

MECHANISM OF ACTION OF ANXIOLYTICS

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Drugs to reduce anxiety have been used by human beings for thousands of years. One of the first anxiolytics and one that continues to be used by humans is ethanol. A detailed description of ethanol's action may be found in Chapter 100. A number of other drugs including the barbiturates and the carbamates (meprobamate) were used in the first half of the 20th century and some continue to be used today. This chapter focuses on current drugs that are used for the treatment of anxiety and approaches that are currently under investigation.

CORTICOTROPIN-RELEASING FACTOR (CRF)

Corticotropin-releasing factor (CRF) is a 41 amino acid peptide that plays an important role in mediating the body's physiologic and behavioral responses to stress (1). Figure 68.1 illustrates that this role of CRF may be mediated by multiple sites of action. As a secretagogue, CRF stimulates the release of adrenocorticotrophic hormone (ACTH) from the pituitary. In addition, CRF plays a neurotransmitter or neuromodulatory role through neurons and receptors distributed in diverse brain regions (2). CRF neurons, localized in the hypothalamic periventricular nucleus, are a major mediator of stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, whereas pathways innervating limbic and cortical areas are thought to mediate the behavioral effects of CRF.

There is a large body of both preclinical and clinical literature implicating a key role of CRF in affective disorders such as anxiety and depression. A significant clinical literature suggests that dysfunctions of CRF in its role as a hormone in the HPA axis or as a neurotransmitter in the brain may contribute to the etiology of a variety of psychiatric

conditions, including anxiety and depression (3). The link between CRF and depression is particularly strong, as numerous clinical studies have demonstrated that depressed patients show elevated cerebrospinal fluid (CSF) levels of CRF, elevated plasma cortisol, and a blunted ACTH response following intravenous CRF. Successful antidepressant treatment was shown to have a normalizing effect on CRF levels. A role of CRF in anxiety disorders has also been postulated, though the clinical evidence is not as strong as it is for depression (4).

Preclinical studies have demonstrated that CRF administered exogenously into the central nervous system (CNS) can produce behaviors indicative of anxiety and depression, for example, heightened startle responses, anxiogenic behaviors on the elevated plus maze, decreased food consumption, and altered sleep patterns. The anxiogenic effects of CRF are not blocked by adrenalectomy, suggesting that they are centrally mediated effects occurring independently of the HPA axis (5). Other studies strengthening the link between CRF and anxiety include recent work by Kalin et al. (6) demonstrating that a "fearful" phenotype in monkeys is associated with increased pituitary-adrenal activity and increased brain CRF levels. Other studies have shown that exposure to early postnatal separation stress in rat pups results in elevated levels of CRF messenger RNA (mRNA) in brain regions including the paraventricular nucleus (PVN) and the central nucleus of the amygdala (7,8).

Molecular Mechanism of Action

A substantial scientific effort has been directed toward characterizing the molecular biology of CRF pathways (9). Perin and Vale (9) first isolated CRF and identified it as a secretagogue for ACTH in primary cultures of rat pituitary cells. CRF activity is shared by two nonmammalian peptides, sauvagine and urotensin I, which share a 50% homology with CRF, and by a new mammalian peptide, urocortin, which has a 45% sequence homology.

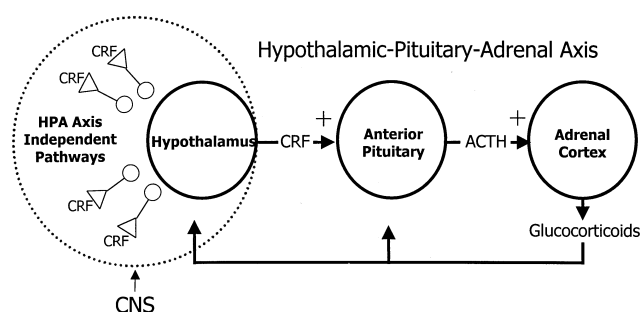


FIGURE 68.1. The role of corticotropin-releasing factor.

CRF acts through two G_s -protein coupled receptors, the CRF-1 and CRF-2 receptor subtypes (9,10). CRF-1 receptors show homology to a number of other neuropeptide receptors, including vasointestinal peptide (VIP) and calcitonin. Three splice variants of the CRF-2 receptor subtype, the CRF-2 α , CRF-2 β , and CRF-2 γ , and two splice variants of the CRF-1 receptor, have been identified (9). Molecular characterization studies have demonstrated that there is approximately a 70% sequence homology between CRF-1 and CRF-2 receptor subtypes. Cloning of the human *CRF-2a* gene revealed that it is 94% identical to the rat CRF-2 α receptor and 70% identical to the human CRF-1 receptor. There is currently no evidence of the existence of the CRF-2 β receptor in humans.

CRF-1 and CRF-2 receptors have different pharmacology and different localizations in the brain and periphery. *In situ* hybridization and receptor autoradiography techniques have been used to map the relative distributions of CRF-1 and CRF-2 receptors in the rat brain (11,12). High expression of CRF-1 receptors was seen in the pituitary, and in a number of brain regions including the PVN of the hypothalamus, cerebral cortex, olfactory bulb, cerebellar cortex, and basolateral and medial amygdala. In contrast, high densities of CRF-2 are found in more circumscribed regions, including the lateral septum, ventromedial nucleus of the thalamus, and choroid plexus. Moderate densities of CRF-2 receptors were reported for the medial amygdala and dorsal raphe nucleus. Further characterization has indicated that the CRF-2 α splice variant accounts for the brain localization of CRF-2 receptors, whereas the CRF-2 β accounts for choroid plexus. Urocortin, rather than CRF, most closely maps to CRF-2 receptors, leading to the suggestion that it may be the endogenous ligand for CRF-2 receptors. Recent work has shown that the distribution of CRF-2 receptors may differ significantly in the nonhuman primate brain relative to the rodent brain such that the CRF-2 subtype may play a more significant role than previously thought (13).

CRF receptors utilize 3',5'-cyclic adenosine monophosphate (cAMP) as a second messenger in the pituitary and brain and can be regulated by chronic activation. Thus,

desensitization following exposure to CRF has been demonstrated both *in vitro* (14) and *in vivo* (15). Furthermore, chronic stress can down-regulate CRF receptors and decrease CRF-stimulated cAMP production in multiple brain areas (16,17). Down-regulation of pituitary CRF receptors following adrenalectomy presumably results from decreased ACTH mediated inhibitory feedback, which produces excess CRF stimulation.

There are a number of pharmacologic agents available for dissecting the functional significance of CRF-1 and CRF-2 receptors. Much work has been carried out using the peptide antagonists α -helical-CRF (9–41) and D-Phe CRF (12–41). However, these compounds have shortcomings in that they do not penetrate the CNS and therefore have to be administered intracerebrally. Furthermore, they do not discriminate between CRF receptor subtypes and therefore do not allow a determination of their relative contributions to behavior. More recently, the development of selective, nonpeptidic antagonists of the CRF-1 receptor such as CP 154,526 (18) have provided important pharmacologic tools for the analysis of CRF-1 receptor function. Mutation studies have demonstrated that peptide and nonpeptide antagonists bind to different domains of the CRF-1 receptor (9). To date, selective CRF-2 antagonists have not been described, though recently, nonpeptide dual antagonists of the CRF-1 and CRF-2 receptors have been described (19).

In addition to CRF receptor subtypes, another potential target for pharmacologic manipulation of CRF is the CRF binding protein (CRF-BP), a 322 amino acid peptide. CRF-BP is a specific carrier for CRF and related peptides found in human plasma and brain. CRF-BP is thought to be a modulator of CRF activity. The CRF-BP is found in high densities in the rat amygdala, cortex, and bed nucleus of the stria terminalis.

Genetically Altered Mouse Models

Studies utilizing transgenic and knockout mouse models have provided important information with regard to the contribution of CRF and CRF receptor subtypes to processes including energy balance, emotionality, cognition, and drug dependence (20). This chapter focuses on the evidence implicating CRF and CRF receptors in anxiety states.

Overexpression of CRF in transgenic mice produced anxiogenic effects using either the black-white box test (21) or the elevated plus maze (22). The latter effect was reversed by central administration of the CRF receptor antagonist α -helical CRF, but not by adrenalectomy, supporting the role of central CRF pathways independent of the HPA axis (22). Studies using antisense directed against CRF in rats have produced evidence of anxiolytic activity (23). Finally, overexpression of CRF-BP is anxiolytic, whereas binding protein knockout mice (in which free CRF levels are elevated) display an anxiogenic phenotype in the elevated plus

maze (24). These data generally support the link between CRF and anxiety.

More recently, several studies have highlighted the importance of the CRF-1 receptor subtype in anxiety. CRF-1 knockout mice demonstrated a diminished anxiogenic response on the elevated plus maze and decreased ACTH and corticosterone responses to restraint stress (25). Similar findings were reported by Timpl et al. (26), using the black-white box anxiety paradigm. Furthermore, inactivation of the CRF-1 receptor with an antisense oligonucleotide was shown to reduce the anxiogenic effect of intraventricularly administered CRF (23). Liebsch et al. (27) provided evidence of anatomic localization by showing anxiolytic activity from CRF-1 antisense that was chronically infused into the central nucleus of the amygdala, an area of the limbic system shown by Michael Davis, Joe LeDoux, and others to be important in mediating fear and anxiety processes. Finally, CRF-2 knockout mice show anxiety-like behavior and are hypersensitive to stress (28), indicating that the CRF-2 receptor has an opposite functional role to that of the CRF-1 receptor. Thus, it could be argued that CRF-2 agonists, rather than antagonists, might be potentially useful as anxiolytic agents.

Another potential use for CRF antagonists is in the treatment of drug abuse. Several lines of evidence suggest that during the period of withdrawal from drugs of abuse such as ethanol, morphine, and cocaine, there is an activation of central CRF pathways. Anxiety is among the many physical symptoms of drug withdrawal, and given the link that has been made between CRF and anxiety, it is not surprising that CRF-1 receptor knockout mice demonstrated decreased anxiety responses during withdrawal from alcohol (26).

Current Drugs in Development

A number of nonpeptidic, small-molecule compounds that show high selectivity for the CRF-1 receptor have been proposed for the treatment of depression, anxiety, and stress disorders (29–31). These include CP-154,526 (Pfizer), and a methylated analogue, antalarmin (Pfizer); SC 241 (DuPont); NBI 30775 (aka R-121919; Janssen-Neurocrine); and CRA 1000 and CRA 1001 (Taisho Pharmaceuticals). An extensive preclinical literature has investigated potential anxiolytic effects of these compounds. Studies using CP-154,526 have demonstrated anxiolytic-like effects in some (32–34) but not all (33,34) preclinical anxiolytic paradigms evaluated. Griebel et al. (33) proposed that high-stress conditions may be required to demonstrate efficacy of CRF-1 receptor antagonists. CP-154,526 produces an anxiolytic effect in the separation-induced vocalization assay (35), an animal model of anxiety in which a preweaning rat pup separated from its litter emits a series of ultrasonic vocalizations that can be dose-dependently suppressed by either benzodiazepine or nonbenzodiazepine anxiolytics (36).

Of the compounds listed above, the compound discovered by Neurocrine Biosciences and licensed by Janssen Pharmaceuticals, R-121919, has proceeded the furthest in clinical evaluation. A recent report (37) describes results from a phase II, open-label, dose-escalating trial in which 20 severely depressed (Hamilton Depression Score >25) patients were administered R-121919 in one of two dose ranges: 5 to 40 mg, or 40 to 80 mg. In the low-dose group, 50% of the patients responded positively to treatment as indicated by a reduction in the Hamilton Depression Score of at least 50%, and 20% were remitters (score ≤8). In the middle-dose group, 80% responders and 60% remitters were reported. In addition, no significant untoward side effects were reported, and basal or stress-induced levels of ACTH or cortisol were unaffected, suggesting that chronic blockade of the HPA axis might not necessarily produce untoward side effects. Although these preliminary data are promising, it is important to bear in mind that they were gathered using an open label design without placebo control. Firm conclusions regarding the efficacy and safety of CRF-1 antagonists in depression and anxiety will require more rigorous double-blind, placebo-controlled trials. R-121919 is no longer under development because of reported elevations in liver enzymes; however, Neurocrine Biosciences has announced that further candidates are being pursued for clinical evaluation.

As mentioned previously, selective CRF-2 antagonists have not been described, though recently, nonpeptide dual antagonists of CRF-1 and CRF-2 receptors have been described (19). As there is contradictory evidence regarding the role of CRF-2 receptors in mediating anxiety, careful preclinical and clinical evaluation of these compounds will be needed to validate the contribution of CRF-2 receptors.

Future Drugs and Directions

As indicated above, a number of drug companies have dedicated significant efforts to identifying potent and selective CRF-1 receptor antagonists suitable for clinical development. To date, no compounds have completed phase II evaluation. Clearly, a challenge for the future will be to achieve this milestone and, in the process, validate with carefully executed clinical trials the concept that CRF-1 receptor antagonists are novel anxiolytics and/or antidepressants. Also, given increasing evidence of the importance of CRF-2 receptors in the human brain, significant efforts should be dedicated to evaluating this target for anxiety. It will be of interest to determine if agonists, rather than antagonists, of the CRF-2 receptor have anxiolytic profiles. Finally, the prospect of identifying additional CRF receptor subtypes, as well as other receptors for peptides, such as VIP, that are involved in the regulation of stress, provides fertile ground for future investigations.

GABA_A RECEPTOR MODULATORS (BENZODIAZEPINES AND RELATED DRUGS)

A majority of the synapses in the mammalian CNS use the amino acids l-glutamic acid, glycine, or γ -aminobutyric acid (GABA) for signaling. GABA is formed by the decarboxylation of l-glutamate, stored in neurons, and released, and its action is terminated by reuptake; GABA's action mimics the naturally occurring inhibitory transmission in the mammalian nervous system. Because of these findings, it has been accepted for over 20 years that GABA fulfills the characteristics of a neurotransmitter (38). Along with l-glutamate, acetylcholine, and serotonin, GABA possesses two different types of receptor conserved across different species and phyla that control both excitation and inhibition. Molecular biological studies of the receptors causing these effects have indicated that GABA's effects on ionic transmission (ionotropic) and metabolism (metabotropic) are mediated by proteins in two different superfamilies. The first superfamily (GABA_A receptors) is a set of ligand-gated ion channels (ligand-gated superfamily) that convey GABA's effects on fast synaptic transmission (39). When a GABA_A receptor is activated, an ion channel is opened (gated) and this allows chloride to enter the cell; the usual result of chloride entry is a slowing of neuronal activity through hyperpolarization of the cell membrane potential. The second superfamily (GABA_B) is slower, mediating GABA's action on intracellular effectors through a seven transmembrane spanning receptor (serpentine superfamily) that modulates the action of certain guanine nucleotide binding proteins (G proteins) (40). Through their activity on other effector systems, G proteins can change second messenger levels, altering signal transduction and gene expression, or open ion channels that are dependent on the G-protein subunit activities (41). Both excitatory and inhibitory activities are possible on a time scale that is longer than GABA_A receptor mediated events. There is extensive heterogeneity in the structure of the GABA_A receptor members of the ligand-gated superfamily. These receptors are the targets of a number of widely used and prescribed drugs for sleep, anxiety, seizure disorders, and cognitive enhancement; they may also contribute to mediating the effects of ethanol on the body.

Structure and Molecular Pharmacology of GABA_A Receptors

It is well established that the GABA_A receptors possess binding sites for the neurotransmitter GABA, as well as allosteric modulatory sites for benzodiazepines, barbiturates, neurosteroids, anesthetics, and convulsants (42–44). The initial cloning of complementary DNAs (cDNAs) coding for the subunits of GABA_A receptors indicated that the chloride channel gated by GABA is intrinsic to the structure of the receptor and that each of the binding sites also possesses

specific requirements for subunit composition (45,46). At present, almost 20 different cDNAs have been identified and classified into six classes based upon sequence homology. Cloned from vertebrates, there are six α , four β , four γ , one δ , one ϵ , and two ρ subunits and some splice variants; the subunits share a basic motif where the amino acids span the membrane four times. This four transmembrane spanning motif is shared with subunits that form other receptor members of the ligand-gated superfamily (39).

Extensive mutagenesis and structural examination has been carried out with the GABA and acetylcholine family of receptors (47,48). Acetylcholine receptors have been shown to possess a pentameric subunit structure with a heterogeneous subunit composition; evidence for this conclusion has been obtained through the use of monoclonal antibodies and through direct electron microscopic visualization of the densely packed receptor in the Torpedo eel. Similar electron microscopic analysis of GABA_A receptors has been carried out (49). It is thought that the native GABA_A receptors also possess such a pentameric structure with general composition of 2 α , 2 β , and one γ subunit forming the majority of the GABA_A receptors in vertebrates. Evidence of this has been more circumstantial, generated by molecular biological and pharmacologic inferences, described below, and by the behavior of solubilized recombinant complexes on sucrose gradient centrifugation in the presence and absence of different subunit specific antibodies (50). The natural receptor in endogenous tissue appears to be also pentameric (51).

Evidence from studies of acetylcholine receptors has also indicated that the second transmembrane spanning sequence forms the actual ion channel of acetylcholine receptors, and that mutations of amino acids at the inner (cellular) side of the membrane are responsible for the ability of specific cation ions to pass through the channel pore (48). The ionic selectivity can be changed by altering the charge of some of these specific amino acids, and the acetylcholine receptor can be forced to gate chloride, rather than sodium, by such changes. Thus, a relatively firm case for the involvement of this spanning region in the formation of the ion channel can be made. Because the core ion pore is highly conserved among the large number of GABA_A receptor subtypes, a number of drugs that interact nonspecifically with all the members of the GABA_A receptor family were identified in the past. These include anesthetic barbiturates, picrotoxin, neurosteroids, and some organic insecticides (42–44). More recently, because the ion channel shows little variation between GABA_A receptor subtypes, it has not been as active a target for pharmaceutical discovery as the convenient allosteric modulatory site for benzodiazepines, drugs discovered by chance almost 40 years ago.

Originally, two subunits of the GABA_A receptor family were cloned and, when expressed in oocytes, were capable of forming a receptor that would gate chloride in response to GABA (45). At that time, some responses were seen to barbiturates, toxins, and benzodiazepines. It is now known

that a full response to the benzodiazepines requires the incorporation of a third subunit, the γ subunit (52). One of the major forms of native GABA_A receptor in vertebrates probably has the structure $\alpha_1\beta_2\gamma_2$, most likely in a 2:2:1 stoichiometry. The α_1 subunit contains the major site that is photoaffinity labeled with the benzodiazepine ³H-flunitrazepam at His 101 (53). For the functional modulation of GABAergic activity by benzodiazepines, in addition to the α subunit, a γ subunit must be incorporated into the complex. Thus, there is reasonable evidence that benzodiazepines and related drugs stimulate GABA activity without opening channels directly; depending on their ability to potentiate GABA activity, they are called full or partial agonists. The binding site for these drugs incorporates a binding site composed of components of both α and γ . Other drugs (called inverse agonists) may occupy the same site to negatively modulate the action of GABA, such as β -carboline derivatives. Yet a third class of compounds exist, drugs such as flumazenil, may occupy the site as antagonists of both agonist and inverse agonists. By themselves, these antagonists have no effect on GABAergic activity and are behaviorally silent. The important allosteric modulatory effects of drugs at the benzodiazepine site were recognized early and the distribution of activities at different receptor subtypes has been an area of intense pharmacologic discovery for many years (39,42–44). The details of some of these findings are described later.

Because the expression of an α or β subunit by itself does not form a functional receptor (54), but expression of both together constitute a functional GABA_A receptor, the binding sites for GABA could be associated with the combination of the two subunits. Systematic mutagenesis of α and β subunits have identified a number of amino acids on both subunits that appear to contribute to the ability of GABA to bind to the GABA_A receptor and modulate chloride conductance (53). Thus, the GABA binding sites are also complex, composed of a binding pocket that is made up of amino acids from both subunits; there are two GABA binding sites per receptor [located at the two identical (or homologous in the case of heterogeneous mixtures of subunits) $\alpha\beta$ interfaces], and electrophysiologic studies indicate that both must be occupied for the chloride channel to open.

Perhaps the most interesting aspect of these mutagenesis studies is the observation that the GABA binding site (two per $\alpha\beta\gamma$ complex) and the benzodiazepine binding site (one site per $\alpha\beta\gamma$ complex) are structurally related to one another; homologous amino acids that contribute to binding in each case are found at similar positions in each subunit. They represent in the case of the GABA binding site $\alpha\beta$ interfaces and in the case of the benzodiazepine binding site the $\alpha\gamma$ interface (Fig. 68.2). By binding to the benzodiazepine binding site, the drugs clearly give a positive allosteric signal to the receptor and/or to the GABA binding sites; this is reflected in a change in affinity at a low-affinity GABA

binding site (43). This signal is analogous to the signal that a single GABA molecule binding to one of the GABA binding sites gives to the receptor and second GABA binding site. The original finding relating GABA and benzodiazepines allosterically showed evidence of a similar signal being transmitted from the occupied GABA binding site to the benzodiazepine site, resulting in a higher affinity state at the benzodiazepine binding site in the presence of GABA at its binding site (55). This type of pseudosymmetric binding site is characteristic of allosteric protein binding interactions and is found also in acetylcholine and in glycine receptors (46,56,57). The theoretical details of such interactions allow one to rationalize how a set of drugs like benzodiazepines could stabilize a channel open state but not be able to open the channel directly. Variations in these binding sites that are dependent on different α , β , and γ subunit amino acid sequences, particularly α and γ as the components of the benzodiazepine binding site, underlie the heterogeneity of GABA_A receptors and point to the possibilities for GABA_A receptor subtype-specific drugs.

Genomic Organization of GABA_A Receptor Subunits

The natural association of $\alpha\beta\gamma$ compositions of GABA_A receptors is also underscored by the genomic organization of the subunits. There is a cluster of the genes coding for α_1 , β_2 , and γ_2 subunits on chromosome 5 and similar clusters of other subunits on other chromosomes such as 4 and 15 (58, 59). They point to the phylogenetic age of GABA_A receptors, and similarities in organization point to the possibility that ancestral GABA_A receptor gene cluster duplications spawned some of the different clusters coding for unique GABA_A receptor isoforms, whereas mutations in individual subunits may have created additional diversity. Such an organization also points to probable coordinated control of subunit production and many interesting aspects of cellular and brain regional regulation of expression yet to be examined. Regional diversity also can mean functional diversity and drugs that affect certain aspects of behavior, but not others.

Functional Activities of Therapeutics at Individual Recombinant Constructs

One reason for the diversity of subtypes of GABA_A receptors is that this is how neurons integrate information and change the behavioral status of the animal. Whereas GABA_A receptors are found on many neurons, particularly local interneurons, some GABA_A receptor subtypes are selectively localized to specific brain regions, specific cell layers within those regions, and even specific parts of cells (60–62). Each subtype has a unique set of electrophysiologic and pharmacologic properties. A number of factors can determine the response properties of GABA_A receptor subtypes. The most

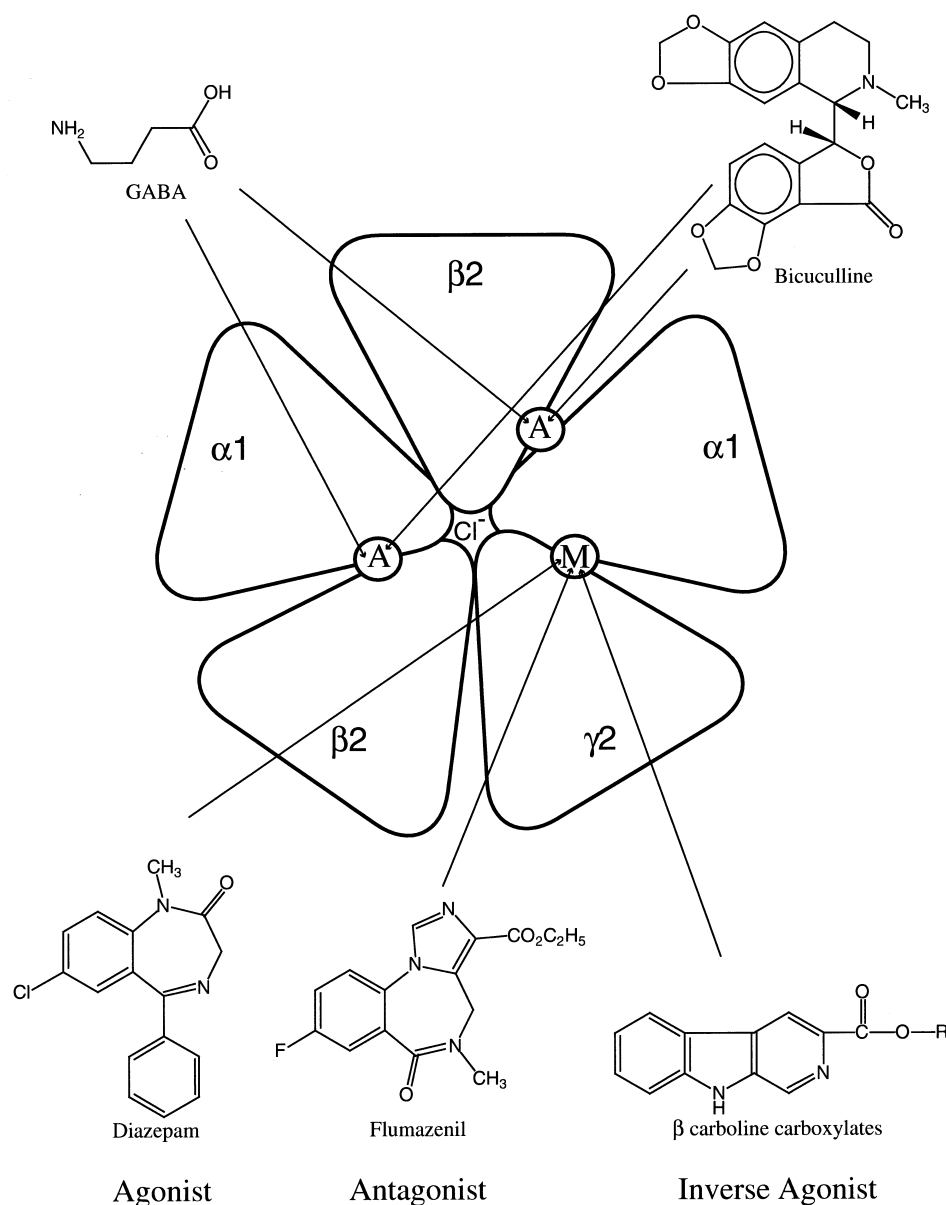


FIGURE 68.2. γ -Aminobutyric acid (GABA) binding site $\alpha\beta$ interfaces.

important determinant is subunit composition. Secondly, these receptor subtypes can have unique profiles of modulation driven by membrane potential, ion gradients, biochemical conditions, and, last but not least, drugs.

Attempts have been made to categorize GABA_A receptors pharmacologically in terms of responses to specific drugs that interact with the benzodiazepine binding site. Thus, in the original nomenclature, two subtypes of GABA_A receptor were described (63). They were called type 1 and type II receptors and were defined in terms of differential affinity for CL 218,872 and a number of other compounds. We now know that type I GABA_A receptors contain the composition $\alpha_1\beta_x\gamma_2$ and are responsive to CL 218,872, a related

compound zaleplon, and zolpidem (64). Both zaleplon and zolpidem are marketed hypnotics. Type II receptors are a mixed heterogeneous class containing $\alpha_2\beta_x\gamma_2$, $\alpha_3\beta_x\gamma_2$, and $\alpha_5\beta_x\gamma_2$. α_4 - and α_6 -containing $\beta_x\gamma_2$ subunit combinations are generally agreed to constitute a separate category of GABA_A receptors due to their lowered affinity for classic benzodiazepine site ligands (65). For example, flumazenil has a very low affinity for these α_4 - and α_6 -containing GABA_A receptor recombinant constructs compared to α_1 -, α_2 -, α_3 -, or α_5 -containing GABA_A receptors (100 nM versus 0.5 nM). The differences in amino acids between the α subunits pointed to the mutagenesis studies described above to delineate GABA and benzodiazepine binding sites.

Thus, affinity differences in the benzodiazepine binding site have been a primary method for differentiating GABA_A receptor subtypes. In contrast to the affinity models of benzodiazepine binding sites, it is not clear that a simple molecular biologically based efficacy model will emerge to simplify our understanding of α_1 -, α_2 -, α_3 -, or α_5 -containing GABA_A receptors.

The GABA_A receptor subtypes with their benzodiazepine receptor sites are an example of a unique situation in biology. Compounds have been synthesized that can allosterically modulate GABA response over a wide range through this site. Modulation of GABA responses has spanned the range of >700% increase in response amplitude to inhibition as great as 60%. Control of efficacy by drugs with subunit specificity can be achieved. One approach of drug companies has been to develop drugs that increase GABA responses less than 100% and develop selectivity for some subunits without increasing responses at other subunit combinations. These have been called subtype-selective partial agonists and will probably represent the next generation of GABAergic modulators to enter the clinic and become drugs.

A number of studies suggest, by circumstantial evidence such as message distribution, genomic localization, and biochemical study, that the major subtype combinations in brain are $\alpha_1\beta_2\gamma_{21}$, $\alpha_2\beta_3\gamma_{21}$, $\alpha_3\beta_3\gamma_{21}$, and $\alpha_5\beta_3\gamma_{21}$. α_1 has been implicated in sedation by virtue of the fact that zolpidem, a marketed hypnotic under the trade name Ambien, is a type I (α_1) selective compound and can cause cognitive deficits (see next subsection). An ideal anxiolytic drug might have limited effects on this subtype while increasing responses at α_2 - and α_3 -containing subtypes, as they are located in the limbic parts of the brain directly implicated in generation and reduction of anxiety. The full examination of subtype selective drugs in humans is in the near future, and it will be interesting to see how these hypotheses fare in clinical trials.

Involvement of GABA_A Receptors in Human Disease and Transgenics

The extensive investigation of the amino acids involved in the binding of GABA and benzodiazepines allows a specific and elegant approach to be made to the *in vivo* investigation of the involvement of GABA_A receptor subtypes with specific neural pathways and specific behavioral activities. This approach can be made either through the examination of chromosomal deletions, the use of specific knockouts of subunit genes, or knock-ins of particular point mutations. These techniques naturally complement the development of drugs with specific activities at GABA_A receptor subtypes.

Some naturally occurring chromosomal deletions of particular GABA_A receptor subunits showed phenotypes of craniofacial deficits, mental retardation, and epilepsy. Deletion of large areas of human chromosome 15, containing α_5 , γ_3 ,

and β_3 subunit genes, results in this phenotype, which causes a human genetic disorder called Prater Willi/Angelman syndrome (66). In a targeted study in mice, animals with the specific deletion of the β_3 subunit shows a similar syndrome with cleft palate and neurologic abnormalities. These studies point to the importance of certain GABA_A receptors in neuronal development (67,68). It is also clear that the deletion of an entire subunit does not result in the rescue of function by substitution of other subunits (69). Other rare genetic disorders of GABA_A receptor function are likely to emerge as our knowledge of the genomic basis of neurologic disorders evolve. A very elegant approach, based on the molecular biological studies described above, has been taken to examine the significance of GABA_A receptor subtypes by replacing an important amino acid for benzodiazepine binding (histidine) found in α_1 , α_2 , α_3 , and α_5 with an arginine characteristic of α_4 and α_6 . Through this conservative mutation, GABA sensitivity is retained, so the receptors function normally, but drug sensitivity is lost (70,71). From these studies, it appears that the α_1 -containing subtypes are important in mediating the anticonvulsant, sedative, and amnesic effects of benzodiazepines, but to a smaller degree the muscle relaxant and anxiolytic effects. These animals are still susceptible to the development of tolerance to the sedative effects. In the near future, we may learn the consequence of deletion of the specific benzodiazepine modulatory sites from the other α subtypes. Thus, from a mechanistic point of view this class of drugs has a well-defined mode of action.

SEROTONIN RECEPTOR MODULATORS AND REUPTAKE INHIBITORS

Preclinical Studies

Serotonin has long been viewed as a neurotransmitter involved in regulating emotional states. Of the 14 or so mammalian serotonin receptor subtypes that have been described in the literature, at least four have been implicated in anxiety in various animal models (72). As reported by Lucki (72) the original hypothesis implicating serotonin in anxiety surfaced from observations that reduced levels of serotonin can produce anxiolytic effects. One of the receptor subtypes implicated in anxiety is the serotonin 1A receptor subtype (5-HT_{1A}), which is an autoreceptor located presynaptically on serotonin neurons. When stimulated, this receptor inhibits the synthesis and secretion of serotonin. The 5-HT_{1A}receptor agonist buspirone exhibits anxiolytic effects in animals and was approved by the Food and Drug Administration (FDA) in 1986 for human generalized anxiety disorder. Other serotonin receptors potentially involved in anxiety include the 5-HT_{2A}, 5-HT_{2C}, and 5-HT₃ receptors. Antagonists for the 5-HT_{2A}receptor, like ritanserin, exhibit anxiolytic effects in some animal models (73,74). Likewise, blockade of the 5-HT_{2C} receptor produces anxiolytic effects in

animals (75) and prevents the anxiogenic effects of m-CPP (76). Finally, the 5-HT₃ receptor antagonist ondansetron was reported to be anxiolytic in some animal models (77).

Recent advances in molecular biology has led to the development of serotonin receptor gene knockout methodology, which generates mice lacking the 5-HT_{1A} receptor, allowing for the evaluation of this receptor subtype in a variety of measurable behaviors. Ramboz et al. (78) reported results consistent with the 5-HT_{1A} agonist's data cited above. Mice lacking this receptor displayed less exploratory activity in an open field and more anxious behavior than the wild types in the elevated plus maze. According to the serotonin hypothesis of anxiety (79), removing the negative feedback control of 5-HT with the 5-HT_{1A} receptor knockout animals should result in increased levels of 5-HT in the synaptic cleft, which would be expected to lead to the anxiogenic behavior. However, Ramboz et al. (78) reported normal levels of 5-HT, which confuses the issues related to anxiety modulation and serotonin levels. As David Julius (80) points out, the interpretation of standard gene knockout experimentation is complicated by the possibility of long-term development changes and this is true with the 5-HT_{1A} knockout animal. So despite the apparent consistency between the 5-HT_{1A} knockout animal and 5-HT_{1A} agonist studies in terms of the behavioral outcomes of each manipulation, the exact role of the 5-HT_{1A} receptor in anxiety is not absolutely clear at this time.

Clinical Studies

In 1986, the FDA approved the 5-HT_{1A} partial agonist for generalized anxiety disorder. This drug was the first to challenge the benzodiazepines for this patient group and was generally perceived as an improvement because of the lack of benzodiazepine side effects. The efficacy of buspirone, however, was not the same as that of the benzodiazepines in terms of its delayed onset of action, and it is generally accepted that when buspirone offers clinical benefit to generalized anxiety disorder (GAD) patients, it takes 3 to 4 weeks to match the efficacy of benzodiazepines such as diazepam and alprazolam (81). The 5-HT_{1A} partial agonist properties of buspirone are believed to account for its clinical effects, but it should be noted that the drug is also a D2 antagonist and is extensively metabolized. One of the major metabolites, 1-pyrimidinylpiperazine (1-PP), may contribute to the pharmacologic activity of buspirone (82). In a double-blind, placebo-controlled study of buspirone in GAD patients (83), the drug was reported to be as efficacious as lorazepam at the end of a 4-week treatment period. After the drugs were discontinued, however, the lorazepam-treated patients worsened whereas the buspirone-treated subjects maintained clinical improvement. Thus, there continues to be evidence that buspirone is effective in GAD.

The development of selective serotonin reuptake inhibitors (SSRIs) in the 1980s and 1990s widely expanded the

treatment for depressive disorders, and these drugs (fluoxetine, sertraline, venlafaxin, paroxetine) have recently made inroads in treating anxiety disorders such as panic, obsessive-compulsive disorder, social phobia, and GAD. Successful treatment of GAD with a class of drugs working through the serotonergic system will come from the SSRIs (84).

Obsessive-compulsive disorder (OCD) is a chronic, disabling anxiety disorder. In a review of the diagnosis and treatment of OCD, Goodman (85) states that the backbone of pharmacologic treatment for OCD is a 10- to 12-week trial with an SSRI in adequate doses. It is clear from a review of the role of the 5-HT_{1A} receptor (86) in OCD that partial agonists such as buspirone are generally ineffective in treating OCD. The authors also note that in studying the potential to augment efficacy of the standard OCD medication, buspirone was not different from placebo as an augmenting agent. Drugs that work through other serotonin receptor subtypes also appear to be ineffective in treating OCD. Thus, drugs modifying the 5-HT_{1A}, 5-HT_{1D}, and 5-HT₃ receptors appear ineffective in treating OCD symptoms and rule out a critical involvement of these receptor subtypes in OCD (87,88).

In the past, tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors, as well as high potency benzodiazepines, have been used to treat patients with panic disorder. The SSRIs have also been added to the list of effective agents for the disorder. In reviewing the pharmacotherapy of panic disorder, den Boer (89) notes that antidepressants are more effective than benzodiazepines in reducing associated depressive symptomatology and are at least as effective for improving anxiety, agoraphobia, and overall impairment. Bell and Nutt (90) remark that SSRIs improve 60% to 70% of panic patients, a similar percentage to those seen with the TCAs.

Like OCD, panic disorder is well treated by SSRIs but does not appear to be effectively treated by receptor specific compounds. Copland et al. (86) reviewed the role of 5-HT_{1A} drugs such as buspirone in panic disorder and reported that buspirone does not significantly treat panic in several well-controlled studies. Using the 5-HT_{1A} receptor agonist flesinoxan, van Vliet et al. (91) reported a worsening of symptoms in panic patients treated with high doses of the drug. It has also been reported that the 5-HT_{2A/2C} antagonist ritanserin had no effects on panic attacks or phobic avoidance, and a similar negative finding has been reported with the 5-HT₃ antagonist ondansetron.

NEUROKININ RECEPTOR ANTAGONISTS

Rationale

There is an extensive literature demonstrating that the peptide tachykinins such as substance P and their associated receptors have a widespread distribution in the brain, spinal cord, and periphery, and may play important roles in

chronic pain and inflammation processes (92–96). In addition, anatomic and physiologic evidence has also indicated that these peptides may affect limbic structures that are involved in the regulation of mood and affect, such as the amygdala, hypothalamus, and periaqueductal gray (97). This notion is supported by early positive clinical findings using a selective neurokinin-1 (NK-1) antagonist for the treatment of depression and anxiety (98).

Molecular Mechanism of Action

Tachykinins collectively refer to small peptides that include substance P (SP), neurokinin A (NK-A), and neurokinin B (NK-B). These peptides show preferential affinity for three receptors, designated NK-1, NK-2, and NK-3, respectively, which are members of the seven-transmembrane, G-protein–coupled family. Of these three receptors, NK-1 and NK-3 are found in the brain, whereas NK-2 is primarily localized peripherally in smooth muscle of the respiratory, urinary, and gastrointestinal tracts. Neurokinin receptors are localized in a number of different brain areas that are implicated in anxiety, including the amygdala, hypothalamus, and locus coeruleus.

Studies assessing the effects of direct administration of neurokinin agonists such as substance P into the nervous system are complicated by the findings that, depending on factors such as the site and dose, opposite effects on behavior may be achieved.

Current Drugs in Development

Numerous NK-1 antagonists have been described in the literature, including MK-869 (Merck) and an analogue, L-760,735 (Merck), SR140333 (Sanofi), CP-122,721 (Pfizer), RP67580 (Rhone-Poulenc), FK-888 (Fujisawa), SDZ NKT 343 (Novartis), and PD 154075 (Parke-Davis). NK-1 antagonists have been reported to demonstrate anxiolytic effects in animal models such as social interaction (99), though these effects are not consistently seen across all compounds (34). Researchers from Merck have reported that vocalizations elicited by maternal separation in guinea pigs are robustly blocked by NK-1 antagonists such as MK-869, an effect that is shared by a range of antidepressant and anxiolytic agents (98).

Of the compounds listed above, the primary indication has been for the treatment of conditions such as pain, chemotherapy-induced emesis, and migraine (94). MK-869, which progressed to phase III trials for emesis, has also been evaluated in a phase II depression trial in which it was reported that, in addition to showing a significant antidepressant effect, MK-869 also showed significant anxiolytic activity that emerged over the course of the 6-week study (98). The data supported the conclusion that NK-1 antagonists might be useful for the treatment of depression and anxiety. Further development of MK-869 for depression

was discontinued, but these early clinical data will undoubtedly lead to further clinical evaluation of NK-1 antagonists.

NK-2 antagonists include SR48968 (Sanofi). Preclinical studies have shown that NK-2 antagonists such as GR159897 and SR48968 have also demonstrated activity in social interaction and exploration anxiolytic models, and activity has been reported in the marmoset monkey using the human “threat” test. Good therapeutic ratios were described for these agents.

NK-3 antagonists described in the literature include osnetant (Sanofi-Synthelabo), talnetant, PD-161182, and PD-157672 (Parke-Davis). The latter two have been designated for the treatment of anxiety disorders, though there have been no reports of clinical trials with any NK-3 antagonist for this indication. It should be noted that preclinical data described to date are sparse, and there is some suggestion that NK-3 agonism may produce an anxiolytic profile. Thus, intraventricular administration of the NK-3 agonist senktide produced anxiolytic effects in mice that could be blocked by administration of the NK-3 antagonist SR 142801, and SR 142801 was found to have some anxiogenic activity (100).

Future Drugs and Directions

Further depression and anxiety clinical trials with centrally active NK-1 antagonists are needed to provide further validation of the role of NK-1 receptors in treating depression and anxiety disorders. In addition, further assessment of the role of NK-2 and NK-3 subtypes is needed to determine the possible relevance, if any, of these receptor subtypes.

GLUTAMATE RECEPTOR AGONISTS AND MODULATORS

Rationale

Glutamate is the major mediator of excitatory neurotransmission in the CNS. Despite this ubiquity, the elucidation of numerous glutamate receptor subtypes with differential localizations in the brain, and the development of selective pharmacologic agents, has led to the realization that glutamate receptors might be viable targets for a number of different neurologic and psychiatric disorders, including anxiety and depression (101–103).

Molecular Mechanism of Action

The molecular biology of glutamate receptors has been the subject of numerous reviews (101,102). Glutamate receptors are classified as either ionotropic or metabotropic. Ionotropic receptors, which mediate fast synaptic transmission, are coupled to cation-specific ion channels and bind the agonists *N*-methyl-D-aspartate (NMDA), α -amino-3-hy-

droxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA). These receptors gate both voltage-dependent and voltage-independent currents carried by Na^+ , K^+ , and Ca^{2+} .

NMDA receptors, which are selectively activated by NMDA, form a receptor/channel complex that is allosterically regulated by several sites. Receptor activation results in depolarization and Ca^{2+} influx. Allosteric binding sites include a strychnine-insensitive glycine site, a polyamine site, a zinc site, and a channel site that binds agents such as MK-801 or phencyclidine to block channel opening. The NMDA receptor has been cloned and has two families of subunits, the NR1, with seven splice variants (1A–1G), and NR2, with four splice variants (2A–2D). NR1 receptors possess the receptor/ion channel complex, whereas the NR2 receptors lack the ion channel and appear to be modulatory. NR2 receptors, however, can form functional heteromeric channels by combining with NR1 subtypes. NMDA antagonists include competitive antagonists at the NMDA receptor such as AP5, CPPene, and CGS 19755; noncompetitive antagonists at the ion channel site such as MK-801 and phencyclidine; and noncompetitive antagonists that bind to the glycine site such as 5,7-dichlorokynurenic acid, L689,560, ACEA 1021, and MDL 105,519 (104).

“Non-NMDA” receptors refer to the class of ionotropic glutamate receptors activated by kainic acid or AMPA, agonists that activate voltage-independent channels that gate a depolarizing current mediated primarily by Na^+ ions. There are four AMPA subunits (GluR1–GluR4) and five KA subunits (GluR5–GluR7 and KA-1–KA-2). Functional channels can be produced by homomeric expression of GluR5 or GluR6 subunits, or by heteromeric expression of KA-1 or KA-2 with GluR5 or GluR6. KA sites are found in the hippocampus, cortex, and thalamus, whereas AMPA receptors are additionally localized in septal and cerebellar sites. Pharmacologic agents for blocking non-NMDA receptors include the competitive antagonists CNQX, DNQX, and NBQX, and the noncompetitive antagonist GYKI 52466.

Metabotropic glutamate receptors (mGluRs) mediate slower synaptic transmission and utilize phosphatidyl inositol (PI) and cAMP as second messengers. Opposite effects on cAMP may be mediated by either G_i or G_s stimulation. Agonists include a conformationally restricted glutamate analogue, 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD). Metabotropic receptors have been classified into three subgroups: group I (mGluR1, mGluR5), which stimulate PI hydrolysis; and two groups that inhibit adenylate cyclase, group II (mGluR2, mGluR3) and group III (mGluR4, mGluR6). Metabotropic receptors are widely distributed throughout the brain, in areas such as the hippocampus, cerebellum, thalamus, olfactory bulb, and striatum, though the precise distribution varies considerably between groups. In addition to the agonist *trans*-ACPD, other agonists that are more specific for receptor subtypes include

LY354740 (group II), L-AP4 (group III), and L-CCG-I (mGluR2). Pharmacologic agents for antagonizing metabotropic glutamate receptors are currently limited.

Genetically Altered Mouse Models

Site-directed mutagenesis studies have indicated that point mutations in the glycine binding site of the NR1 subunit result in mice that have reduced glycine affinity and have an anxiolytic profile as seen by decreased natural aversion to an exposed environment (105). These data supported other pharmacologic lines of evidence (see below), indicating that blockade of the glycine site can have anxiolytic actions.

Current Drugs in Development

LY354740 is an orally active group II metabotropic receptor agonist (106) currently in clinical development. Preclinically, the compound has anxiolytic activity in fear-potentiated startle (107), the elevated plus maze (107), conflict testing (108), the four-plate test (108), and in a lactate-induced panic attack model (109). LY354740 has also been shown to decrease withdrawal signs seen during naloxone-precipitated morphine withdrawal (110).

Future Drugs and Directions

Competitive and noncompetitive NMDA antagonists have primarily undergone clinical evaluation for the treatment of stroke and trauma (103). Unfortunately, clinical development for many of these compounds was halted because of severe side effects, including psychotic-like symptoms. The potential for these side effects has been a major deterrent for using NMDA antagonists for the treatment of psychiatric disorders. Although there are currently no drugs reported to be in development, preclinical studies have suggested that selective antagonists of the strychnine-sensitive glycine site can have anxiolytic properties with reduced side-effect potential relative to competitive and noncompetitive NMDA antagonists such as AP5 and MK-801 (36,111). Recent work has supported such a profile (112,113).

Antagonists of AMPA receptors have also been proposed to have anxiolytic actions in preclinical models. Thus, LY326325 was shown to have anxiolytic activity in the elevated plus maze and in a conflict test (punished drinking) in rats (114), and CNQX injected directly into the periaqueductal gray produced anxiolytic effects on the elevated plus maze (115). The antagonists CNQX and GYKI 52466 were also able to block the anxiogenic responses produced by bicuculline injected into the basolateral amygdala (116).

CCKB ANTAGONISTS

Cholecystokinin (CCK) is a peptide found extensively both in the gut (where it was originally identified) and in the

brain (117). CCK exists in multiple forms, the most predominant of which is CCK octapeptide (CCK8) and, to a lesser extent, CCK tetrapeptide (CCK4) (118). CCK is colocalized with a number of different neurotransmitters, including serotonin, dopamine, GABA, substance P, neuropeptide Y, and VIP. CCK-like immunoreactivity has been demonstrated in anatomic regions that include the amygdala, cerebral cortex, hippocampus, striatum, hypothalamus, and spinal cord (119). There are two subtypes of CCK receptor, CCKA (sulfated CCK) and CCKB (unsulfated CCK) (120). CCKA receptors are localized in the nucleus accumbens, posterior hypothalamus, and area postrema. CCKB receptors are localized in cortex, olfactory bulb, nucleus accumbens, and other brain areas (121).

A number of selective antagonists for the CCKB receptor have been synthesized, including LY288513 (Lilly), PD 135158 (Parke-Davis), L-365,260 (Merck), and CI-988. Griebel (34) reviewed the extensive literature on the effects of CCK antagonists in models of anxiety and concluded that the results were contradictory and did not lead to a clear conclusion. L-365,260 and CI-988 have been evaluated clinically in panic disorder, but neither of these agents had significant anxiolytic effects (122–124).

CONCLUSION

Many different neurotransmitter receptor systems have been shown to modulate anxiety and possess anxiolytic effects. This is not surprising because many of these transmitters control anatomic circuitry important in anxiety. The next generation of marketed anxiolytics will be determined more by their side-effect profile than by their anxiolytic activity. It will be interesting to see which of the mechanisms described in this chapter will provide the most useful anxiolytics in human populations.

ACKNOWLEDGMENT

Drs. Tallman, Cassella, and Kehne are all full-time employees of Neurogen Corporation.

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