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CCK and NPY as anti-anxiety treatment targets: promises, pitfalls, and strategies

Review Article

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Summary. Short CCK peptides elicit panic attacks in humans and anxiogenic-like effects in some animal models, but CCK receptor antagonists have not been found clinically effective. Yet CCK overactivity appears to be involved in submissive behaviour, and CCK_B receptor expression and binding are increased in suicide victims and animal models of anxiety. Preliminary data suggest that involvement of CCK and its receptor subtypes in anxiety can be better described when focusing on distinct endophenotypes, and considering environmental contingencies and confounds originating from interactions with dopamin-, opioid- and glutamatergic neurotransmission. In contrast, NPY is an anti-anxiety peptide with robust effects in various animal models when administrated into several brain regions. Studies with non-peptide antagonists selective for receptor subtypes have revealed the role of endogenous NPY in active coping. At least Y_1, Y_2 and Y_5 receptors in various brain regions are involved, with the strongest evidence for contribution of Y_1.

Keywords: Cholecystokinin – Neuropeptide Y – Anxiety – Panic – Suicide – Brain circuits

Introduction

Pathological anxiety is probably based on exaggerated activity in the brain fear circuits. If so, the neuropeptide systems should become ideal targets for anxiety treatment, as it is likely that the physiological significance of peptides increases with increasing neuronal activity (Hökfelt et al., 2000). Two peptides which raised early hopes for the arrival of novel and more anxioselective pharmacological treatments are cholecystokinin (CCK) and neuropeptide Y (NPY). Furthermore, as all major classes of psychopharmacological drugs had been discovered by serendipity, these peptides seemed to herald the time of psychopharmacology capable of designing new classes of drugs on the basis of understanding of the pathogenetic principles underlying psychiatric disorders. These peptide systems have yet not yielded in treatments so far; nevertheless, leading peptide pharmacologists have continued to express and explain their cautious optimism (e.g., Griebel, 1999). This review will provide a brief description of the background of the CCK and NPY hypotheses of anxiety, and discuss approaches which could eventually lead to the long-awaited success.

The advent of CCK as the "anxiety peptide"

The idea that peptides could serve as selective messengers in specific emotive circuits of the brain emerged in the eighties (Panksepp, 1986). The first initially promising candidate for an anxiety peptide, diazepam-binding inhibitor (DBI), prepared the pharmacologists for a peptide with a role in emotional regulation (Marx, 1985; Alho et al., 1985). At variance with the elusive DBI, CCK emerged as another strong candidate for an anxiety peptide. The endogenous CCK system was well defined by use of immunocytochemical techniques (Rehfeld, 1985) and its function could be readily examined by use of agonists and antagonists selective for the well-described CCK receptor subtypes (CCK_A and CCK_B or, respectively, CCK_1 and CCK_2 receptors).

Importantly, evidence for a role of CCK in anxiety converged from both human and animal studies. After the serendipitous discovery of Jens Rehfeld that CCK-4 can

elicit anxiety, dyspnea and depersonalization (described in Rehfeld, 1992), psychiatrists at the McGill University in Montreal realized that the CCK-4-induced syndrome very much resembles a panic attack. De Montigny demonstrated that CCK-4 can indeed reliably induce panic attacks in healthy volunteers (De Montigny, 1989), and Bradwejn and collaborators carried out a series of thorough studies which provided evidence that patients with panic disorder experience panic attacks after CCK-4 more frequently than healthy volunteers, are sensitive to lower doses of this peptide, and that the episodes of panic induced by CCK-4 are indistinguishable from the symptoms of the disorder (Bradwejn et al., 1990, 1991a, b). Other investigators were able to confirm these findings using pentagastrin or CCK-5 which similarly to CCK-4 selectively stimulates CCK_B receptors (Abelson et al., 1991; Van Megen et al., 1994). This clear evidence for a major role of CCK in panic disorder, and possibly in anxiety in general, gained simultaneous support from a number of animal studies (see below).

CCK systems and pharmacology

Several biologically active molecular forms of CCK that are derived from a 115-amino-acid precursor molecule exist, ranging from 4 to 58 amino acids in length. In the brain, the sulphated form of CCK octapeptide (CCK-8) is by far the most abundant form. This is also the minimal configuration active at the CCK_A receptors, whereas shorter CCK-peptides and desulphated CCK-8 activate both CCK receptor subtypes. CCK levels are high in the cerebral cortex, caudate-putamen, hippocampus and amygdala, intermediate in the thalamus, hypothalamus and olfactory bulb, and low in pons, medulla and spinal cord (Beinfeld et al., 1981).

Both identified CCK receptors belong to the superfamily of G protein linked receptors and 48% of their structure is identical. CCK_B receptor is also the receptor for gastrin. More than one CCK_B-like receptor subtype or affinity state has been suggested to exist on the basis of different in vivo pharmacological profiles of receptor agonists (Bellier et al., 2004) and radioligand binding assays (Harper et al., 1999). High densities of CCK receptors have been found in the striatum, cerebral cortex, olfactory bulb and olfactory tubercle; the levels are moderate in the hippocampus, substantia nigra, periaqueductal grey matter, and pontine nuclei, and low in thalamus, hypothalamus, and spinal cord. The majority of the CCK receptors in the brain are of the CCK_B subtype, but CCK_A receptors are more prevalent than originally described.

The most commonly used CCK receptor agonists include CCK-8 and caerulein which stimulate both receptor subtypes. Of the selective CCK_B receptor agonists, CCK-4 is the most popular tool, but CCK-5 or pentagastrin has similar properties. Other agonists include BC264, BC197, BC254 (Wang et al., 2005). Non-peptide agonists have only recently been synthetised for both CCK receptor subtypes (Shilling and Feifel, 2002; Kopin et al., 2003), and should facilitate further understanding of the receptor systems in anxiety substantially. It was, however, the synthesis of non-peptide CCK receptor subtype selective antagonists that strongly supported the studies on the role of CCK in anxiety. Benzodiazepine derivatives devazepide and L-365,260, selective for the CCK_A and CCK_B receptor subtypes, respectively, were the first potent tools, followed by dipeptoid, ureidoacetamide and pyrazolidinone derivatives (Griebel, 1999), and by now there is a wide selection of compounds for comparative analysis.

Anxiety, GABA and CCK: an attempt of rational psychopharmacology

Observations from animal experiments that could be interpreted as resulting from an anxiogenic effect of exogenously applied CCK peptides started to accumulate immediately after the discovery of CCK in the brain (see Harro et al., 1995), but the first claim of CCK as an anxiogenic stimulus in animals appeared in 1988 (Csonka et al., 1988). This was immediately followed by a number of studies demonstrating that CCK-peptides were anxiogenic and CCK receptor antagonists anxiolytic in several animal tests of anxiety, and that CCK receptor characteristics are related to anxiety states (Harro et al., 1989, 1990a, b, c; Hughes et al., 1990). Indeed, a possible role of CCK in anxiety was easily deducible from the general knowledge on chemical neuroanatomy and neuropharmacology. Regarding our team, we were unaware of the panicogenic properties of CCK in humans or any suggestions of its anxiogenic effect in animals when the studies were initiated, but we were doing research on GABA receptor pharmacology and the author was focusing on the mechanism of action of anxiogenic drugs such as beta-carbolines. A neighbouring group was pursuing the cholecystokinin hypothesis of schizophrenia which had been evoked by the seminal study of Hökfelt and collaborators (1980) on co-localization of CCK with dopamine in mesotelencephalic neurons. Researchers on both of these tracks noticed that CCK was increasingly found to be associated with GABA-ergic neurotransmission (see Harro et al., 1995 for overview and references). Neuroanatomically, GABA

and CCK are localized in the same neurons in several brain regions. GABA inhibits the release of CCK, and the non-competitive $GABA_A$ receptor antagonist picrotoxin was found to augment the release of the peptide, thus suggesting a tonic GABA-ergic control over CCK. Bradwejn and de Montigny (1984) had shown in electrophysiological studies in rats that benzodiazepine anxiolytics could inhibit CCK-elicited neuronal firing. Regarding pharmacological effects on behaviour, there was evidence that motor impairments caused by large doses of diazepam could be potentiated by CCK receptor antagonism. As to the effects of CCK itself, besides being a satiety factor the peptide was described to elicit promnestic and aversive effects, which are common with anxiogenic drugs. The sedative properties of CCK, when measured in novel environments, could also be viewed as partly related to an anti-exploratory effect. When we examined the effect of a number of substances against anti-exploratory effects of anxiogenic drugs reducing GABA-ergic neurotransmission, such as DMCM and pentylenetetrazol, in mice and rats, then proglumide, at that time a popular non-peptide CCK receptor antagonist, provided a dose-dependent and complete blockade of their anxiogenic effects. Furthermore, caerulein, a CCK receptor agonist, was extremely potent in reducing plus-maze activity, eliciting this anxiogenic-like effect at doses well below the known sedative range (Harro et al., 1989, 1990). The emergence of new non-peptide CCK antagonists highly selective for receptor sybtypes allowed to identify the CCK_B receptor with the anxiogenic effect (Hughes et al., 1990; Harro and Vasar, 1991; Singh et al., 1991). Many investigators reported potent anxiolytic-like effects of selective CCK_B receptor antagonists, others found that CCK-4 and CCK-5 elicit anxiogenic-like effects and these are blocked by CCK_B antagonists. Research in humans also demonstrated that CCK_B antagonists blocked CCK-induced panic attacks both in healthy volunteers (Lines et al., 1995) and in panic disorder patients (Bradwejn et al., 1994), thus reinforcing the emerging theory.

Besides the rapidly increasing number of animal studies implicating neuronal CCK in pathological anxiety, much information was piling up suggestive of the possible mechanisms by which CCK could contribute to the pathogenesis of anxiety. A review of the promising evidence from studies on rodents, non-human primates and human subjects concluded that the only important missing piece of information was the clinical efficacy of CCK_B receptor antagonists (Harro et al., 1993). Herewith the success story ended, as this evidence was not delivered. Different selective CCK_B antagonists failed to be effective in patients with either generalized anxiety disorder or panic disorder (Adams et al., 1995; Kramer et al., 1995; Van Megen et al., 1997; Pande et al., 1999). There have been suspicions that the CCK antagonists used in these negative studies are pharmacokinetically or pharmacodynamically inadequate, but this is not substantiated by any unequivocal data. Furthermore, comprehensive behavioural analyses of the effects of CCK_B receptor agonists and antagonists revealed that even in mouse and rat models the anxiolytic effects are unreliable (Dawson et al., 1995; Johnson and Rodgers, 1996).

Had the writing already been on the wall?

Hindsight may well be considered a poor practice, and entirely useless in science, but analysis of the emergence of knowledge may assist us in preventing future over-enthusiastic attempts to preferentially select information consistent with a fashionable theory. A more cautious analysis of preclinical evidence could possibly have foreseen that CCK receptor antagonists will not show such a robust anti-anxiety effect as optimistically expected. Indeed, any experimenter who has observed animals moving around in a novel environment after treatment with a benzodiazepine or a CCK receptor antagonist would easily distinguish the behavioural effects of the former but not these of the latter. I have remarked in a previous review (Harro et al., 1995) that studies which have been able to demonstrate an anxiolytic-like effect of a CCK_B receptor antagonist in elevated plus-maze have all used animals with relatively lower baseline anxiety than those that could not find any effect of the drug on its own, which is counterintuitive for a potent anxiolytic drug. CCK_B receptor blockade thus appears to increase locomotor and possibly exploratory activity, but not reduce anxiety. This has been corroborated by Daugé and collaborators (2001) who did not find any increase in anxiety in CCK_B receptor deficient mice in tests such as plus-maze or conditioned suppression of motility, but found a robust increase in locomotor activity and impairment of spontaneous alternation behaviour which suggests impairments in attention or memory. Thus, human and animal data have indeed been similar in that CCK_B agonist induced anxiety is reduced by CCK_B antagonists, but evidence for anxiolytic effect of CCK_B receptor blockade remains controversial. Our original data that proglumide attenuated the anxiogenic effects of drugs reducing $GABA_A$ receptor function were not reproduced with other CCK receptor antagonists, and proglumide is rather a functional antagonist with poor CCK receptor binding in vitro. Further

studies in several laboratories indicated that the reliability of the anxiolytic-like effects of CCK_B antagonists is limited in all paradigms It also became apparent that neither are the CCK receptor agonists equally active in all laboratory settings, and increasing the dose of the CCK-peptide (e.g., the use of high doses of caerulein) to achieve a reduction in activity may lead to unwanted sedation through CCK_A receptors or other unspecific cognitive effects through CCK_B receptors. Obviously, there is much more in e.g, the plus-maze behaviour than just anxiety: neither a CCK_B antagonist nor a benzodiazepine anxiolytic reduced the immediate early gene stimulation by plus-maze exposure (Hinks et al., 1996). The variability between different laboratories has not received an adequate explanation, but should not left unattended. Thus, recent careful studies which have demonstrated the high sensitivity of anxiety tests to environmental variables (Wahlsten et al., 2003) suggest that the underlying neurobiology should and can be explored, and CCK systems are a potential source of variation. Preferably such future studies should consider even the issue that measures of affective behaviour are most sensitive to the unidentified variables associated with the person of experimenter (Chesler et al., 2002).

Further clinical evidence for a role of CCK in anxiety

As noted in a recent review by Bourin and Dailly (2004), the state of the pharmacological studies may be disappointing but evidence for CCK anomalies in neuropsychiatric disorders with an anxiety component continues to emerge. There is evidence for differences in the CCK-ergic neurotransmission in panic disorder, as Lydiard et al. (1992) found decreased levels of CCK in the CSF of panic disorder patients, and Akiyoshi et al. (1996) described enhanced CCK-4-induced calcium mobilization in T cells from patients. In 105 patients with major depressive disorder, significant negative correlations were present between CCK levels and certain depression and anxiety parameters (Löfberg et al., 1998). Individual differences in anxiety sensitivity and C-peptide secretion have been found to contribute to the differences in sensitivity to pentagastrin-induced emotionality changes, as measured by galvanic skin response, between healthy volunteers (Radu et al., 2003). Using another psychometric scale, Aluoja and colleagues (1997) reported that dysfunctional attitudes predicted the number of symptoms after CCK-4 challenge in healthy volunteers, whereas baseline anxiety and anxiety sensitivity was associated with reactions to placebo but not to CCK-4. Genetic analyses have suggested CCK-related genes, among others, as contributors to higher risk of anxiety disorders (Maron et al., 2005). Some polymorphisms of the CCK_B receptor gene have been described to be associated with panic disorder. In particular, patients and controls were found to differ with regard to a CT repeat polymorphism, the patients being over-represented among the carriers of the longer repeat alleles (Hosing et al., 2004).

Recently, Sherrin and colleagues (2004) compared CCK_B receptor gene expression in ten suicide victims and ten matched controls by quantitative PCR and demonstrated increased gene expression in suicide victims in prefrontal and cingulate cortex and cerebellum. This finding confirms the results of our study which demonstrated increased CCK receptor binding in frontal and cingulate cortex of suicide victims (Harro et al., 1992).

Brain circuitries involved in the anxiogenic effects of CCK

Involvement of cortical CCK in anxiety is also supported by animal studies. Mice and rats selected on the basis of anxiety as measured in plus-maze display neurochemical differences in the GABA system (Rägo et al., 1988; Harro et al., 1990a) and rats with higher anxiety in the plus-maze test have higher levels of CCK_B receptor binding in frontal cortex (Harro et al., 1990a). Subsequently, others found that rats with higher plus-maze anxiety rather have lower CCK_B receptor binding in the amygdala (Wunderlich et al., 2002), but these animals went through training and testing in fear-potentiated acoustic startle response before sacrifice, so the results may not be directly comparable. Parenthetically, it should be noted that CCK receptor binding by means of labelling with an agonist is not without problems because at room temperature the fluidity of membranes does not allow to characterize receptor – G protein interaction, while at higher temperature CCK receptors are inactivated at a rate too high to allow saturation binding (Rinken et al., 1998). Nevertheless, the results of the study on suicide victims using RT-PCR can be taken as an independent validation of the CCK receptor binding experiments in suicide victims. In animal studies, CCK receptor binding, always in cortex, was found to be higher in response to administration of anxiogenic drugs (Harro et al., 1989), during diazepam withdrawal period (Harro et al., 1990b), and in stressful laboratory conditions (Harro et al., 1996). When these results were obtained, the animals had always been observed before sacrifice as moving very little and assuming a crouching or cowering position, having clearly a

very passive if not hopeless appearence. CCK_B receptor expression has subsequently been measured in more or less anxious rats, as selected in the plus-maze test, and found to be higher in anxious rats in cortex and cerebellum (Wang et al., 2005). Thus, overexpression of cortical CCK_B receptors in anxiety has also been confirmed in animal studies by use of independent methods. Differences in anxiety between animal strains have not been explained by the molecular characteristics of the CCK_B receptor gene, but again, differences at the expression level have been found (Wang et al., 2005). PVG hooded rats that are more responsive to cat exposure have higher CCK_B receptor expression in comparison with Sprague–Dawley rats in cortex and hippocampus (Wang et al., 2003). Thus, cortical CCK_B receptors have been found highly expressed in anxious subjects using a number of different approaches. If the decreased levels of CCK in the CSF of patients with panic disorder (Lydiard et al., 1992) reflect a decrease in CCK release in the involved brain regions in subjects with emotional dysbalance, the upregulation of cortical CCK_B receptors could be a quite universal mechanistic result of the reduced supply of the messenger. This has not been shown yet experimentally. However, a recent study which generated transgenic mice overexpressing CCK_B receptors in the forebrain demonstrated that this alteration produced animals more anxious than the wild-type (Chen et al., 2006). The transgenic animals were reported not to display any significant neuronal developmental or morphological changes, but were behaviourally more anxious in the open field, social interaction, and conditioned fear test. Importantly, a low dose of diazepam attenuated anxiety in mice with overexpression of CCK_B receptors. Since these animals had similar CCK mRNA expression as the wild-type, these results support the notion that CCK_B receptor regulation may be more important for anxiety than levels of different CCK peptides (Harro et al., 1996).

Neurochemical shifts occur in the cerebral cortex also after systemic administration of anxiogenic CCK-peptides. Most of this evidence suggests an effect through 5-HT-ergic neurotransmission. Thus, the increase in extracellular 5-HT levels in prefrontal cortex in guinea-pigs elicited by aversive environment is potentiated by CCK_B receptor stimulation (Rex et al., 1994). However, it should be noted that, in rats, the anxiogenic effect of CCK is reduced after long-term enhancement of 5-HT function (To and Bagdy, 1999).

Converging lines of evidence suggest that cerebellum is another site of the anxiety-related CCK-ergic functions. Thus, not only is CCK receptor expression higher in the cerebellum both in anxious rats (Wang et al., 2005) and suicide victims (Sherrin et al., 2004), but the cerebellar vermis was the most clearly activated brain region in healthy volunteers during CCK-4 elicited panic attacks (Benkelfat et al., 1995).

Amygdala is strongly implicated in the anxiogenic-like effects of CCK. Local application of pentagastrin into the amygdala potently enhanced acoustic startle response, and this effect was antagonized by two CCK_B receptor antagonists (Frankland et al., 1997). Anxiety-increasing effects elicited by CCK in amygdala have also been reported by others (Belcheva et al., 1994). Lower CCK_B receptor binding in the basolateral amygdala of more anxious rats has thus been interpreted as a downregulation compensatory for increased CCK activity (Wunderlich et al., 2002). In amygdala, but also in a number of other brain regions, GABA and CCK exist in the same parvalbumin-negative population of interneurons which also express cannabinoid CB_1 receptors (Marsicano and Lutz, 1999). The rather contrasting effects of cannabinoids and CCK may thus have a mutually antagonistic interaction at the level of GABA-ergic neurons.

CCK mRNA expression has been found increased by chronic stress in hippocampus and thalamus (Giardino et al., 1999). This increase was sensitive to sertraline in the CA1/CA2 region but not in CA3 or thalamus.

Administration of CCK-4 raises immediate early gene expression, among other brainstem sites, in the periaqueductal grey matter (Singewald and Sharp, 2000), and microinjection of CCK-8 into dorsal periaqueductal grey matter (DPAG) elicited an anxiogenic-like effect in the elevated plus-maze test (Netto and Guimaraes, 2004). In the latter study, the effect of CCK-8 was prevented by PD 135,158, a CCK_B receptor antagonist. Furthermore, microinjection of CCK-8 into the DPAG induced, via CCK_B receptors, Fos immunoreactivity in a number of brain areas involved in emotional regulation, such as the raphe nuclei, superior colliculus, lateral septum, medial hypothalamus, and medial amygdala.

Anxiogenic-like effects of CCK-4 can be blocked by CRF receptor blockade, but not vice versa (Wang et al., 2005). Thus, CCK-mediated anxiety-related mechanisms operate upstream the CRF systems implicated in anxiety. Similar regulation is present in the NPY-ergic mechanisms in which case dysfunction of the endogenous anxiolytic mechanisms leads to anxiety that can be attenuated by CRF receptor blockade (Kask et al., 1997, see below).

A variety of anxiogenic drugs, including CCK-4, elicits a similar pattern of immediate early gene expression in the brainstem areas such as rostral dorsolateral and caudal ventrolateral periaqueductal grey matter, locus coeruleus,

dorsal raphe, nucleus of the solitary tract, ventrolateral medulla, lateral and external medial parabrachial nucleus (Singewald and Sharp, 2000). These areas are obviously among the prime candidates for the beginning of anxiety circuit activation. Nevertheless, in the quoted study the effects of caffeine, a drug popular among humans and a relatively mild anxiogenic, were similar to all other substances, including the strongest anxiogenics such as beta-carbolines. Thus, at least some anxiogenic compounds may not be anxiogenic in all conditions, and the activation of some brain areas by anxiogenics probably does not reflect effects on negative emotionality. Anxogenics have in common other, e.g., promnestic effects.

In which conditions is the CCK signal anxiogenic?

This question will not receive the final answer in the present review, but it is to be borne in mind that both anxiogenic and anxiolytic-like effects of CCK can be demonstrated, and by now the crucial question is what determines the nature of the effect. Perhaps similarly to the activating effects of anxiogenic drugs, the changes in CCK levels or release need not always have emotional consequences, even if observed in stressful conditions. CCK expression can be increased by psychoactive drugs with very different types of action, including benzodiazepine anxiolytics (Rattray et al., 1993; Brodin et al., 1994; Pratt and Brett, 1995). CCK mRNA expression is increased by novel odors irrespective of whether the predator odor component was included or not (Hebb et al., 2003). Restraint stress and yohimbine both increase CCK release in the frontal cortex, and this effect is reversible with diazepam, but also with 5-HT_{1A} agonists (Becker et al., 1999). While 5-HT_{1A} agonists are clinically anxiolytic, they do not possess this activity when given acutely; thus even in case restraint stress induced CCK release could serve as a screening test for anxiolytics, this effect does not explain the mechanism of anti-anxiety action. Release of CCK upon environmental challenges depends upon the brain region and the nature of the challenge. Daugé and colleagues (2003) demonstrated a late decrease of extracellular CCK levels in hippocampus after a brief confinement of animals to the open arm of elevated plus-maze. This stressful manipulation, which reduced performance in a spatial memory task, also reduced significantly the large increase in CCK release that was elicited by the spatial memory task itself. Thus, these experiments support the notion that CCK release can be necessary for active coping.

The theory that CCK is a universal coping signal which would misbehave in case of predisposition to anxiety (Harro et al., 1995) is supported by accumulating evidence, but the available information is still not sufficient for developing a mechanistic model of such a predisposition. Indeed, it would make little evolutionary sense to have a mediator of pathological anxiety in the brain, and the CCK-ergic mechanisms involved in emotional responses either represent coping mechanisms that fail or are a part of a larger pathogenic pathway (Panksepp and Harro, 2004).

Large changes in extracellular and tissue levels of CCK-8-like immunoreactivity have been found in the prefrontal cortex after stressors that are part of most experimental conditions (Radu et al., 2001). These changes have been found to occur time-dependently in both directions. CCK levels were found to differ in rats placed into the cages of resident animals dependent upon the action of the resident towards the intruder: When friendly encounters had occurred, CCK levels were lower in a few brain areas, including posterior cortex, if compared to the intruders that were attacked and emitted 22 kHz distress calls (Panksepp et al., 2004). In another study, CCK levels were found different in a number of brain regions after an extended play-fighting which had turned unpleasant as measured by the emitted ultrasound spectrum; similarly to the previously described investigation, CCK levels were higher in the posterior cortex in the distressed condition, and these levels correlated well with a behavioural measure of distress (Burgdorf et al., 2006). A follow-up of this investigation could measure CCK levels – and release – immediately after the stage of play-fighting when it is still pleasant to the animals. An important study by Wiertelak et al. (1992) which found that CCK_B receptor blockade prevented the perception of the safety of the environment strongly emphasizes the dual nature of the CCK-ergic signal. Another interesting idea regarding the role CCK plays in coping with environment can be derived from the study which demonstrated that low doses of the CCK receptor agonist ceruletide, while not significantly influencing preattentive processing of stimuli or general cortical arousal, increased event-related potentials in a manner suggesting an improvement in selective attention (Schreiber et al., 1995). Enhancement of selective attention would be required in conditions of exposure to novel, potentially threatening or rewarding stimuli. Learned fear (but not confrontation with the aggressive resident in learned safety) increases CCK release in the prefrontal cortex (Becker et al., 2001). In this social defeat paradigm, the defeated animals are hyperalgesic, and CCK_B receptor blockade can reduce this hyperalgesia (Andre et al., 2005). The painful stimulus clearly increased CCK

release only in the previously stressed animals. Both acute morphine and chronic chlordiazepoxide suppressed CCK release; chlordiazepoxide did eliminate pain response but attenuated the stress-associated hyperalgesia. It can be concluded that prefrontal CCK release is mediating the anxiety component in pain syndrome. After chronic stress, CCK_B receptors in the paraventricular thalamus assume control over hormonal responses to acute novel stressors (Bhatnagar et al., 2000). As mentioned before, chronic stress can increase thalamic CCK mRNA expression (Giardino et al., 1999). Thus, the role of CCK in adapting with environmental signals depends upon the previous experiences of the subject, which may determine whether the net effect of CCK release is anxiety, panic, or perceived safety.

Confounding factors in the CCK and anxiety studies, and further directions

Animal studies on CCK and anxiety can easily become confounded by motivational effects related to interactions with dopamin- and glutamatergic mechanisms. Both are important in adaptive responses to novelty signals. Therefore the modulatory effects that endogenous CCK which is released during such encounters (Ladurelle et al., 1995) exerts over these other neurochemical mechanisms have the potential to be channeled into behaviours which can be interpreted as anxiety-related. Sometimes they probably are, but in other instances may rather reflect activation of neural substrates of other psychological domains. As an example, the CCK_B receptor knockout mice are likely to display increased activity in some locomotion-dependent anxiety tests not because of reduced anxiety but abnormally increased locomotor activity (Daugé et al., 2001). CCK modulates via both CCK_A and CCK_B receptors the dopaminergic neurotransmission which is crucial for the expression of psychomotor stimulation and the sensitization to psychostimulants (Wunderlich et al., 2000). Novelty-reactive neurobiological mechanisms play an important role in shaping the efficacy of drugs that have addictive properties due to their dopamine-potentiating effects (Piazza et al., 1991). CCK release triggered by novel aspects in experimental conditions clearly interferes with the motivational component of ongoing behaviour, but a comprehensive model of this interaction has been slow to emerge due to the complexity of details so far revealed. CCK_B receptor blockade does, in different paradigms, disinhibit fear-conditioned reduction of behavioural activity (Adamec et al., 1997; Farook et al., 2001). It should be examined whether this is due to a reduction in anxiety as an emotionally valenced phenomenon or direct disinhibition of acquired suppression of locomotion.

Further studies in this area should e.g., consider explicitly the possibility that CCK-dependent behaviours are highly associated with environmental contingencies. Dopamine-CCK interactions in the nucleus accumbens are important in conditioned reinforcement (Philips et al., 1993). When repeated administration of amphetamine together with very low doses of devazepide or L-365,260 was contingent with behavioural testing, both the augmenting and inhibitory effects of endogenous CCK acting through CCK_A and CCK_B receptors, respectively, on amphetamine stimulation and sensitization were revealed more clearly than in earlier studies which did not administer the drugs in stringent association with testing environment (Alttoa and Harro, 2004). Interaction of CCK-ergic mechanisms with endogenous opioid peptides (Kõks et al., 1998) provides another important issue which may both contribute to the pathogenesis of anxiety but also confound animal studies; this issue has very recently been reviewed thoroughly (Hebb et al., 2005). Available data are too indirect to support unequivocal interpretations, but the apparent intricate balance between the memory-enhancing and anxiety-provoking effects of CCK and the amnesic and anxiolytic effects of opioid peptides implies interference in encoding salient environmental cues.

Recent discovery of the orexin peptides and their contribution to arousal may explain another long-standing controversy in behavioural pharmacology of CCK. A significant minority of researchers consistently found that in their setting, not CCK_B but CCK_A receptor antagonists behaved as anxiolytics (e.g., Bickerdike et al., 1994 vs Matto et al., 1997). Orexins are involved in generation of anxiety both in mice and rats (Suzuki et al., 2005), and CCK excites orexin neurons, this effect being mediated by CCK_A receptors (Tsujino et al., 2005). CCK_A receptor-mediated neurotransmission seems to be necessary to maintain active behaviour in a repeatedly explored environment, as very low doses of devazepide can in such conditions reduce activity in rats (Kõiv and Harro, unpublished). Against this background, it is easy to imagine that when there is a high endogenous tone of orexins, CCK_A receptor blockade should be or at least appear anxiolytic. However, this remains to be experimentally demonstrated. The possibility thus exists that in some generalized anxiety patients, the receptor that should be blocked is rather the CCK_A receptor.

In conclusion, manipulations at CCK receptors may be beneficial only in rather specific conditions, when certain but yet ill-defined variables which emerge from the

individual's genes, past experiences and the immediate environment converge in a specific manner. For treatment strategies, this necessitates either careful preselection of patients who are responsive, or use of the CCK-ergic drugs together with other therapies, or combining CCK receptor activity with other neurochemical effects in a single ''dirty'' drug. Eventual success of any of these strategies is, for the time being, purely hypothetical, and subject to explicit experimental investigation.

NPY: an endogenous tranquillizer?

Neuropeptide Y was identified in the laboratory of Viktor Mutt at the Karolinska Institutet in Stockholm, where the amino acid sequence of CCK also had been specified. It may come as a surprise, but the number of hits in a PubMed search for ''NPY and anxiety'' is only about half of that of ''CCK and anxiety''. The anxiolytic properties of the ''anti-anxiety peptide'' were revealed about the same time as the anxiogenic-like effects of CCK, but limitations to further studies such as the absence of non-peptide receptor ligands and tools for experimental research in humans hindered the developments. It should nevertheless be noted that adding other related search words such as ''depression'' or ''alcohol'' would make the comparison between representations of CCK and NPY in literature more equal, because there is much preclinical evidence for the role of NPY in depression and alcoholism (Redrobe et al., 2002; Heilig, 2004a). These possibly related roles of NPY are, nevertheless, beyond the scope of this review. This abundant neuropeptide is very highly conserved in evolution, and it is the most powerful endogenous substance to counteract anxiety and the behavioural effects of stress (Heilig, 2004b). Indeed, NPY-treated animals have the appearance of as if treated with benzodiazepines.

NPY systems and pharmacology

NPY and related peptides and NPY receptor subtypes are widely distributed in the mammalian brain, but each has a specific location pattern (see Dumont et al., 2000; Kask et al., 2002). In brief, NPY is a highly conserved 36-amino acid peptide. Other two structurally related members of the NPY system are peptide YY (PYY) and pancreatic polypeptide (PP). Being one of the most abundant peptides in the brain, NPY is involved in a variety of physiological functions. Highest levels of expression are in the hypothalamus, locus coeruleus, septum, nucleus accumbens and periaqueductal grey. Moderate levels of NPY are found in the amygdala, hippocampus, neocortex, basal ganglia, and thalamus. NPY is also present in the major neural tracts of the brain, suggesting that it is being used by many long intracerebral pathways.

NPY is acting through several subtypes of G protein linked receptors. Both the peptide and receptor families are very complex and the presence of peptides and receptor proteins varies significantly along the evolutionary tree (Larhammar and Salaneck, 2004). Seven NPY receptor genes have been identified: Y1, Y2, Y4, Yb, Y5, Y6, and Y7. In mammals, Yb and Y7 are not found, and Y6 is functional only in a few species. Y_1 receptors have been detected in high quantities in the superficial layers of the cortex, in thalamus and in brainstem nuclei. Y_2 receptors are most prominent in the hippocampus, septum, and the brainstem. The PP-preferring Y_4 receptors are largely restricted to the medial pre-optic area, paraventricular nucleus of the hypothalamus, interpeduncular nuclei and area postrema. Y_5 receptors are predominantly found in the limbic structures and brainstem. Of the most studied receptors, Y_1 receptors are largely postsynaptic. Y_2 receptors are located presynaptically on NPY-ergic neurons, and control the release of NPY (King et al., 1999).

The majority of studies on anxiety have been carried out using different NPY-related peptides, with different receptor selectivity profiles. Recently, a number of non-peptide subtype-selective receptor antagonists have been developed (Holmes et al., 2003). These are mentioned in Table 1 and results obtained with these compounds form the backbone of the discussion below.

Evidence for the anti-anxiety efficacy of administrated and endogenous NPY in animal models

NPY and other Y_1 receptor stimulating peptides, administered ICV, decrease the preference of the rats for the closed arms and increase the time spent on open arms in the elevated plus-maze (Heilig et al., 1989; Broqua et al., 1995); as to the learned fear, fear-potentiated startle is also reduced. NPY produces dose-dependent anticonflict/anxiolytic-like effects in the Geller-Seifter test of operant responding (Heilig et al., 1992; Britton et al., 1997), which is an established animal model of anxiety especially suitable for detecting the effects of anxiolytics which reduce GABA-ergic function. Similarly, NPY can markedly increase the number of electric shocks accepted in the Vogel's punished drinking test (Heilig et al., 1989). At the doses applied, NPY was reported not to affect pain sensitivity in a shock threshold test, or thirst. Transgenic rats with enhanced expression of NPY are also resistant to

Table 1. Comparison of CCK and NPY systems involved in anxiety

	CCK	NPY
Endogenous agonists	CCK-8, possibly others	NPY, possibly others
Receptors cloned	CCK_A, CCK_B (CCK_1, CCK_2)	Y_1, Y_2, Y_4, Y_5, Y_6, Y_7, Y_b
Suggested receptors	CCK_B subtypes	Y_3
Agonists	caerulein (ns)	[$Leu^{31}Pro^{34}$]NPY (Y_1,Y_4, Y_5)
	CCK-4	$NPY_{13–36}$ (Y_2, Y_5)
	CCK-5	$NPY_{3–36}$ (Y_5, Y_1)
	SR146131 (CCK_A)	PYY (Y_2, Y_5, Y_1)
	BC264 (CCK_B)	HumanPP (Y_4, Y_5)
Antagonists	proglumide (low affinity)	BIBP3226 (Y_1)
	devazepide (CCK_A)	BIBO3304 (Y_1)
	L-365,260 (CCK_B)	1229U91 (Y_1)
	CI-988 (CCK_B)	BIIE0246 (Y_2)
	LY288513 (CCK_B)	CGP71683A (Y_5)
	PD135158 (CCK_B)	L152804 (Y_5)
Effects of agonists in animals		
– systemic administration	CCK_A no effect	lack of suitable ligands
	CCK_B anxiogenic	
ICV administration	CCK_B anxiogenic	Y_1 anxiolytic
		Y_2 anxiogenic or anxiolytic
–		Y_5 anxiolytic
Effects of antagonists in animals		
– systemic administration	contradictory evidence	lack of suitable ligands
		Y_5 anti-anxiolytic
– ICV administration	contradictory evidence	Y_1 anxiogenic
		Y_2 anxiolytic
Effects in humans		
– agonists	CCK_B panicogenic	no suitable ligand
– antagonists	no effect	no suitable ligand
Changes in suicide victims		
– peptide levels	–	lower (frontal cortex, caudate)
– receptor binding	CCK_B higher in cortex	–
– receptor expression	CCK_B higher in cortex and cerebellum	–
Behavioural tests revealing anxiety-related effects		
– exploration-based	yes	yes
– punished responding	no	yes
– potentiated startle	yes	yes
– social interaction	contradictory	yes
– defensive behaviour	weak effect	not studied
Brain regions implicated		
– cerebral cortex	+	–
– amygdala	+ (basolateral)	+ (central, basolateral)
– periaquaductal grey matter	+	+
– cerebellum	+	–
– septum	–	+ (dorsocaudal, ventral)
– hippocampus	+ (CA1, CA2)	+
– locus coeruleus	–	+
– thalamus	+ (paraventricular)	–
Interactions with other neurotransmitter systems		
– GABA	hippocampus, cortex, amygdala	amygdala (Y_1)
– Serotonin	lateral prefrontal cortex	–
– Noradrenaline	cerebral cortex	locus coeruleus (Y_2)
– CRF	CCK-4 anxiogenic effect blocked at CRF receptors	Y_1 blockade induced anxiety blocked at CRF receptors
– Glutamate	hippocampus (relevance to anxiety?)	hippocampus (relevance to anxiety?)
– Opioid	CCK-4 anxiogenic effect blocked by naloxone	NPY anxiolytic effect blocked by naloxone
– Dopamine	ventral tegmental area, nucleus accumbens (relevance to anxiety?)	ventral tegmental area, nucleus accumbens (relevance to anxiety?)

Only the most often used or unique receptor ligands are mentioned, ns denoting nonselectivity between known receptor subtypes. + Evidence for implication; – no clear evidence for implication. ? denotes questionable relevance of the association. See text for references

stress (Thorsell et al., 2000), and the effect of NPY in anxiety tests is stronger in rat substrains with reduced dipeptidyl-peptidase IV activity, presumably because of a slower degradation of the peptide (Karl et al., 2003). While NPY is a prominent cotransmitter of noradrenaline, the anxiolytic effect of NPY does occur even after extensive destruction of the nerve terminals projecting from the locus coeruleus (Kask et al., 2000) which otherwise impairs certain cognitive capacities and makes the animals more reactive and anxious in response to novelty. Comparatively, as described above, a CCK_B antagonist failed to reduce anxiety elicited by locus coeruleus denervation, which is sensitive to benzodiazepines (Harro et al., 1995).

The position of NPY as an endogenous anxiolytic is further strengthened by experiments demonstrating that inactivation or blockade of NPY receptors can serve as an anxiogenic stimulus. Before NPY receptors could have been pharmacologically blocked, Wahlestedt and colleagues (1993) demonstrated by use of the antisense technique that reduction of NPY receptor expression increases the plus-maze anxiety in rats. This study also confirmed that Y_1 is the receptor subtype mediating the anxiolytic action of NPY, as experiments with different NPY-like peptides had previously suggested. When the first non-peptide selective antagonist of NPY Y_1 receptors, BIBP3226 became available, ICV administration of this compound was found to have an anxiogenic effect in the elevated plus-maze (Kask et al., 1996). A low dose of diazepam completely normalized plus-maze exploration of BIBP3226-treated rats, thus confirming the anxiety-enhancing nature of the effect. Concerns about neurotoxicity and limited specificity of BIBP3226 probably do not relate to most of its actions in this study and others described below, because of the low doses of the compound used in anxiety paradigms (Kask et al., 2002). Another Y_1 receptor antagonist, BIBO3304, also displayed an anxiogenic profile in the open field while its inactive enantiomer did not (Kask and Harro, 2000). By use of BIBO3304, the anxiolytic effect of NPY in amygdala was also attributed to Y_1 receptors (Sajdyk et al., 1999). The anxiogenic effect of NPY Y_1 receptor antagonists in animal tests of anxiety is robust and much better reproducible than that of CCK receptor agonists. Blockade of Y_1 receptors is not only acutely anxiogenic, but produces learned aversion in the place conditioning paradigm (Kask et al., 1999).

Clinical evidence for a role of NPY in anxiety

The position of NPY in our understanding of the pathogenesis of anxiety is very strong as compared to CCK as far as animal models are concerned. Unfortunately, in case of NPY, the evidence for an anxiolytic effect in humans is lacking, as there has been no compound available for the critical experiment. Nevertheless, peripheral measures of NPY-ergic mechanisms suggest that low levels of NPY are associated with increased risk of mood and anxiety disorders (Holmes et al., 2003). Recent suicide attempt has been associated with lower plasma levels of NPY (Westrin et al., 1999). NPY levels have been found lower in frontal cortex and caudate nucleus in suicide victims as compared to age-matched subjects who died a sudden natural or accidental death (Widdowson et al., 1992); no difference between victims of suicide and controls was found in temporal cortex and cerebellum. Several studies have described lower NPY levels in plasma, CSF or brain in patients with major depression or bipolar disorder, and it is possible that these findings also reflect the anxiety component of affective disorders (see Heilig, 2004b for review).

NPY and NPY receptor subtypes in anxiety-related brain circuits

The effects of NPY receptor agonists and antagonists are observable after ICV administration, but many attempts have been made to localize the anxiety mechanisms that are controlled by NPY. Focus has been on the amygdala, where indeed administration of NPY has an anxiolytic effect. The initial suggestions of the primary involvement of the central nucleus are currently being re-evaluted to emphasize the role of lateral/basolateral amygdala (Heilig, 2004b). NPY-peptides can be anxiolytic when given to a number of other brain regions as well, such as locus coeruleus (Kask et al., 1998a) and dorsocaudal lateral septum (Kask et al., 2001). However, studies with receptor antagonists provide a better understanding of the brain regions where endogenous neurotransmitter is being released during anxiogenic challenges to reduce their behavioural consequences. When BIBP3226 was injected into a number of brain regions involved in stress response such as the central amygdala, paraventricular nucleus of the hypothalamus, locus coeruleus, and dorsal periaqueductal grey matter (DPAG), the blockade of Y_1 receptors had an anxiogenic effect in elevated plus-maze only when given to the DPAG (Kask et al., 1998b). This suggests that DPAG is a brain region where exposure to novel and potentially threatening stimuli elicits neurochemical changes which include NPY release to maintain active coping. The active dose in DPAG was considerably – an order of magnitude – lower than the dose effective after ICV administration. That Y_1 receptors in DPAG are ger-

mane to anxiety was further confirmed in a subsequent experiment in which two structurally unrelated NPY Y_1 receptor antagonists were found to elicit an anxiogenic-like effect in the social interaction test after local administration to the DPAG (Kask et al., 1998c).

That BIBP3226 did not elicit anxiety response when given to amygdala was quite unexpected and led to a conclusion that in this brain region, like in several others mentioned above, NPY-ergic mechanisms control anxiety phasically but not tonically (Kask et al., 2002). However, BIBP3226 had an anxiogenic effect in amygdala in the recent study by Primeaux and colleagues (2005) in a dose which was within the range inefficient in our study. Nevertheless, the drug was given bilaterally. If all other conditions could be considered comparable, then these studies raise the interesting possibility that release of NPY and Y_1 receptor activation in one amygdala is sufficient for preventing neophobia and anxiety.

GABA and NPY coexist in the neurons of the amygdaloid complex and NPY appears to influence local GABAergic activity via Y_1 receptors. Y_1 receptor expression in the amygdala was found to be increased by chronic administration of benzodiazepine receptor agonists and reduced by repeated inverse agonist treatment (Eva et al., 2004). Thus, in the amygdala, long-term GABA receptor modulation is coupled with Y_1 receptor mediated neurotransmission. NPY gene expression in amygdala is influenced by both acute and chronic stress, but in opposite ways: while a single episode of restraint stress rapidly downregulates NPY mRNA (Thorsell et al., 1998), repeated restraint stress exposure which leads to behavioural habituation is associated with increased NPY expression in amygdala (Thorsell et al., 1999).

As mentioned above, transgenic rats with enhanced expression of NPY are more resistant to stress, even though this does not show in mild stress conditions and requires relatively severe stressors such as restraint and electric shocks to to be presented (Thorsell et al., 2000). Interestingly, the brain regions contributing to the anxiolytic effect of NPY in pharmacological studies did not show any significant increase in NPY expression. The largest increase was observed in the hippocampal fields CA1 and CA2. Increase in the expression of NPY in hippocampus is a robust finding in studies of repeated presentation of stimuli which induce seizure activity (e.g., Fetissov et al., 2003). How this change in the NPY systems, apparently paralleling increased resistance to stress, relates to cognitive information processing in anxiety requires further studies.

In septum, the anxiolytic-like effect of NPY is regionally specific, being observed after administration to the dorsocaudal lateral septum but not to the largely cholinergic medial septum (Kask et al., 2001). In locus coeruleus, the anxiolytic-like effect of NPY follows Y_2 receptor profile (Kask et al., 1998a). As presynaptic Y_2 receptors on A6 neurons inhibit noradrenergic neurotransmission in the locus coeruleus projection areas (Heilig, 2004b), this effect is possibly related to a reduction of noradrenergic stimulation, which is anxiogenic in conditions perceived as adverse (Harro and Oreland, 2001).

Above, a speculative explanation was offered to the findings demonstrating a contribution of CCK_A instead of CCK_B receptors to anxiety. Thus, it was proposed that anxiety-producing orexin overactivity (Suzuki et al., 2005) can be elicited by CCK_A receptors (Tsujino et al., 2005). It should be noted that NPY tonically inhibits orexin neurons and orexin-induced arousal (Fu et al., 2004). We did not observe an anxiogenic effect of BIBP3226 when microinjected into hypothalamus (Kask et al., 1998b), but these injections were not targeted to the regions of orexin neurons and, perhaps more importantly, orexin neurons are tonically controlled not only by Y_1 but also by Y_2 or Y_5 receptors (Fu et al., 2004).

While the majority of studies have focused on the role Y_1 receptors play in anxiety, evidence is accumulating to implicate other receptor subtypes. NPY release is controlled in part by autoreceptors of the Y_2-subtype (King et al., 1999). Antagonism of these receptors should indirectly and perhaps in a more subtle manner augment the Y_1-mediated tranquillizing effect of NPY. Indeed, there is evidence for Y_2 receptor-mediated anxiogenic effect in the basolateral amygdala (Sajdyk et al., 2002). This result is compatible with the evidence obtained with mice lacking NPY Y_2 receptors: these animals were less anxious in three anxiety tests based on exploratory behaviour (Redrobe et al., 2003; Tschenett et al., 2003). These knockout mice appear, however, to be amazingly active in several behavioural paradigms, displaying very limited immobility in the forced swim test, and thus caution should be exercised in attributing these behavioural changes to an absence of anxiety in terms that make the data translatable to human conditions. Some evidence exists for an anxiolytic action of NPY mediated via the Y_5 receptor subtype. This mechanism has been described to mediate anxiety in the basolateral amygdala as measured in the social interaction test (Sajdyk et al., 2002), and the Y_5 receptor has also been implicated in the plus-maze anxiety in a study where the drugs were given ICV (Sorensen et al., 2004).

Expectations for, and limitations to, the use of the NPY-ergic anti-anxiety mechanism

NPY signalling in a broad network of neural circuits appears necessary for active coping in acutely stressful situations, and adaptive changes in these circuits during chronic stress increase resilience of the organism. Hence, the most obvious candidate drug would possess Y_1 receptor agonistic properties, but to be clinically useful, such a chemical compound has to possess good oral bioavailability and pass the blood–brain-barrier.

It has been suggested that NPY has a role in dampening the possible anxiety responses to CRF (Heilig et al., 1994) which is a universal signal of stress and thus normally useful (see Harro and Oreland, 2001 for discussion). Indeed, pretreatment with NPY in the basolateral amygdala reduces the anxiogenic-like effect of urocortin (Sajdyk et al., 2004). The antagonism between neural mechanisms which use CRF and NPY, respectively, can also work in the other way, the lack of the NPY signal being anxiogenic but possible to be overruled with reducing the effect of CRF. Thus, the blockade of CRF receptors by pretreatment with α-helical $CRF_{9–41}$ eliminated the anxiogenic effect of NPY receptor blockade (Kask et al., 1997). The stress response is a well-balanced physiological strategy involving multiple components and at present only a trial-and-error approach can answer the question whether pharmacological manipulations at NPY receptors can bring clinical benefits and in which instances. The hopes appear to be well justified, however. What may be of bigger concern is the other roles NPY plays in the CNS, which are hard to leave alone with a NPY-related anti-anxiety treatment.

NPY-systems are manipulated by presentation of several natural reinforcers and NPY is the most potent appetite enhancer known. The appetite enhancing action of endogenous NPY released by appropriate stimuli is in part mediated by Y_1 receptors (Kask et al., 1998d). Therefore a nonpeptide Y_1 receptor agonist, if developed, is not likely to serve as a useful anti-anxiety drug, unless in some way well balanced by another action of the drug preventing its influence in orexigenic pathways. Indeed, a messenger well conserved in evolution which promotes feeding, especially by increasing craving for energy-rich food, and simultaneously reduces fear of novel areas and even serves as an antinociceptive signal may well be the ultimate SEEKING peptide in terms of Panksepp's theory of the evolution of emotions (Panksepp, 1998). Along these lines, it could be speculated that the Y_1 receptor mediated anti-anxiety effect of NPY is part of a natural general food-seeking and feeding response: in search of food, reduction in anxiety and enhanced drive for motion (Kask and Harro, 2000) are advantageous. Indeed, diet restriction has an anxiolytic-like effect on rat behaviour in elevated plus-maze (Buyse et al., 2001). The area postrema-lesioned rat, a model of food motivated behaviour, overconsumes palatable foods, has lower anxiety, and shows increased NPY expression not only in the hypothalamus but also in the amygdala and hippocampus (Miller et al., 2002). It should be recalled that benzodiazepine anxiolytics also have appetite-increasing effect, but this is not comparable to that of NPY. Interestingly, when rats were given diazepam, BIBP3226 failed to reduce NPY-induced eating that was otherwise completely blocked by the Y_1 antagonist (Kask et al., 1998d). The orexigenic signal of NPY is mediated through different NPY receptor subtypes (e.g., Y_5 receptors). Hence this finding would support a speculation that one role of the Y_1 receptor in feeding response is to eliminate the negative influence anxiety may have on consummatory behaviour.

Among other mechanisms involved, NPY mediated reduction of orexin/hypocretin mediated arousal (Fu et al., 2004) may contribute to its anti-anxiety effects but also reduce necessary day-time alertness as a side effect. An alternative is to exploit the other NPY receptor mechanisms, but there is so far less conclusive evidence about their role in anxiety. A recent study which revealed the role of only Y_1 but not other NPY receptor subtypes in NPY-mediated fear-conditioned alterations of heart rate (Tovote et al., 2004) further emphasizes the significance of the best characterized NPY receptor subtype in the anxiety syndrome. Nevertheless, one could speculate that while NPY has a universal anti-stress effect via Y_1-receptors, more specific anxiety-related effects could be brought about via other receptors. In particular, available evidence suggests that Y_2-antagonists could serve as more selective anti-anxiety compounds.

On the other hand, despite of the fact that NPY Y_1 receptor mediated mechanisms are crucial for many behavioural functions besides dampening excessive arousal, Y_1-stimulating action may be beneficial for a carefully selected subset of patients with excessive worry and eating problems or chronic pain syndrome. Alternatively, Y_1-selective receptor agonism could be useful as a component in a complex pharmacological effect which includes control over food intake exploiting other mechanisms. These strategies require a non-peptide NPY Y_1 receptor agonist with adequate pharmacokinetic properties, but even before such a receptor ligand is made available, preclinical studies could examine the combination of Y_1 stimulation with neurochemical manipulations restrict-

ing feeding. The role of other NPY receptor subtypes should be further clarified using different animal models comparatively, and because NPY and NPY receptors prominently feature in preclinical studies of depression, studies on NPY might help to reveal what is behind the frequent comorbidity of anxiety and mood disorders. Indeed, symptoms of anxiety and depression usually coexist, appear both associated with dysfunction of stress-responsive systems, and thus the separation of affective and anxiety disorders in its present form may be not useful for understanding the biological basis of mental health (Sullivan and Kendler, 1998).

To conclude, further efforts to clarify the role of CCK and NPY in fear and anxiety, if focused on specific neural circuits, environmental contingencies and particular psychopathological syndromes, make a promising lead to novel treatment options and better strategies for selecting treatment for an individual patient.

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