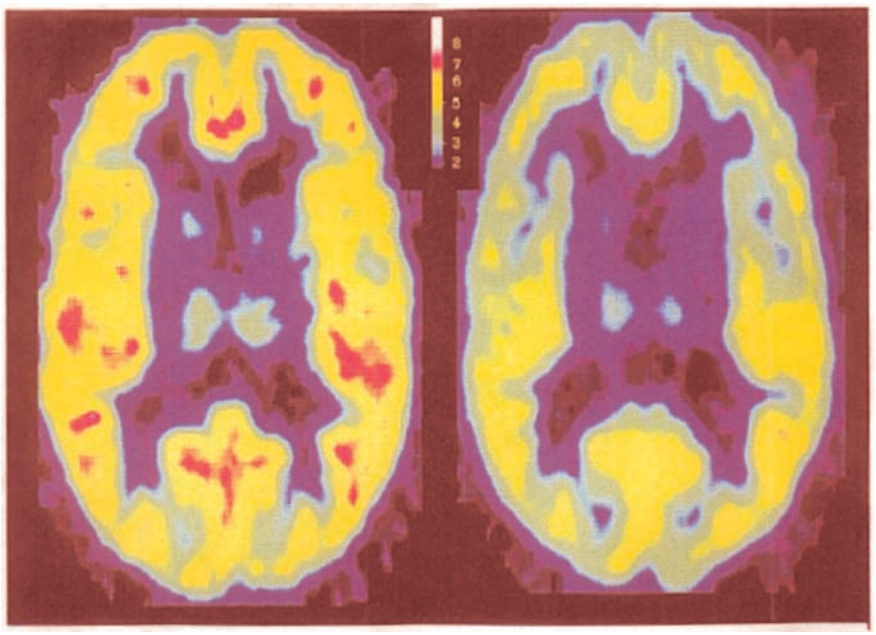

Fig. 4 Position emission tomography (PET) scans of the brains of patients with panic disorder (right) show a significant global reduction in binding to the benzodiazepine antagonist flumazenil compared with 'normal' brains (left).

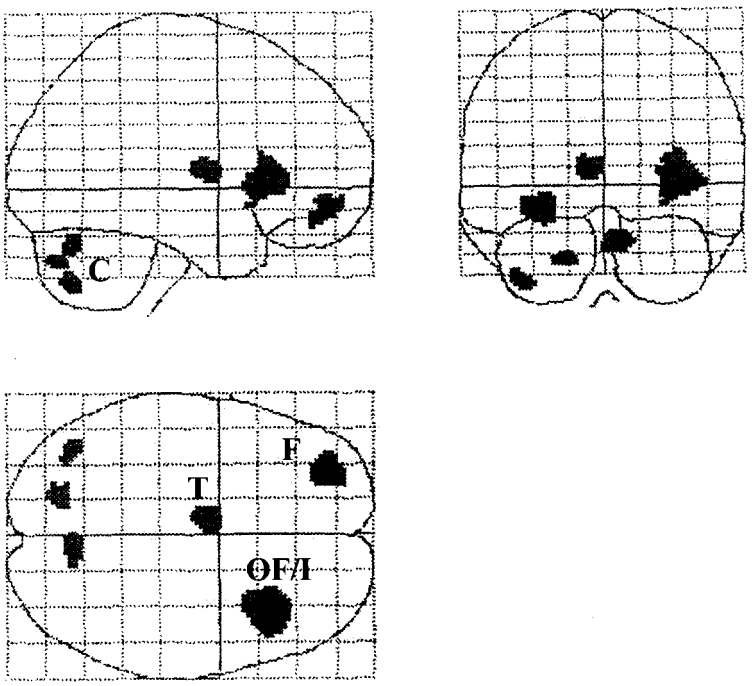

Fig. 5 Water positron emission tomography (PET) scan imaging shows that the anxiolytic effects of the benzodiazepines (in this case, midazolam) relate to significant modulation in brain metabolism in the insula/orbitofrontal cortex (OF/I), frontal cortex (F), thalamus (T) and cerebellum (C).

taken in drawing too many conclusions from these small studies, but they do strengthen the case that abnormalities in basal or adaptive inhibitory neuromodulation are of pathological significance in anxiety disorders.

ROLE OF GABA IN MEMORY AND ANXIETY

As well as its apparent involvement in anxiety, the central GABAergic system is also critically involved in cognitive processes, especially memory. Many human and animal studies show that benzodiazepine receptor agonists impair memory formation. Conversely drugs that decrease GABA function can have memory-enhancing properties. Since anxiety conditioning involves both the experience of anxiety and the formation of an association (a form of memory) it can be postulated that inhibition of GABAergic transmission is a powerful mechanism in predisposing an organism to increased and sometimes inappropriate anxiety reactivity. Conversely, increased GABAergic stimulation would be powerfully anxiolytic by inhibiting both the experience of anxiety and the aversive reinforcement.

There may be many clinical sequelae from this relationship. Traumatic memories, such as those which occur in post-traumatic stress disorder, are extremely deeply encoded, probably because of the high level of emotion and arousal at the time of the original insult as well as the negative reinforcement on re-experience of the events. Anxiety leads to rapid and profound learning of escape and avoidance behaviours, as in agoraphobia following a panic attack, or anxious avoidance after being bitten by a dog. Apart from animal studies, clinical evidence of reduced GABA in the genesis of anxiety comes from the use of pentylenetetrazol as a convulsant agent before electroconvulsive therapy was introduced in clinical practice. Pentylenetetrazol acts by blocking GABA$_A$ receptor function and has been reported to produce extreme anxiety, traumatic memories and extreme avoidance behaviour when used clinically (reviewed in Kalueff & Nutt, 1997). Recently, Goddard *et al* (2001) have reported decreased cortical GABA levels in patients with panic disorder using magnetic resonance spectroscopy.

INSIGHTS FROM MOLECULAR BIOLOGY

Each of the five sub-units of the GABA$_A$–benzodiazepine receptor complex is a different protein. These can be classified into families according to their structural similarities. The principal families have been coded the α, β and γ sub-units. GABA binds to the β sub-unit, whereas benzodiazepines and related drugs, for example, zopiclone, zolpidem and zaleplon, bind to a site on the α sub-unit (Doble, 1999).

At the last count, 19 subtypes coded by different genes had been found in

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mammals. Six different isoforms of the α sub-unit have so far been identified and different subtypes have different sensitivities to benzodiazepine receptor ligands. Receptors with the α_6 sub-unit, for example, are essentially insensitive to all hypnotic/anxiolytic benzodiazepine agonists. The type of γ sub-unit is also critical as only the γ_2 sub-unit gives $GABA_A$–benzodiazepine receptor which is responsive to benzodiazepines. The genes that encode for these various subtypes seem to occur in clusters, suggesting that there is some coherence in the expression of receptor complexes with different forms of the sub-units. The most important and most prevalent $GABA_A$–benzodiazepine receptor in the brain is made up from α1, β2 and γ2 sub-units, encoded by the same cluster of genes on chromosome 5.

IDENTIFYING THE ROLE OF RECEPTOR SUB-UNITS

Using 'knock-out' (gene deletion) and 'knock-in' (gene alteration) technologies in mice it is possible to produce $GABA_A$–benzodiazepine receptors that are deficient in various types of sub-unit. The first such experiments were carried out by Mohler and colleagues who knocked out the gene for the γ_2 sub-unit. Homozygous mice with both the γ_2 genes knocked out are not viable, but heterozygotes survive to adulthood and breed. However, their $GABA_A$–benzodiazepine receptors have half the usual complement of the γ_2 sub-unit and so are less sensitive to benzodiazepines. Moreover, these mice exhibit symptoms of hypervigilance and anxiety (see Fig. 6) and so represent a genetically defined model of trait anxiety that closely mimics pharmacological and behavioural features of human anxiety disorders (Crestani *et al*, 1999). Further, these mice show decreases in flumazenil binding throughout the brain that are similar to the decreases shown in humans with panic disorder (see 'Imaging studies in anxiety', above).

Knock-out mice with $GABA_A$–benzodiazepine receptors deficient in other types of sub-unit have also been produced. When the gene for the expression of the β_3 sub-unit is knocked out, for instance, it produces mice that are hyperactive, have poor motor coordination and have spontaneous seizures. They have an attenuated response to GABA and it would seem that the β_3 sub-unit has been replaced by a β_2 sub-unit. Interestingly, there is also reduced sensitivity to anaesthetic agents, including halothane, enflurane, etomidate and the benzodiazepine, midazolam, the first direct evidence that GABA–benzodiazepine receptors may be important in mediating the anaesthetic effects of these agents.

More recently, knock-in technology has been used to produce mice in which the α_1 subtype gene has been mutated to become insensitive to benzodiazepines but still responsive to GABA (knock-in mutations). In these mice the sedative actions of benzodiazepines are abolished but the anxiolytic, anticonvulsant and hypnotic ones remain (Rudolph *et al*, 1999; Tobler *et al*, 2001). Subsequent mutation of two remaining subtypes has shown that the anxiolytic effect is lost if the α_2 but not the α_3 subtype is mutated (Low *et al*, 2000). The localisation of the α_2 subtype in the limbic system supports the role of this circuit in anxiety. This growing evidence for the specificity of benzodiazepine actions being mediated via receptor subtypes is leading to the search for subtype-selective drugs such as α_2 or α_3 agonists as non-sedating anxiolytics and α_5 inverse agonists (which act mainly in the hippocampus) as memory enhancers (Lingford-Hughes *et al*, 2001).

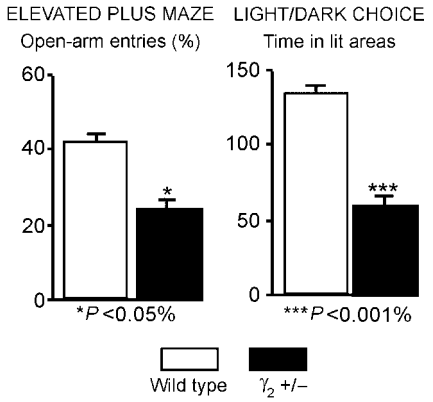

Fig. 6 Mice heterozygous for the γ_2 subtype of the γ sub-unit represent a genetically defined model of trait anxiety, closely reproducing the molecular, pharmacological and behavioural features of human anxiety disorders. The figure shows significant differences from control animals in two separate measures of anxiety. Such studies suggest that $GABA_A$–benzodiazepine receptor dysfunction could be a causative factor for a heightened harm-avoidance behaviour and a hypersensitivity to negative associations in patients. (Data from Crestani *et al*, 1999.)

CURRENT CLINICAL ISSUES

Because the benzodiazepines may cause dependence and withdrawal after chronic use (see Fig. 7) and owing to their potential for misuse, there are controls on their use in most countries. This control can result in a significant number of patients being denied a therapeutic option that could be appropriate and effective. Recently, there has been an effort to reassess the place of this class of drugs in clinical practice and provide a more balanced view of their relative advantages and disadvantages (Williams & McBride, 1998). A clearer understanding of the mechanisms underlying dependence will also enable us to develop treatment regimes

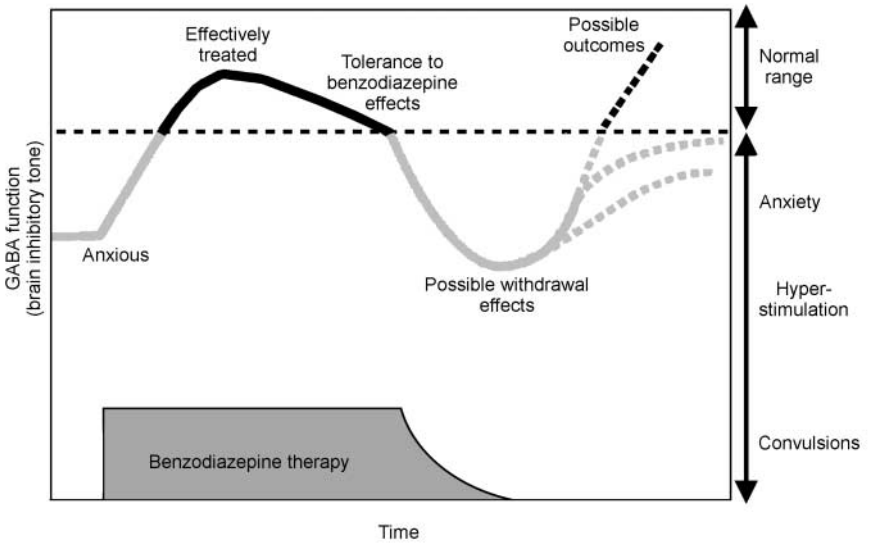

Fig. 7 Schematic diagram of changes in $GABA_A$ receptor function during benzodiazepine treatment and withdrawal.

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for using current drugs which will optimise benefits and minimise any unwanted effects. We may also be able to identify individuals who are more liable to develop dependence. As discussed above, there is evidence to suggest that the sensitivity of the GABA$_A$–benzodiazepine receptor to various ligands is not fixed and that the set-point – where drugs bind, but have no effect – can be moved (Fig. 3). A shift in the set-point of the receptor in the inverse agonist direction has been seen in studies in animals following chronic benzodiazepine use and could explain both tolerance to benzodiazepine effects and withdrawal symptoms such as rebound insomnia, anxiety and seizures when the treatment is stopped (Little *et al*, 1988).

WHY SHOULD RECEPTOR SENSITIVITY CHANGE?

One possibility is that there is substitution of one sub-unit subtype for another. In rats given benzodiazepines chronically, the common α_1 and γ_2 sub-units are down-regulated, while rarer sub-units are elevated proportionately (Holt *et al*, 1999). It is suggested that transcription of the gene cluster on chromosome 5 (which encodes for α_1 β_2 γ_2 sub-units) is inhibited on chronic benzodiazepine administration, while the transcription of the gene cluster on chromosome 15 is upregulated (Holt *et al*, 1999). In certain brain regions, the chromosome-5-encoded receptor sub-unit proteins are replaced by those encoded in chromosome 15, which show less sensitivity. When benzodiazepines are present this manifests as tolerance, but when they are stopped GABA function is effectively reduced, leading to withdrawal.

NEW THERAPEUTIC HORIZONS

Both neuroimaging and molecular biology are giving us fresh insights into the role of the GABA$_A$–benzodiazepine receptor in anxiety. The latest data confirm the importance of the GABAergic system in the pathogenesis of anxiety states and help explain the important role of drugs that bind to the GABA$_A$–benzodiazepine receptor in treating such disorders. Molecular biological and gene manipulation studies have given major clues to the mechanisms involved in tolerance, dependence and withdrawal. This research should ultimately enable us to develop regimens for using current drugs which will optimise their benefits and to find new drugs with enhanced anxiolytic effects and with fewer undesirable side-effects. It would also benefit patients with other conditions responsive to GABA$_A$–benzodiazepine ligands, such as seizures, spasticity and insomnia.

CLINICAL IMPLICATIONS

- The benzodiazepines work at specific receptor sites on the GABA$_A$ receptor complex in the mammalian brain, and subtypes of these receptors mediate different actions of these drugs.
- Abnormalities of these benzodiazepine receptors may underlie some anxiety disorders.
- Drugs targeted at specific receptor subtypes may offer the hope of anxiolytics without unwanted side-effects.

LIMITATIONS

- Imaging studies need replication and extension to other anxiety disorders.
- Mutations in benzodiazepine receptor genes have not yet been linked to human anxiety.
- The effects of new anxiolytics in mouse may not be replicated in humans.

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(First received 7 August 2000, final revision 26 January 2001, accepted 31 January 2001)

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https://doi.org/10.1192/bjp.179.5.390 Published online by Cambridge University Press