

The GABA-Benzodiazepine Receptor Complex: Structure, Function, and Role in Anxiety

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Benzodiazepines bind to a specific site on the γ -aminobutyric acid (GABA)-benzodiazepine receptor complex. This complex has been implicated in the pathophysiology of anxiety by numerous preclinical and clinical studies. Preclinical studies have shown that there are multiple molecular forms of this receptor complex, that these genetically coded variations are linked to specific actions of the benzodiazepines, and that receptors are located in neuroanatomical areas known to mediate the anxiety response in animals and humans. Human studies have shown that patients with pathologic anxiety have anomalous responses to drugs that specifically bind to these receptors and have reduced numbers of benzodiazepine receptors in key brain areas that regulate anxiety responses. More recent preclinical studies suggest that molecular alterations in this receptor complex may produce findings in animals similar to those observed in anxious humans. Finally, chronic treatment with benzodiazepines causes the development of tolerance, which may be associated with molecular changes and a pharmacologic response profile similar to that observed in pathologically anxious humans.

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One of the most ubiquitous neurotransmitters in the central nervous system (CNS) is γ -aminobutyric acid (GABA), which operates in more than a third of CNS synapses. As the major inhibitory neurotransmitter, GABA stands in dynamic balance with the excitatory neurotransmitter glutamate, and together these 2 systems modulate neuronal excitability. GABA operates through 3 major receptor subtypes: GABA-A and GABA-C, which mediate rapid effects via a ligand-gated chloride ion channel, and GABA-B, which mediates slower effects via G protein-coupled effects on calcium and potassium channels.¹ The GABA-benzodiazepine receptor, also known as the GABA-A receptor and the benzodiazepine receptor, is composed of a complex glycoprotein that also contains binding sites for the various benzodiazepine compounds whose potent anxiolytic effects have formed a “pharmacologic bridge” that points to this receptor as playing a potential role in the pathophysiology of anxiety and a clear-cut role in its treatment.²

This article will review 3 main areas related to the role of the GABA-benzodiazepine receptor complex in anxiety: how the molecular nature of this receptor complex may relate to disparate sedative, anxiolytic, and amnestic effects of the benzodiazepines; how animal and human studies identify functional and structural alterations of this complex associated with anxiety states; and how functional and structural changes in this complex that occur with chronic benzodiazepine treatment may relate to various problematic aspects of benzodiazepine tolerance in humans. It must be emphasized that the pathophysiology of anxiety is currently poorly understood and most certainly involves a cascade of neurotransmitters, neuropeptides, receptors, second messengers, other intracellular signaling mechanisms, immediate early genes, and longer-term changes in gene expression.³ Hence, the focus on this receptor complex is only meant to highlight its potential role as part of this intricate interplay of factors and events.

MOLECULAR NATURE OF THE GABA-BENZODIAZEPINE RECEPTOR COMPLEX

The GABA-benzodiazepine receptor complex is a pentameric structure composed of 5 distinct glycoprotein subunits that span a lipid bilayer and form a cylindrical structure whose center constitutes an ion channel.^{1,2,4} Activation of the GABA-benzodiazepine receptor causes an increase in the influx of chloride ions, which in turn results in the membrane hyperpolarization that is responsible for neuronal inhibition. Benzodiazepines do not independently activate this process but rather facilitate the action

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of GABA by increasing the frequency of ion channel opening.⁵ A number of other psychoactive compounds, including barbiturates, anesthetic steroids, and even alcohol, also act at this receptor complex in slightly different ways at different sites but have the same effect of enhancing neuronal inhibition.

A series of studies has established that there are several molecular families of subunits: α with 6 isoforms, β with 3 isoforms, γ with 3 isoforms, θ with 1 isoform, and ρ with 3 isoforms.⁶ The receptor complex is known to consist of 2 α subunits alternating with 2 β subunits and a single γ subunit.⁷ Each receptor complex has 2 GABA binding sites but only 1 benzodiazepine binding site. The GABA binding sites are located at the intersection of the 2 alternating α and β subunit pairs, while the single benzodiazepine binding site is located at the intersection of the 1 pairing of the α and γ subunits. This latter phenomenon is consistent with evidence that a γ subunit is required for benzodiazepine action, but not for GABA action, which can still occur with reengineered receptors that lack a γ subunit.⁴

Although the potential permutations and combinations of different subunit types seem extraordinarily large, most receptors seem to be composed of 1 of 4 α subunits (1, 2, 3, or 5), 2 β subunits (2 or 3), and 1 γ subunit.⁴ Thus a number of possible different subtypes are available, with each subtype potentially differing in its affinity for GABA, its chloride channel kinetics, and its affinity for different benzodiazepine ligands. Evidence suggests that a majority of these receptor complexes are actually composed of α_1 subunits in combination with β_2 and γ_2 subunits.⁴ The 2 α_1 -2 β_2 -1 γ_2 receptor subtype is thought to be responsible for the sedating properties of benzodiazepines,^{8,9} consistent with the location of this subtype in the thalamus, brainstem, and cerebellum and consistent with evidence that the commonly used hypnotic zolpidem has a particularly high affinity for receptors with the α_1 isoform. Interestingly, all 3 of these subunits are coded for on a single chromosome, chromosome 5. In contrast, receptors containing isoforms α_2 , α_3 , and α_5 are more heavily concentrated in subcortical and limbic areas and thought to be responsible for other pharmacodynamic actions of benzodiazepines. A recent study¹⁰ nicely documented the importance of the α_2 isoform for anxiolytic effects. In 2 different rat models of anxiety, this study successively eliminated the α_2 , α_3 , or α_5 subunit from the receptor complex and showed that the anxiolytic effect of diazepam was preserved despite the absence of α_3 and α_5 but disappeared when the α_2 subunit was eliminated. Finally, receptors containing either isoforms α_4 or α_6 have much lower affinity for benzodiazepines and are insensitive to their effects.

The considerable variety of receptor subtypes provides a molecular substrate for altered functioning of the GABA-benzodiazepine receptor complex. Such alterations could occur both naturally, possibly underlying the proclivity to anxiety that is clearly present in a proportion of individuals

and also seems to have genetic underpinnings, and in response to environmental change and administration of benzodiazepine medications.

THE GABA-BENZODIAZEPINE RECEPTOR COMPLEX IN ANXIETY

Neuroanatomic Sites of Action and the Neuroanatomy of Anxiety

GABA-benzodiazepine receptors are widely distributed throughout the CNS, with particular concentration in the spinal cord, brainstem, thalamus, cerebellum, subcortical areas including hippocampus and amygdala, and cortex. Studies^{3,11} of the neurocircuitry of anxiety have implicated in particular the amygdala, hippocampus, and medial prefrontal cortical areas as important way stations subserving anxiety signaling. GABA-benzodiazepine receptors are specifically concentrated in these areas and quite likely play an important role in modulating anxiety.¹² In the amygdala, GABAergic circuits modulate key excitatory circuits subserved by glutamate as well as corticotropin-releasing factor, and it is likely that some of the immediate anxiolytic effects of benzodiazepines occur at this site.¹³

Effects of GABA-Benzodiazepine Receptor Agonists and Antagonists in Patients With Anxiety Disorders

A series of studies have experimentally administered doses of benzodiazepine agonists as well as doses of the normally inert benzodiazepine antagonist flumazenil in an attempt to determine if effects in patients with anxiety disorders differed from those in normal volunteers, which might indicate that the GABA-benzodiazepine receptor complex is functioning differently in anxious patients. My group¹⁴ administered 4 logarithmically increasing doses of intravenous diazepam or placebo on alternate days to 2 groups of subjects: control subjects and medication-free and largely benzodiazepine-naïve patients with panic disorder. Effects of diazepam at each of the 4 time points on self-rated sedation, memory, and saccadic eye movement velocity (SEV) were compared. SEV was selected as a principal pharmacodynamic measure because this measure was not affected by anxiety, so that anxious patients would have both normal SEV at baseline and no alteration in SEV as a result of the reduced anxiety that might be produced by diazepam.¹⁵ Our study¹⁴ showed that panic disorder patients had reduced effects for all pharmacodynamic measures, and these effects were not due to differences in diazepam blood levels achieved during the infusion. This finding suggested that panic disorder was possibly associated with subsensitive GABA-benzodiazepine receptors. A smaller pilot study¹⁶ found similar results in individuals with generalized anxiety disorder, and this finding was subsequently replicated in panic disorder patients and extended to patients with

obsessive-compulsive disorder.¹⁷ These findings, along with 2 separate studies showing that reduced benzodiazepine sensitivity is associated with the anxiety-associated personality traits of harm avoidance¹⁸ and neuroticism,¹⁹ suggest that agonist subsensitivity is diagnostically non-specific and quite likely related to a common state of being pathologically or abnormally anxious.

At the same time this study from my group¹⁴ was published, a similarly designed study²⁰ was performed in panic disorder and control patients, but flumazenil was administered rather than diazepam. Although flumazenil had the expected absence of pharmacodynamic effect in normal volunteers, it unexpectedly provoked panic attacks in panic disorder patients. This anxiogenic effect was replicated at 1 but not both doses of flumazenil by a second study,²¹ although 2 subsequent studies^{22,23} in panic disorder patients, using different designs, did not replicate this effect. Because the major effect of note is a subjectively reported anxiety state, without a more objective correlate other than the expected cardiovascular concomitants of anxiety, differences in design and subject psychological set are likely to contribute to self-report and possibly confound some attempts at replication. Furthermore, a small pilot study²⁴ even showed that flumazenil had an anxiolytic effect in normal volunteers subjected to a stressful simulation of public speaking. Nonetheless, findings that anxious patients have reduced effects when given benzodiazepine agonists, along with the presence in some studies of unanticipated anxiogenic effects when given normally inert benzodiazepine antagonists, prompted a novel theory of how receptor functioning in anxious patients might be altered.

Theory of Altered GABA-Benzodiazepine Receptor Set Point

Publication of the original flumazenil study by Nutt et al.²⁰ was accompanied by a theoretical interpretation that the normal spectrum of GABA-benzodiazepine receptor ligand activity could be altered in states of pathologic anxiety. There are a spectrum of ligands for this receptor that normally have full agonist, partial agonist, neutral antagonist, partial inverse agonist, and full inverse agonist activities. Inverse agonists would dampen rather than potentiate GABAergic activity at the GABA-benzodiazepine receptor. Hosts of animal studies²⁵ have documented that these agents have potent anxiogenic effects, and a study²⁶ infused 5 German volunteers with the experimental inverse agonist FG-1742 and observed severe induction of anxiety and panic consistent with this theory. Given that this is the spectrum of ligand activity in nonanxious people, it can be hypothesized that in anxious patients the spectrum of activity is shifted so that agonists act like partial agonists, partial agonists would not be active (this has not been tested), antagonists that are normally inert would act like partial inverse agonists, and so on.

Although this theory nicely explains the aforementioned findings,¹⁴⁻²⁶ the reason for this receptor spectrum shift is harder to pinpoint. Nutt et al. originally hypothesized that this effect could be due to 1 of 3 phenomena associated with anxiety: a deficiency in a naturally occurring benzodiazepine agonist, an excess of a naturally occurring benzodiazepine inverse agonist, or a naturally occurring alteration in the protein conformation of the receptor,²⁰ perhaps mediated by changes in genetic expression of different subunit isoforms in response to environmental or developmental events.²⁷ There are limited data confirming the presence of a naturally occurring benzodiazepine compound, although endogenous benzodiazepine-like substances have been isolated from the human brain²⁸ as well as in states of hepatic encephalopathy.²⁹ Of course, neurosteroids, variously derived from progesterone, exist in the brain; some clearly have positive allosteric modulating properties at the receptor, i.e., agonist-like effects, and others have inverse agonist-like properties.³⁰ Unfortunately, the 1 study that has examined neurosteroids in anxious patients showed that patients with panic disorder had higher levels of key agonist-like neurosteroids,³¹ which runs counter to this hypothesis. Although early studies isolated a substance called tribulin that was thought to be anxiogenic from the urine of anxious patients,³² the structure of tribulin has yet to be determined. Early studies also had isolated an inverse-agonist compound called diazepam binding inhibitor from brain³³ as well as other peripheral tissues. Unfortunately, altered levels of this compound were never found to be consistently associated with pathologic anxiety states, and the compound was subsequently found to be more closely linked with the mitochondrial GABA-benzodiazepine receptor. Up to this time, no other inverse-agonist candidates have been identified.²⁷ Because the data after 2 decades still do not support an anxiety-associated alteration in the levels or amounts of endogenous agonist-like or inverse agonist-like substances, the third hypothesis by Nutt et al.²⁰ about a basic alteration at the receptor level mediated by changes in composition of the receptor provides perhaps the best potential explanation for the receptor spectrum shift in anxious patients.

Neuroimaging Findings in Patients With Anxiety Disorders

The implications derived from the aforementioned pharmacologic challenge studies¹⁴⁻²⁴ have been more directly confirmed by a series of neuroimaging studies. Nutt's group in England followed up their original work with a C11 flumazenil positron emission tomography study in unmedicated panic disorder patients.³⁴ This study showed that, compared with controls, there was a general reduction in benzodiazepine binding measured with the flumazenil ligand, with the largest decreases in right orbitofrontal cortex and insula, 2 areas repeatedly impli-

cated in the anatomic circuitry that mediates anxiety responses. This finding has been replicated by another group using iomazenil and single photon emission computed tomography methodology in panic disorder patients, with decreases found in the hippocampus and precuneus and particular reductions in the prefrontal cortex in subjects who were having a panic attack.³⁵ Another study³⁶ has shown similar reductions in benzodiazepine binding in the left temporal cortex in patients with generalized anxiety disorder, while a third study³⁷ has shown reductions in benzodiazepine binding in patients with posttraumatic stress disorder (PTSD), also in the prefrontal cortex. Finally, using magnetic resonance spectroscopy, a recent study³⁸ demonstrated reduction in GABA levels in occipital cortex. The potential relationship of these decreased GABA levels to GABA-benzodiazepine receptor complex functioning is not known.

Potential Molecular Substrates for Pathologic Anxiety States

The findings reviewed in this section indicate that human anxiety appears to be associated with reduced density of GABA-benzodiazepine receptors, reduced amounts of cortical GABA, reduced functioning of this receptor complex manifested as reduced sensitivity to benzodiazepines, and perhaps a “shift” in the activity of ligands along the agonist-antagonist-inverse agonist spectrum. Crestani et al.³⁹ developed an interesting mouse model of anxiety that employed a γ subunit “knockout.” Because homozygotes without a γ subunit would not be viable, heterozygotes were used, with these mice having only half the usual complement of γ_2 subunits for their GABA-benzodiazepine receptor complex. These mice were found to have reductions in brain GABA-benzodiazepine receptor binding, reduced sensitivity to benzodiazepines, increased anxiety in the elevated plus maze, increased trace aversive conditioning (i.e., more easily conditioned to noxious stimuli), and a reversal of maze anxiety behavior with diazepam. This model suggests not that alterations in γ subunit pharmacology underlie human anxiety, but rather that some kind of molecular alteration in the subunits or more probably isoforms of subunits for the receptor complex may well underlie the findings reviewed here and contribute to the generation of pathologic anxiety states.

An interesting finding has recently been published linking the 5-HT_{1A} knockout mouse model of anxiety with GABA-benzodiazepine receptor alterations. Mice lacking the 5-HT_{1A} receptor display marked anxiety in a variety of experimental paradigms.⁴⁰ This study⁴⁰ showed that mice lacking the 5-HT_{1A} receptor have reduced sensitivity to diazepam, reduced GABA-benzodiazepine receptor binding in the amygdala, and reduced expression of the α_1 and α_2 subunits in the amygdala. Thus the findings reviewed here, rather than being primary, could be secondary to alterations in another transmitter system.

An extensive body of literature has implicated stressful early life experiences in the development of anxiety in both animal models and human developmental psychopathology. Caldji et al.⁴¹ measured expression of γ_2 subunit messenger RNA (mRNA) in the amygdala of anxious mice as a function of genetic strain and child rearing experience. These anxious mice had the full complement of DNA, unlike the knockout mice in the Crestani et al. study,³⁹ but only expressed half as much γ_2 mRNA in their amygdala as normal mice. In this study, the authors then used a cross-fostering paradigm that allowed anxious mice to be raised by normal mothers and normal mice to be raised by anxious mothers. The study found that γ_2 benzodiazepine subunit mRNA expression could be normalized when anxious mice were raised by normal mothers, which was accompanied by a reduction in anxiety as well. In contrast, although subunit expression was correspondingly lowered in normal mice raised by anxious mothers, these mice continued to be behaviorally normal (i.e., these reductions were not accompanied by the expected increase in fear level). This finding is consistent with evidence that learning and reconditioning can be helpful in ameliorating certain anxiety states and demonstrates a potential molecular mechanism for this. Failure to develop anxiety despite lowering of message expression in the amygdala in mice raised by anxious mothers suggests that additional mechanisms besides GABA-benzodiazepine receptor alterations contribute to the pathophysiology of anxiety (see the 5-HT_{1A} knockout mice study⁴⁰ reviewed earlier).

Finally, similar to the SEV findings implicating reduced agonist sensitivity,¹⁴ Iwata et al.⁴² showed that a genetic substitution of the GABA-benzodiazepine receptor α_6 subunit was associated with differential sensitivity to diazepam effects on smooth pursuit eye movement accuracy or gain in children of alcoholics at risk for alcoholism. This finding documents that in humans, changes in receptor subunit composition may be associated with changes in pharmacodynamic response to receptor agonists. Although this study involved smooth pursuit gain—an eye movement task that was not measured in the original panic disorder study¹⁴—and because the α_6 form of receptor is mostly expressed in the cerebellum, which mediates effects of smooth pursuit eye movements, the results of Iwata et al.⁴² are quite consistent with the known anatomy of eye movements and receptor subunit distribution as well as with the known pharmacology of benzodiazepine agonists.

Although these findings all suggest that GABA-benzodiazepine receptor subunit composition could be altered in states of pathologic anxiety and possibly serve as a mediating, if not causal, mechanism, this link has not yet been established. One interesting study in patients with PTSD found an association of this condition with a heterozygous state of the alleles for the β_3 subunit of the GABA-benzodiazepine receptor.⁴³ Unfortunately, this

association was both relatively weak and not consistent with the previously reviewed preclinical data, which have focused mostly on alterations in α and γ subunits.

THE GABA-BENZODIAZEPINE COMPLEX: CHANGES WITH CHRONIC TREATMENT

Perhaps the most controversial aspect of treatment with benzodiazepines has been problems of physiologic dependence and withdrawal, which have fueled misrepresentations of these drugs as addictive and spawned numerous anecdotes of patients with anxiety disorders deteriorating clinically after being prescribed these medications.⁴⁴ The case presented in the article by Stewart⁴⁵ in this supplement is such an example: an individual without a clear-cut history of substance abuse seems to develop escalating tolerance to the effects of these medications, requiring higher and/or more frequent doses, which result, not in improvement, but in further worsening. What is known about the molecular mechanisms of tolerance? Do anxious patients manifest a uniquely different way of developing tolerance from controls without anxiety? Because the latter group of patients would not be likely to use these medications chronically, this question is difficult to address definitively from an empirical perspective, although I will try to shed some light on this. The only study that has actually documented the response of normal volunteers to benzodiazepine withdrawal did not highlight, but rather buried in a single sentence, the surprising and important finding that after 6 weeks of diazepam treatment, only 1 in 20 women experienced a transient withdrawal syndrome.⁴⁶

Holt et al.⁴⁷ have performed an interesting study examining how chronic benzodiazepine treatment differentially alters GABA-benzodiazepine receptor subunit expression. By including a novel partial agonist, abecarnil, as well as the full agonist diazepam, they also facilitated interpretation of their results using the receptor spectrum shift hypothesis by Nutt et al.²⁰ In their study, Holt et al.⁴⁷ focused on 3 subtypes of receptors, distinguished by their α subunit isoform (isoforms 1, 2, and 5). After chronic treatment with diazepam, the mRNA expression of receptor subtypes with α_1 isoform was reduced, while expression of receptor subtypes with α_2 and α_5 isoforms was increased. Interestingly, after chronic abecarnil treatment, while α_1 isoform receptors were reduced, there were no increases in the other 2 receptor subtypes. Since animal studies⁴⁸ show that chronic abecarnil treatment is associated with markedly reduced tolerance and withdrawal reactions in animals, the Holt et al.⁴⁷ findings suggest that these 2 receptor subtypes might underlie differences in tolerance and withdrawal traditionally thought to be associated with partial benzodiazepine agonists. Another study⁴⁹ used a rat cell culture preparation and found that exposure to 5 days of diazepam reduced expression of α_1 and γ_2 subunit proteins, but that actual withdrawal was additionally associ-

ated with an increase in the α_4 subunit. This study suggests the possibility that the neurobiological mechanisms responsible for withdrawal are not merely the accumulated mirror image of those associated with tolerance, although there is reason to believe that many of the mechanisms associated with both are similar.

Although differences in GABA-benzodiazepine receptor subunit composition and expression most likely contribute to differences in tolerance, other studies have suggested that changes in the glutamate system are also important in the development of benzodiazepine tolerance. Allison and Pratt⁵⁰ showed that changes in α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor subunit mRNA in multiple brain regions are potently involved in withdrawal reactions. This is of course consistent with the dynamic balance between GABAergic inhibitory and glutamatergic excitatory systems that was briefly alluded to in the introduction.

In humans, tolerance to benzodiazepines is associated with reduced sensitivity to agonist administration and, as such, appears to be an exaggeration of the small reductions in agonist sensitivity demonstrated by my group a decade ago.¹⁴⁻¹⁸ Using the same SEV paradigm, we⁵¹ tested panic disorder patients who were being treated chronically with alprazolam and compared the effects observed in them following intravenous diazepam with those observed in the benzodiazepine-naive panic patients. Interestingly, there was a much wider distribution of sensitivity values, with some treated patients having sensitivities similar to benzodiazepine-naive panic patients, while others had markedly reduced sensitivity. These 2 subgroups appeared to be distinguished not by the characteristics of their treatment (i.e., higher doses of benzodiazepine or longer duration of treatment), as one might expect, but rather by characteristics of their illness. The most insensitive (i.e., tolerant) patients were the most symptomatic, with more frequent panic attacks and more severe phobic avoidance. This finding suggests that some patients might be more prone to develop tolerance than others and that these patients will respond poorly to these medications. However, the doses received by all these patients, who were taking average alprazolam doses of 2 mg/day with a range of 1 to 4 mg/day, were still much lower than doses recommended by some experts. Hence, these patients might require higher doses and continue to exhibit symptoms when they do not receive the appropriate dose. Nonetheless, this finding still begs the question: why does the GABA-benzodiazepine receptor complex of some patients function in such a manner that they require much higher doses of these medications?

CONCLUSION

An emerging body of evidence suggests that the GABA-benzodiazepine receptor system plays an impor-

tant role in the modulation and mediation of pathologic anxiety states. The plasticity and multiple forms of this receptor may well underlie dimensional variations in proclivity to anxiety in humans as well as play a role in anxious states that develop either as a result of early life stress or as a consequence of severe stress in later years. Treatment of anxiety with benzodiazepines is highly effective, although controversy still persists about the appropriate role of long-term treatment with these agents and whether problems with tolerance and dependence relate to underlying alterations in the receptor complex that either preceded chronic benzodiazepine treatment or developed as a consequence of it.

Drug names: alprazolam (Xanax and others), diazepam (Valium and others), flumazenil (Romazicon and others), zolpidem (Ambien).

Disclosure of off-label usage: The author has determined that, to the best of his knowledge, no investigational information about pharmaceutical agents has been presented in this article that is outside U.S. Food and Drug Administration–approved labeling.

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