Developing Small Molecule Nonpeptidergic Drugs for the Treatment of Anxiety Disorders: Is the Challenge Still Ahead?

Thomas Steckler

Contents

Abstract Neuropeptide systems have been considered a major opportunity for the development of novel treatment approaches for anxiety disorders based on preclinical evidence and neurochemical alterations seen in anxiety disorders. This excitement was further facilitated by the fact that drugs acting at these systems, such as CRF1 antagonists, NK1 antagonists, NK3 antagonists or CCK2 antagonists, may have unique properties not seen with drugs affecting more classical mechanisms involved in anxiety. Consequently, there have been major efforts to develop such small-molecule, nonpeptide receptor ligands. A number of these molecules have been tested in the clinic, either in trials where levels of anxiety served as a secondary measure, or in a few studies with patients suffering from anxiety disorders. But unfortunately, and despite all the efforts of the field as a whole, we still lack convincing clinical proof-of-concept for any of the neuropeptidergic approaches in patients. It must, therefore, be concluded that neuropeptide targets remain a promising approach for the development of the next generation drugs to treat anxiety disorders, but that they continue to be high-risk targets for drug development.

T. Steckler
Johnson & Johnson PRD, Turnhoutseweg 30, 2340 Beerse, Belgium
e-mail: tsteckle@prdbe.jnj.com

M.B. Stein and T. Steckler (eds.), *Behavioral Neurobiology of Anxiety and Its Treatment*, Current Topics in Behavioral Neurosciences 2, DOI 10.1007/7854_2009_14,

Keywords Corticotropin-releasing factor · CRF1 antagonist · Substance P · Neurokinin · NK1 Antagonist · NK3 Antagonist · Cholecystokinin · CCK2 Antagonist · Atrial natriuretic peptide · ANP · Oxytocin · Clinical trial · Proof-of-concept

1 Introduction

Drugs that target neuropeptide systems – antagonists, full or partial agonists, positive allosteric modulators, or inverse agonists – could represent an alternative approach to treat anxiety disorders. Characteristics that should make neuropeptide systems attractive as drug targets are, first, their generally more discrete neuroanatomical localization when compared to monoamines and GABA and glutamate and, second, their state-dependent effects, i.e., neuropeptides need conditions of relatively high neuronal firing to be released at quantities which activate their receptors, while classical neurotransmitters are released by low neuronal frequency bursts (Hokfelt et al. 2003). Based on the latter, we can assume that antagonists, i.e., compounds that block binding of the endogenous peptide to the receptor recognition site, are more active under conditions of increased peptide release than under basal conditions. In other words, antagonistic drugs could normalize pathological anxiety associated with increased peptide release, while leaving normal physiology in the absence of neuropeptide release relatively unaffected, thus minimizing target-related side effect burden (Steckler 2008).

When it comes to agonists, the latter advantage of antagonists may be lost as they may stimulate the neuropeptide receptor in the absence of the endogenous ligand. Thus, full or partial agonists acting at the orthosteric (i.e., endogenous ligand) binding site of the peptide receptor, will fully or partially mimic the effects of the endogenous peptide. Nevertheless, there are a spectrum of anxiety disorders, presumably with different underlying pathophysiology, and those drugs could be of relevance if there is a decrease in peptide release and consequent peptidergic hypoactivity associated with an anxiety disorder. Likewise, agonists may be of use under conditions where the peptidergic system is not directly involved in the pathophysiology of the anxiety disorder, but where it is known preclinically that activation of the specific neuropeptide receptor will lead to a reduction in anxiety and arousal, thereby providing an indirect treatment approach. However, the ability of those compounds to stimulate the receptor in the absence of the endogenous ligand imposes the inherent risk that the receptor is tonically overstimulated, as well as the potential for the development of receptor desensitization and internalization, and hence tolerance development to the drug after chronic treatment. Compared to full agonists, this risk may be somewhat mitigated with a partial agonist leading to submaximal receptor activation and hence reduced relative efficacy when compared to a full agonist, but still exists.

Numerous selective and nonselective agonists acting at neuropeptide receptors have been described. An example for a partial agonist is the drug diltiazem, an atypical L-type calcium channel blocker used since long to treat cardiac arrhythmias,

which has recently been discovered to also be a partial agonist at the ghrelin receptor (Ma et al. 2007). Ghrelin is a 28-amino acid gastrointestinal peptide with orexigenic properties. In the brain, ghrelin binds to the growth hormone secretagogue receptor (GHS1A) (Dornonville de la Cour et al. 2001; Kojima et al. 1999; Tschop et al. 2000), where one of its functions, besides regulating food intake, may be to increase anxiety-related behavior (Asakawa et al. 2001; Carlini et al. 2002, 2004; Kanehisa et al. 2006). Interestingly, some anxiolytic efficacy has been reported for diltiazem in panic disorder patients (Balon and Ramesh 1996), although this may be more attributable to the calcium channel blockade than the partial ghrelin agonism (especially as the animal literature would suggest the latter would lead to an increase, not a reduction, in anxiety).In contrast to the full and partial agonists, positive allosteric small-molecule nonpeptidergic modulators (PAMs) bind at a site different from the binding pocket of the endogenous ligand, thereby facilitating the effects of the neuropeptide, but (assuming that there is no endogenous ligand for the allosteric binding site) should have no effect on their own. Those molecules may overcome part of the problems inherent to orthosteric agonism as they again lead to phasic effects only in the presence of the endogenous neuropeptide ligand and not to tonic activation, and hence reduce the risk of overstimulation and tolerance development. Examples for such allosteric drugs acting at neuropeptide systems have been reported, e.g., again for the ghrelin receptor (Holst et al. 2005).

Yet another class of compounds is the inverse agonists. To understand this concept, it must be acknowledged that some receptors can be constitutively active. That means that the receptor signals in the absence of the endogenous ligand, i.e., there is a basal tone of receptor signaling that is further increased by phasic release and binding of the peptide. Inverse agonists will reduce the tonic signaling of the receptor in the absence of the endogenous peptide ligand. Once again using the ghrelin system as an example, it is noteworthy that the ghrelin receptor is constitutively active (Holst et al. 2003) and that inverse ghrelin agonists (albeit peptidergic) have been described (Holst et al. 2006). Such inverse agonists could be particularly useful under conditions where an anxiety disorder is caused by an increase in constitutive firing rate of a neuropeptide receptor. They will also be of benefit if there is increased peptide release associated with an anxiety disorder, but in contrast to the antagonists, may lead to unintended hypoactivity of the system.

Other means to interact with peptidergic systems could be the inhibition of peptide synthesis or breakdown. Zinc metallopeptidases, e.g., are enzymes that rapidly degrade enkephalins in the brain. Consequently, blockade of these peptidases increases extracellular concentrations of enkephalins (Nieto et al. 2005). Enkephalins in turn reduce anxiety-related behavior in animals and, indeed, anxiolytic-like effects were observed in mice following intraperitoneal administration of a zinc metallopeptidase inhibitor (Nieto et al. 2005).

Yet another strategy to modulate neuropeptide systems could be to alter the availability of the neuropeptide at its target by freeing it from its binding protein in cases where such proteins exist. For example, it has been shown that corticotropin-releasing factor (CRF) can be displaced from its binding protein by specific CRF

binding protein inhibitors (Behan et al. 1995). In this specific example, of course, one would not expect anxiolytic activity as CRF is well known to increase anxiety-related behavior (e.g., Steckler 2008).

Thus, there are several strategies that would allow one to manipulate neuropeptide systems pharmacologically and to affect anxiety states. As a caveat, however, it must also be recognized that at least some of these peptide systems are centrally and peripherally involved in major homeostatic mechanisms, such as the control of food intake and regulation of pituitary or cardiovascular function. Thus, a close monitoring or safety and tolerability profiles of drugs that target neuropeptides is warranted. Other issues that proved to be a challenge for the development of compounds that act at neuropeptide systems relates to their physicochemical properties. At least for some targets it has proved to be very difficult to synthesize compounds that show good solubility, bioavailability, brain penetrance, and metabolic stability while maintaining high efficacy and selectivity (Steckler 2008). Nevertheless, the pharmaceutical industry has succeeded in developing small molecule, nonpeptidergic compounds acting at various neuropeptide systems with acceptable drug-like characteristics and safety profile that could be of benefit for the treatment of anxiety disorders, examples of which will be discussed below.

2 Is there Clinical Proof-of-Concept with Compounds Acting at Neuropeptide Systems?

No drug acting at neuropeptidergic systems has yet been approved for the treatment of anxiety disorders. However, a number of compounds have been evaluated in clinical trials.

2.1 CRF_1 Antagonists

Possibly, the neuropeptide target that received most attention over the last years is the CRF_1 receptor. Several review articles have summarized the preclinical evidence indicating potent anxiolytic-like properties of small molecule, nonpeptidergic CRF_1 antagonists in animals (e.g., Holmes et al. 2003; Landgraf 2005; Steckler 2005, 2008; Steckler and Dautzenberg 2006; Steckler and Holsboer 1999; Zorrilla and Koob 2004) and the picture that emerges is that these compounds are particularly efficacious in animals that are in states of heightened anxiety induced by pharmacological or emotional stressors. Sites where CRF1 antagonists seem to affect anxiety-related behavior in animals are the amygdala (Bakshi et al. 2002) and the bed nucleus of the stria terminalis (Sahuque et al. 2006), key brain areas

involved in anxiety (Canteras et al. 2009). But despite intense activity in the field for many years, there are only a few published studies investigating the effects of CRF_1 antagonists in man and none has been published on the effects of CRF_1 antagonists in patients suffering from anxiety disorders. Those studies that investigated the effects of CRF1 antagonists in stress-related psychiatric disorders focused on depression, which is another disorder where CRF1 antagonists may be of benefit. Part of the reason for a focus on depression rather than anxiety may be a strategic decision of the pharmaceutical industry based on indication sequencing, because depression is generally considered to be commercially a more attractive therapeutic indication.

Studying the effects of CRF_1 antagonism with NBI-34041 in 24 healthy male volunteers, Ising et al. (2007) reported an attenuation of the psychosocial stress-induced activation of the HPA axis. However, NBI-34041 failed to affect the stress-induced increases in emotionality as measured by the State–Trait–Anxiety Inventory (STAI-X1). In contrast, some beneficial effects of CRF_1 antagonism on anxiety was suggested by data from an earlier study in depressed patients. In this open label study in 20 patients, significant reductions in both depression and anxiety scores were reported (Zobel et al. 2000). Of note, this study was primarily designed as a safety, dose-escalation study and did not include a placebo group or an active control. More recently, however, lack of efficacy was reported for the CRF_1 antagonist CP-316,311 in a 6-week randomized, placebo controlled trial in depressed patients, which used the Hamilton Depression scale as a primary endpoint but also included a secondary efficacy analysis on the Hamilton Anxiety Scale (HAM-A) (Binneman et al. 2008). Based on these three clinical studies, evidence for anxiolytic effects of CRF_1 antagonists in man is inconclusive at best, which contrasts the substantial preclinical evidence supporting a role of CRF_1 antagonists as anxiolytic drugs. However, it should also be noted that the entry criteria for anxiety (based on HAM-A) were not high, suggesting that anxiety was moderate, which may not be sufficient to detect potent anxiolytic effects

Therefore, a counter argument would be that no CRF_1 antagonist has been tested in patients with primary diagnosis of anxiety disorder and that these compounds may show efficacy in these patients. Indeed, there is evidence for abnormalities in the CRF system in certain types of anxiety disorders: a relatively consistent finding reported in several studies is an increase in cerebrospinal fluid (CSF) levels of CRF and a blunted ACTH response to CRF challenge in post-traumatic stress disorder (PTSD) (see Nemeroff et al. 2006; Steckler 2008, for reviews). A blunted response to CRF challenge has also been reported in panic disorder patients (reviewed in Gold et al. 1988; Holsboer et al. 1987; Steckler et al. 2008) and, more recently, Smoller et al. 2003, 2005) found an association between the CRF gene and behavioral inhibition in children at risk for panic disorder. Likewise, abnormalities of the CRF system have been described in obsessive-compulsive disorder (OCD) (Fossey et al. 1996). Taken together, this would suggest that drugs normalizing CRF activity, such as CRF_1 antagonists, could be of benefit for the treatment of PTSD and panic disorder, and possibly also in OCD. Until the relevant clinical trials have been performed, the jury is still out.

2.2 NK_1 Antagonists

Another target that received substantial interest in the past is the neurokinin 1 (NK_1) receptor for which the endogenous ligand is substance P. This interest was strongly triggered by a seminal paper published in 1998, where it was shown that the NK_1 antagonist aprepitant (MK-869) had anxiolytic-like properties in a guinea pig pup model measuring separation-induced distress vocalizations comparable to the anxiolytics diazepam (a benzodiazepine) and buspirone (a 5-HT_{1A} agonist) (Kramer et al. 1998). The amygdala has also been suggested to be the site of action where NK1 antagonists elicit anxiolytic-like effects in animals (Rupniak et al. 2003). Aprepitant was as efficacious as the selective serotonin reuptake inhibitor (SSRI) paroxetine in improving scores on both the Hamilton Anxiety (HAM-A) and Depression (HAM-D) scales in depressed patients with comorbid anxiety (Kramer et al. 1998). Subsequently, an improvement in HAM-A score was also reported with another NK_1 antagonist, LY-759274, in depressed patients (Kramer et al. 2004). Interestingly, in a human positron emission tomography (PET) study, aprepitant at the dose that was efficacious in depression also blocked more than 90% of the NK_1 receptors and also displaced the NK_1 ligand [F^{18}]SPA in the amygdala (Hargreaves 2002). However, in five subsequent Phase III studies, aprepitant failed to significantly improve HAM-A (and HAM-D) scores in depressed patients. In two of those studies, paroxetine was used as active comparator and showed a significant benefit on HAM-A scores in one (Keller et al. 2006), which casts some doubts on the therapeutic benefit of NK_1 antagonism in the treatment of anxiety symptoms in depressed patients and in the treatment of depression in general. These data also raise more general questions about how predictive preclinical animal models and Phase II data are of efficacy in Phase III studies (Steckler et al. 2008).

As for CRF_1 antagonists, it could be argued, however, that the real value of NK_1 antagonists for the treatment of anxiety disorders would only become apparent once such compounds have been tested in the appropriate patient population. A recent study reported that fear provocation in individuals with specific phobia was associated with a decreased uptake of a NK_1 PET ligand in the amygdala, possibly because of an increase in endogenous substance P release triggered by the fear-provoking stimuli (Michelgard et al. 2007). This hypothesis is corroborated by preclinical findings, showing that stress exposure leads to increased substance P release in the medial nucleus of the amygdala and that direct injections of a NK_1 antagonist into the medial nucleus attenuated stress-induced anxiety-related behavior in rats (Ebner et al. 2004). These findings argue in favor of a role for NK_1 antagonists in the treatment of at least some types of anxiety. In fact, the NK_1 antagonist GR205171 has been reported to improve social phobia comparable to the SSRI citalopram in patients. In this study, symptom improvement was paralleled by a reduction in regional cerebral blood flow in response to a psychosocial stressor in the rhinal cortex, parahippocampal–hippocampal regions, and also in the amygdala (Furmark et al. 2005). These are

encouraging results, but experience with NK_1 antagonists in the field of depression should be taken as a note of caution. Certainly, one would like to see confirmation of these findings in patients suffering from social phobia or other phobias in other studies (ideally Phase III trials) before efficacy of NK_1 antagonism in these disorders is taken for granted.

2.3 NK_3 Antagonists

Compared to the NK_1 receptor antagonists, far less information has been released on the potential anxiolytic properties of NK_2 and NK_3 antagonists in man, although these two NK receptor subtypes have also been suggested to play a role in the modulation of anxiety. Only one study reported failure of the NK_3 antagonist osanetant to improve panic symptoms in panic disorder patients challenged with cholecystokinin tetrapeptide (CCK-4), a peptide frequently employed to elicit panic-like symptoms in healthy volunteers and panic disorder patients (Kronenberg et al. 2005).

2.4 CCK_2 Antagonists

Based on challenge studies with CCK-4 or with the cholecystokinin receptor 2 (CCK_2) antagonist pentagastrin (a synthetic analogue of CCK-4), linkage studies, measurements of CSF levels of CCK and preclinical data obtained from animal tests, the CCK system has also been suggested to play an important role in anxiety disorders, in particular in panic disorder (see Steckler et al. 2008, for review). A number of CCK_2 antagonists have been tested in both healthy volunteers and patients suffering from panic disorder or generalized anxiety disorder, but evidence for anxiolytic activity is conflicting. Although initial studies with the CCK_2 antagonist L-366,260 suggested antipanic effects, based on studies in healthy volunteers receiving an intravenous challenge with pentagastrin (Lines et al. 1995), or in panic patients challenged with CCK-4 (Bradwejn et al. 1994), those findings were not confirmed by others (Kramer et al. 1995; Sramek et al. 1994–1995). Likewise, lack of efficacy was reported for the CCK_2 antagonist CL-988 in a number of studies in healthy volunteers following CCK-4 or lactate challenge (Bradwejn et al. 1995, Cowley et al. 1996) and in patients with panic disorder (Pande et al. 1999; Van Megen et al. 1997) or generalized anxiety disorder (Adams et al. 1995; Goddard et al. 1999). Part of the issue here may be the fact that these compounds in general show relatively low bioavailability. Unfortunately, and unlike to the situation in the NK1 field, no PET ligand exists that could be used to demonstrate central occupancy of CCK4 receptors in human to come to real conclusions. However, as it stands one has to assume that CCK_2 antagonists are not clinically effective for the treatment of anxiety disorders.

In addition to these small molecule approaches, anxiolytic-like activity has been seen following administration of some peptidergic compounds in man.

3 Atrial Natriuretic Peptide and Oxytocin

Intravenous infusions of panic disorder patients with atrial natriuretic peptide (ANP) resulted in anxiolytic effects following challenge with CCK-4 (Strohle et al. 2001; Wiedemann et al. 2001), although it is unclear how this effect was mediated as peripherally administered ANP is not brain penetrant. It has been suggested that the effects of CCK challenge could be peripherally or centrally mediated; e.g., via activation of CCK receptors expressed at vagal nerve endings or vasodilatation of cerebral arteries, both leading to secondary central effects, or in brain areas lacking a tight blood–brain barrier (Cano et al. 2003; Crawley and Corwin 1994; Rinaman et al. 1995; Sanchez-Fernandez et al. 2003). Likewise, ANP could alter measures of anxiety by either peripheral or central effects, thereby counteracting the effects of CCK-4.

Anxiolytic effects were also reported following intranasal administration of oxytocin to healthy volunteers following psychosocial stress (Heinrichs et al. 2003). Moreover, an increase in trust was seen following oxytocin administered via this route (Kosfeld et al. 2005). Intranasal administration has been suggested to result in brain penetration of a number of peptides in humans (Born et al. 2002), although direct proof in man is lacking for oxytocin. In fact, plasma oxytocin levels also increase markedly after intranasal application (Landgraf 1985), raising the possibility that the anxiolytic-like effects of oxytocin might be peripherally mediated. But in support of – primary or secondary – central effects, functional magnetic resonance imaging (fMRI) studies showed that the fear-induced activation of the amygdala (Kirsch et al. 2005) and the amygdala response to emotional faces (Domes et al. 2007) were reduced in volunteers receiving intranasal oxytocin. Clinical trials of intranasal oxytocin treatment in social phobia are underway.

A number of other small-molecule, nonpeptidergic compounds with potential for the treatment of anxiety disorders have been proposed, including vasopressin V_{1b}, neuropeptide Y Y_2, melanocortin MC_4, angiotensin AT_1, galanin GAL_3, bombesin BB_1, melanin-concentrating hormone MCH_1 and orexin OX_1 antagonists, as well as oxytoxin, δ opiate and orphanin FQ OFQ_1 agonists, but those compounds have only been tested preclinically yet. Even more neuropeptide systems have been associated with anxiety-related behavior preclinically for which at present no small molecule, nonpeptidergic compounds with anxiolytic-like properties have been reported (reviewed in Steckler 2008). A detailed discussion of these data would be beyond the scope of this chapter.

4 Is Efficacy Enough?

Summarizing the clinical trial data, one has to conclude that the evidence for neuropeptide-based therapies for anxiety disorders is still very limited, despite major efforts by the pharmaceutical industry for more than 20 years. The only

class of small molecule, nonpeptidergic compounds that showed a preliminary clinical proof-of-concept is the NK_1 antagonists, but more studies with positive outcome need to be published before firm conclusions can be drawn. Moreover, requirements are already or can be expected to increase and just showing efficacy may not be sufficient to bring a new anxiolytic to the market in the future. What will be required is the demonstration of superiority! This might be superior efficacy in particular anxiety disorders where efficacy of current medication is limited, e.g., in specific phobias or PTSD. It can be faster onset of action, for e.g., in obsessive compulsive disorder, where benzodiazepines do not work and the onset of efficacy with antidepressant drugs is delayed. It can also be an improved side effect profile, i.e., lack of cognitive impairment, sedation, ataxia, dependency, or withdrawal complications as is the case with the benzodiazepines, or lack of nausea, sexual dysfunction and weight gain, as is seen with the SSRIs (Steckler et al. 2008). However, none of these have so far been convincingly demonstrated in clinical trials with compounds targeting neuropeptide systems in patients suffering from an anxiety disorder. Moreover, given the involvement of many peptide systems in major homeostatic mechanisms, as mentioned above, there is a risk that other, mechanism-based side effect profiles will occur. For example, nephrotoxic and hepatotoxic effects were seen with the CRF_1 antagonist antalarmin in a 90-day toxicity study in rats (Horn et al. 2008). It is unclear whether these findings are structurally related to the molecule or mechanistically to CRF_1 receptor blockade, but from these findings kidneys and liver must be considered target organs for antalarmin toxicity in humans (Horn et al. 2008). Of note, elevated liver transaminases were also reported in 3 out of 20 patients treated with the CRF_1 antagonist R121919 (Zobel et al. 2000), which is structurally relatively close to antalarmin. Moreover, implantation loss of rat fetuses has been reported following treatment of pregnant rats with antalarmin during the early stages of pregnancy, which may be related to the mechanism of action, i.e., CRF1 receptor-mediated (Makrigiannakis et al. 2004). Likewise, adverse effects can be expected with some other neuropeptide targets, such as weight gain with MC_4 receptor antagonists (Kask et al. 1998; Skuladottir et al. 1999), blood pressure lowering effects with AT_1 antagonists, or proconvulsive properties with brain penetrant δ-opioid agonists (Comer et al. 1993; Yajima et al. 2000), to name just a few.

Clearly, this is not meant to devalue the potential of neuropeptidergic targets as novel therapeutic approaches to treat anxiety disorders, because the points mentioned above are not unique to the peptide field, but it points to the fact that one might be faced with new issues when developing novel drugs that act on these targets, some being more obvious than others, and that are not necessarily seen with current anxiolytic medication. The importance of this is the fact that novel anxiolytic drugs would be expected to have a clean side effect profile as possible, making a close evaluation of this profile mandatory.

5 Conclusion

Coming back to the initial question: Is the challenge still ahead when developing small molecule nonpeptidergic drugs for the treatment of anxiety disorders? From a review of the literature the answer is clearly "yes." It is evident that neuropeptide systems represent a major opportunity for the development of novel treatment approaches for anxiety disorders as drugs acting at these systems may have unique properties not seen with other targets. There is a huge literature providing evidence for an important role of neuropeptides in the mediation of anxiety-related behavior, both in animals and man. It is also evident that drugs acting at those targets might overcome some of the problems inherent in today's anxiolytic medication. However, the approach remains a high risk and, unless there is unambiguous proof-of-concept in patients, highly speculative.

References

Adams JB, Pyke RE, Costa J, Cutler NR, Schweizer E, Wilcox CS, Wisselink PG, Greiner M, Pierce MW, Pande AC (1995) A doubleblind, placebo-controlled study of a CCK-B receptor antagonist, CI-988, in patients with generalized anxiety disorder. J Clin Psychopharmacol 15:428–434

Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Fujimiya M, Katsuura G, Makino S, Fujino MA, Kasuga M (2001) A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. Neuroendocrinology 74:143–147

Bakshi VP, Smith-Roe S, Newman SM, Grigoriadis DE, Kalin NH (2002) Reduction of stress-induced behaviour by antagonism of corticotropin-releasing factor 2 (CRF2) receptors in lateral septum or CRF1 receptors in amygdala. J Neurosci 22:2926–2935

Balon R, Ramesh C (1996) Calcium channel blockers for anxiety disorders? Annals Clin. Psychiatry 8:215–220

Behan DP, Heinrichs SC, Troncoso JC, Liu XJ, Kawas CH, Ling N, De Souza EB (1995) Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. Nature 378:284–287

Binneman B, Feltner D, Kolluri S, Shi Y, Qiu R, Stiger T (2008) A 6-week randomized, placebo-controlled trial of CP-316, 311 (a selective CRH1 antagonist) in the treatment of major depression. Am J Psychiatry 165:617–620

Biro E, Sarnyai Z, Penke B, Szabo G, Telegdy G (1993) Role of endogenous corticotropin-releasing factor in mediation of neuroendocrine and behavioural responses to cholecystokinin octapeptide sulfate ester in rats. Neuroendocrinology 57:340–345

Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL (2002) Sniffing neuropeptides: a transnasal approach to the human brain. Nature Neurosci 5:514–516

Bradwejn J, Koszycki D, Couetoux du Tertre A, Van Megen H, Den Boer J, Westenberg H (1994) The panicogenic effects of cholecystokinintetrapeptide are antagonized by L-365, 260, a central cholecystokinin receptor antagonist, in patients with panic disorder. Arch Gen Psychiatry 51:486–493

Bradwejn J, Koszycki D, Paradis M, Reece P, Hinton J, Sedman A (1995) Effect of CI-988 on cholecystokinin tetrapeptide-induced panic symptoms in healthy volunteers. Biol Psychiatry 38:742–746

Brawman-Mintzer O, Lydiard RB, Bradwejn J, Villarreal G, Knapp R, Emmanuel N, Ware MR, He Q, Ballenger JC (1997) Effects of the cholecystokinin agonist pentagastrin in patients with generalized anxiety disorder. Am J Psychiatry 154:700–702

Cano V, Caicoya E, Ruiz-Gayo M (2003) Effect of peripheral cholecystokinin receptor agonists on c-Fos expression in brain sites mediating food consumption in rats. Neurosci Lett 343:13–16

Canteras NS, Resstel LB, Bertoglio LJ, de Padua Carobrez A, Guimaraes FS (2009) Neuroanatomy of anxiety. In: Steckler T, Stein M (eds) Behavioral neurobiology of anxiety and its treatment. Curr Top Behav Neurol Springer, Heidelberg

Carlini VP, Monzon ME, Varas MM, Cragnolini AB, Schioth HB, Scimonelli TN, De Barioglio SR (2002) Ghrelin increases anxiety-like behavior and memory retention in rats. Biochem Biophys Res Commun 299:739–743

Carlini VP, Varas MM, Cragnolini AB, Schioth HB, Scimonelli TN, De Barioglio SR (2004) Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. Biochem Biophys Res Commun 313:635–641

Comer SD, Hoenicke EM, Sable AI, McNutt RW, Chang KJ, Mosberg HI, Woods JH (1993) Convulsive effects of systemic administration of delta opioid agonist BW373U86 in mice. J Pharmacol Exp Ther 267:888–895

Cowley DS, Adams JB, Pyke RE, Cook J, Zaccharias P, Wingerson D, Roy-Byrne PP (1996) Effect of CI-988, a cholecystokinin-B receptor antagonist, on lactate-induced panic. Biol Psychiatry 40:550–552

Crawley JN, Corwin RL (1994) Biological actions of cholecystokinin. Peptides 15:731–755

Domes G, Heinrichs M, Glascher J, Buchel C, Braus DF, Herpertz SC (2007) Oxytocin attenuates amygdala responses to emotional faces regardless of valence. Biol Psychiatry 62:1187–1190

Dornonville de la Cour C, Bjorkqvist M, Sandvik AK, Bakke I, Zhao CM, Chen D, Hakanson R (2001) A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. Regul Pept 99:141–150

Ebner K, Rupniak NM, Saria A, Singewald N (2004) Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. Proc Natl Acad Sci USA 101:4280–4285

Fossey MD, Lydiard RB, Ballenger JC, Laraia MT, Bissette G, Nemeroff CB (1996) Cerebrospinal fluid corticotropin-releasing factor concentrations in patients with anxiety disorders and normal comparison subjects. Biol Psychiatry 39:703–707

Furmark T, Appel L, Michelgard A, Wahlstedt K, Ahs F, Zancan S, Jacobsson E, Flyckt K, Grohp M, Bergstrom M, Pich EM, Nilsson LG, Bani M, Langstrom B, Fredrikson M (2005) Cerebral blood flow changes after treatment of social phobia with the neurokinin-1 antagonist GR205171, citalopram, or placebo. Biol Psychiatry 58:132–142

Goddard AW, Woods SW, Money R, Pande AC, Charney DS, Goodman WK, Heninger GR, Price LH (1999) Effects of the CCK(B) antagonist CI-988 on responses to mCPP in generalized anxiety disorder. Psychiatry Res 85:225–240

Gold PW, Pigott TA, Kling MA, Kalogeras K, Chrousos GP (1988) Basic and clinical studies with corticotropin-releasing hormone. Implications for a possible role in panic disorder. Psychiatric Clin North Am 11:327–334

Hargreaves R (2002) Imaging substance P receptors (NK1) in the living human brain using positron emission tomography. J Clin Psychiatry 63(Suppl 11):18–24

Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U (2003) Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry 54:1389–1398

Hokfelt T, Bartfai T, Bloom F (2003) Neuropeptides: opportunities for drug discovery. Lancet Neurol 2:463–472

Holmes A, Heilig M, Rupniak NMJ, Steckler T, Griebel G (2003) Neuropeptide systems as therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 24:580–588

Holsboer F, von Bardeleben U, Buller R, Heuser I, Steiger A (1987) Stimulation response to corticotropin-releasing hormone (CRH) in patients with depression, alcoholism and panic disorder. Horm Metabol Res Suppl 16:80–88

Holst B, Cygankiewicz A, Halkjar JT, Ankersen M, Schwartz TW (2003) High constitutive signaling of the ghrelin receptor-identification of a potent inverse agonist. Mol Endocrinol 17:2201–2210

Holst B, Brandt E, Bach A, Heding A, Schwartz TW (2005) Nonpeptide and peptide growth hormone secretagogues act both as ghrelin receptor agonist and as positive or negative allosteric modulators of ghrelin signaling. Mol Endocrinol 19:2400–2411

Holst B, Lang M, Brandt E, Bach A, Howard A, Frimurer TM, Beck-Sickinger A, Schwartz TW (2006) Ghrelin receptor inverse agonists: identification of an active peptide core and its interaction epitopes on the receptor. Mol Pharmacol 70:936–946

Horn TL, Harder JB, Johnson WD, Curry PT, Parchment RE, Morrissey RL, Mellick PW, Calis KA, Gold PW, Rice KC, Contoreggi C, Charney DS, Cizza G, Glaze ER, Tomaszewski JE, McCormick DL (2008) Integration of in vivo and in vitro approaches to characterize the toxicity of Antalarmin, a corticotropin-releasing hormone receptor antagonist. Toxicology 248:8–17

Ising M, Zimmermann US, Kunzel HE, Uhr M, Foster AC, Learned-Coughlin SM, Holsboer F, Grigoriadis DE (2007) High-affinity CRF1 receptor antagonist NBI-34041: preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. Neuropsychopharmacology 32:1941–1949

Kanehisa M, Akiyoshi J, Kitaichi T, Matsushita H, Tanaka E, Kodama K, Hanada H, Isogawa K (2006) Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats. Prog Neuro-Psychopharmacol Biol Psychiatry 30:1403–1407

Kask A, Rago L, Wikberg JE, Schioth HB (1998) Evidence for involvement of the melanocortin MC4 receptor in the effects of leptin on food intake and body weight. Eur J Pharmacol 360:15–19

Keller M, Montgomery S, Ball W, Morrison M, Snavely D, Liu G, Hargreaves R, Hietala J, Lines C, Beebe K, Reines S (2006) Lack of efficacy of the substance p (neurokinin1 receptor) antagonist aprepitant in the treatment of major depressive disorder. Biol Psychiatry 59:216–223

Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, Gruppe H, Mattay VS, Gallhofer B, Meyer-Lindenberg A (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 25:11489–11493

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660

Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E (2005) Oxytocin increases trust in humans. Nature 435:673–676

Kramer MS, Cutler NR, Ballenger JC, Patterson WM, Mendels J, Chenault A, Shrivastava R, Matzura-Wolfe D, Lines C, Reines S (1995) A placebo-controlled trial of L-365, 260, a CCKB antagonist, in panic disorder. Biol Psychiatry 37:462–466

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hale JJ, Mills SG, MacCoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR, Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NM (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281:1640–1645

Kramer MS, Winokur A, Kelsey J, Preskorn SH, Rothschild AJ, Snavely D, Ghosh K, Ball WA, Reines SA, Munjack D, Apter JT, Cunningham L, Kling M, Bari M, Getson A, Lee Y (2004) Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression. Neuropsychopharmacology 29:385–392

Kronenberg G, Berger P, Tauber RF, Bandelow B, Henkel V, Heuser I (2005) Randomized, double-blind study of SR142801 (Osanetant). A novel neurokinin-3 (NK3) receptor antagonist in panic disorder with pre- and posttreatment cholecystokinin tetrapeptide (CCK-4) challenges. Pharmacopsychiatry 38:25–29

Landgraf R (1985) Plasma oxytocin concentrations in man after different routes of administration of synthetic oxytocin. Exp Clin Endocrinol 85:245–248

Landgraf R (2005) Neuropeptides in anxiety modulation. Handb Exp Pharmacol 169:335–369

Lines C, Challenor J, Traub M (1995) Cholecystokinin and anxiety in normal volunteers: an investigation of the anxiogenic properties of pentagastrin and reversal by the cholecystokinin receptor subtype B antagonist L-365, 260. Br J Clin Pharmacol 39:235–242

Ma JN, Schiffer HH, Knapp AE, Wang J, Wong KK, Currier EA, Owens M, Nash NR, Gardell LR, Brann MR, Olsson R, Burstein ES (2007) Identification of the atypical L-type Ca^{2+} channel blocker diltiazem and its metabolites as ghrelin receptor agonists. Mol Pharmacol 72:380–386

Makrigiannakis A, Zoumakis E, Kalantaridou S, Chrousos G, Gravanis A (2004) Participation of maternal and fetal CRH in early phases of human implantation: the role of antalarmin. Curr Drug Targets Immune Endocr Metabol Disord 4:75–78

McCann UD, Slate SO, Geraci M, Roscow-Terrill D, Uhde TW (1997) A comparison of the effects of intravenous pentagastrin on patients with social phobia, panic disorder and healthy controls. Neuropsychopharmacology 16:229–237

Mezey E, Reisine TD, Skirboll L, Beinfeld M, Kiss JZ (1985) Cholecystokinin in the medial parvocellular subdivision of the paraventricular nucleus. Co-existence with corticotropin-releasing hormone. Ann N Y Acad Sci 448:152–156

Michelgard A, Appel L, Pissiota A, Frans O, Langstrom B, Bergstrom M, Fredrikson M (2007) Symptom provocation in specific phobia affects the substance P neurokinin-1 receptor system. Biol Psychiatry 61:1002–1006

Nemeroff CB, Bremner JD, Foa EB, Mayberg HS, North CS, Stein MB (2006) Posttraumatic stress disorder: a state-of-the-science review. J Psychiatric Res 40:1–21

Pande AC, Greiner M, Adams JB, Lydiard RB, Pierce MW (1999) Placebo-controlled trial of the CCK-B antagonist, CI-988, in panic disorder. Biol Psychiatry 46:860–862

Rinaman L, Hoffman GE, Dohanics J, Le WW, Stricker EM, Verbalis JG (1995) Cholecystokinin activates catecholaminergic neurons in the caudal medulla that innervate the paraventricular nucleus of the hypothalamus in rats. J Comp Neurol 360:246–256

Rupniak NM, Webb JK, Fisher A, Smith D, Boyce S (2003) The substance P (NK1) receptor antagonist L-760735 inhibits fear conditioning in gerbils. Neuropharmacology 44:516–523

Sahuque LL, Kullberg EF, Mcgeehan AJ, Kinder JR, Hicks MP, Blanton MG, Janak PH, Olive MF (2006) Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: role of CRF receptor subtypes. Psychopharmacology 186:122–132

Sanchez-Fernandez C, Gonzalez C, Mercer LD, Beart PM, Ruiz-Gayo M, Fernandez-Alfonso MS (2003) Cholecystokinin induces cerebral vasodilatation via presynaptic CCK2 receptors: new implications for the pathophysiology of panic. J Cereb Blood Flow Metabol 23:364–370

Skuladottir GV, Jonsson L, Skarphedinsson JO, Mutulis F, Muceniece R, Raine A, Mutule I, Helgason J, Prusis P, Wikberg JE, Schioth HB (1999) Long term orexigenic effect of a novel melanocortin 4 receptor selective antagonist. Br J Pharmacol 126:27–34

Smoller JW, Rosenbaum JF, Biederman J, Kennedy J, Dai D, Racette SR, Laird NM, Kagan J, Snidman N, Hirshfeld-Becker D, Tsuang MT, Sklar PB, Slaugenhaupt SA (2003) Association of a genetic marker at the corticotropin-releasing hormone locus with behavioral inhibition. Biol Psychiatry 54:1376–1381

Smoller JW, Yamaki LH, Fagerness JA, Biederman J, Racette S, Laird NM, Kagan J, Snidman N, Faraone SV, Hirshfeld-Becker D, Tsuang MT, Slaugenhaupt SA, Rosenbaum JF, Sklar PB (2005) The corticotropin-releasing hormone gene and behavioral inhibition in children at risk for panic disorder. Biol Psychiatry 57:1485–1492

Sramek JJ, Kramer MS, Reines SA, Cutler NR (1994–1995) Pilot study of a CCKB antagonist in patients with panic disorder: preliminary findings. Anxiety 1:141–143

Steckler T (2005) CRF antagonists as novel treatment strategies for stress-related disorders. In: Steckler T, Kalin NH, Reul JMHM (eds) Handbook of stress and the brain, part 2: integrative and clinical aspects. Elsevier, Amsterdam, pp 373–407

Steckler T (2008) Peptide receptor ligands to treat anxiety disorders. In: Blanchard RJ, Blanchard DC, Griebel G, Nutt D (eds) Handbook of anxiety and fear. Elsevier, Amsterdam, pp 157–221

Steckler T, Dautzenberg F (2006) Corticotropin-releasing factor antagonists in affective disorders and drug dependence – an update. CNS Neurol Disorders Drug Targets 5:147–165

Steckler T, Holsboer F (1999) Corticotropin-releasing hormone receptor subtypes and emotion. Biol Psychiatry 46:1480–1508

Steckler T, Stein MB, Holmes A (2008) Developing novel anxiolytics: improving preclinical detection and clinical assessment. In: McArthur R, Borsini F (eds) Animal and translational models for CNS discovery, vol 1. Elsevier, Amsterdam, pp 117–132

Strohle A, Kellner M, Holsboer F, Wiedemann K (2001) Anxiolytic activity of atrial natriuretic peptide in patients with panic disorder. Am J Psychiatry 158:1514–1516

Tschop M, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407:908–991

Van Megen HJ, Westenberg HG, Den Boer JA, Slaap B, Van Es-Radhakishun F, Pande AC (1997) The cholecystokinin-B receptor antagonist CI-988 failed to affect CCK-4 induced symptoms in panic disorder patients. Psychopharmacology 129:243–248

Van Vliet IM, Westenberg HG, Slaap BR, Den Boer JA, Ho Pian KL (1997) Anxiogenic effects of pentagastrin in patients with social phobia and healthy controls. Biol Psychiatry 42:76–78

Wiedemann K, Jahn H, Yassouridis A, Kellner M (2001) Anxiolyticlike effects of atrial natriuretic peptide on cholecystokinin tetrapeptide-induced panic attacks: preliminary findings. Arch Gen Psychiatry 58:371–377

Yajima Y, Narita M, Takahashi-Nakano Y, Misawa M, Nagase H, Mizoguchi H, Tseng LF, Suzuki T (2000) Effects of differential modulation of mu-, delta- and kappa-opioid systems on bicuculline-induced convulsions in the mouse. Brain Res 862:120–126

Yehuda R, Giller EL, Southwick SM, Lowry MT, Mason JW (1991) Hypothalamic-pituitary-adrenal dysfunction in posttraumatic stress disorder. Biol Psychiatry 30:1031–1048

Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, Holsboer F (2000) Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. J Psychiatric Res 34:171–181

Zorrilla EP, Koob GF (2004) The therapeutic potential of CRF1 antagonists for anxiety. Exp Opin Invest Drugs 13:799–828