Amino Acids (2006) 31: 241–250
DOI 10.1007/s00726-006-0333-y

Amino Acids
Printed in Austria

Corticotropin-releasing factor, vasopressin and receptor systems in depression and anxiety

Review Article

M. E. Keck

Division of Psychiatry Research, University Hospital of Psychiatry Zurich, Zurich, Switzerland

Received December 5, 2005
Accepted March 6, 2006

Summary. Affective disorders tend to be chronic and life-threatening diseases: suicide is estimated to be the cause of death in 10–15% of individuals with major depressive disorders. Major depression is one of the most prevalent and costly brain diseases with up to 20% of the worldwide population suffering from moderate to severe forms of the disease. Only 50% of individuals with depression show full remission in response to currently available antidepressant drug therapies which are based on serendipitous discoveries made in the 1950s. Previously underestimated, other severe depression-associated deleterious health-related effects have increasingly been recognized. Epidemiological studies have provided substantial evidence that patients with depression have a 2–4-fold increased risk both of developing cardiovascular disease and of mortality after experiencing a myocardial infarction. The majority of patients suffering from affective disorders have measurable shifts in their stress hormone regulation as reflected by elevated secretion of central and peripheral stress hormones or by altered hormonal responses to neuroendocrine challenge tests. In recent years, these alterations have increasingly been translated into testable hypotheses addressing the pathogenesis of illness. Refined molecular technologies and the creation of genetically engineered mice have allowed to specifically target individual genes involved in regulation of corticotropin releasing factor (CRF) and vasopressin (AVP) system elements. The cumulative evidence makes a strong case implicating dysfunction of these systems in the etiology and pathogenesis of depression and pathological anxiety. Translation of these advances into novel therapeutic strategies has already been started.

Keywords: Corticotropin releasing factor (CRF) – Vasopressin (AVP) – Depression – Anxiety – CRF receptor antagonist – R121919 – NBI 30775 – CRF receptor type 1 – CRF receptor type 2 – Transgenic mice – Conditional knockout

A spotlight on the clinical situation

Hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) system in affective disorders is the most prominent and well-documented neuroendocrine change in these diseases (Tichomirowa et al., 2005), and it has been proposed that this feature is in some way integral to the pathogenesis and maintenance of psychopathology (Holsboer, 1999a, b, 2000; Nemeroff, 1996; Nemeroff and Owens, 2002). Accordingly, hyperactivity of central neuropeptidergic circuits such as corticotropin releasing factor (CRF; also termed corticotropin releasing hormone, CRH) and vasopressin (AVP) neuronal systems is thought to play a causal role in the etiology and symptomatology of mood and anxiety disorders (Gold et al., 1984; Hökfelt et al., 2000; Holsboer, 2000). This is reflected by increased numbers of CRF- and AVP-secreting neurons in the hypothalamus and locus coeruleus of depressed patients (Bissette et al., 2003; Purba et al., 1996; Raadsheer et al., 1994), increased levels of CRF in the cerebrospinal fluid, and characteristic changes in neuroendocrine challenge tests, such as the combined dexamethasone/CRF challenge test (Holsboer, 1999a, b, 2000). In major depression, the combined dexamethasone/CRF test, in which dexamethasone-pretreated subjects receive a single dose of CRF, has proven to be the most sensitive tool for the detection of altered hypothalamic-pituitary-adrenocortical (HPA) regulation (Heuser et al., 1994). The more severe the patient's depression, i.e. patients with psychotic features and melancholia, the more robust the HPA hyperactivity (Nemeroff, 1996). In recent years, a considerable amount of evidence has been accumulated suggesting that normalization of the HPA system might be the final step necessary for stable remission of the disease (Barden et al.,

1995; Holsboer, 2000; Ising et al., 2005; Zobel et al., 1999). Antidepressant drugs, in turn, have been shown to attenuate and normalize HPA system abnormalities (De Bellis et al., 1993; Heuser et al., 1996; Kling et al., 1994; Nemeroff et al., 1991). Although – with respect to stress hormone regulation – the clinical picture is less clear and exact pathophysiological mechanisms underlying anxiety disorders such as panic disorder have not been identified yet, distinct biological hypothesis have been posited and preclinical and clinical studies point towards the importance of stress hormone regulation as one major pathological condition in anxiety (Keck et al., 2005; Schreiber et al., 1996). Taken together, the clinical findings available so far support the hypothesis that both, depression and anxiety disorders, share impaired HPA system regulation, supporting the notion that the impairment is causally involved in the pathophysiology of these clinical conditions.

It has to be emphasized, however, that although these findings derived from peripheral HPA assessments in depressed patients led to the concept of CRF and AVP hyperactivity, it is now clear that central CRF/AVP neuropeptidergic circuits other than those driving the peripherally accessible HPA system may well be overactive and act on behaviorally relevant extra-pituitary brain sites.

Hypothalamic-pituitary-adrenocortical (HPA) system – a stress-responsive system

Stress has repeatedly been demonstrated to precipitate major depression and to influence its severity and course (Kessler et al., 1994). In the field of neurobiology, stress is an internal or external cue that disrupts the homeostatic status of a subject. One major neuroendocrine system underlying an individual's capacity to cope with stress is the HPA axis. CRF is the primary hypothalamic hypophysiotropic hormone, which regulates both basal and stress-induced release of pituitary corticotropin (ACTH) and is the major constituent of the HPA system (Vale et al., 1981). ACTH from the anterior pituitary, subsequently leads to release of glucocorticoid hormones (GC, cortisol in humans and corticosterone in rats and mice) from the adrenal cortex. At the pituitary level, the effects of CRF are amplified by AVP, which, after prolonged stress, is increasingly co-expressed and co-secreted from parvocellular hypothalamic CRF neurons (Antoni, 1993; Keck et al., 2000, 2002). These parvocellular neurons project to the anterior pituitary where CRF and AVP are released into hypophyseal portal blood vessels to activate the HPA system by triggering ACTH release from pituitary corticotropes through activation of CRF 1 receptors (CRF1) and AVP 1b (V1b) receptors. Furthermore, independently of their action at the pituitary level, these two neuropeptides act as neurotransmitters and neuromodulators in several behaviorally relevant brain areas such as the amygdala and the hippocampus.

Several research groups proposed a hypothesis relating aberrant stress hormone regulation to causality of depression (for review: Buckingham, 2006; Holsboer, 1999a, b, 2000; Korte, 2001; Nemeroff, 1996; Nemeroff and Owens, 2002). The, for example, ''corticosteroid receptor hypothesis'' submits that corticosteroid receptor signaling is impaired in depression (Holsboer, 2000). Specifically, based on the finding of increased CRF and AVP levels which account for a large number or the signs and symptoms prevalent in depression and anxiety disorders, it can be hypothesized that corticosteroid receptor signaling, exerting a negative feedback on AVP and CRF gene expression, is defunct (Keck and Holsboer, 2001; Reul and Holsboer, 2002).

CRF and family

Beyond CRF, three other mammalian neuropeptides of the CRF family are known, urocortin 1 (Ucn 1; Vaughan et al., 1995), urocortin 2 (Ucn 2; Reyes et al., 2001), and urocortin 3 (Ucn 3; Lewis et al., 2001) the latter of which is an N-terminally shortened sequence of stresscopin (Hsu and Hsueh, 2001). CRF peptides share four amino acids with each other and secondary structure is thought to determine biological activity (Dautzenberg and Hauger, 2002). Human Ucn 2 lacks the standard consensus site required for proteolytic cleavage and C-terminated amidation, which is a prerequisite for biological potency (Dautzenberg and Hauger, 2002). It has to be kept in mind, therefore, that human Ucn 2 may not be processed into a biologically active peptide in vivo (Hauger et al., 2003).

Two non-mammalian CRF-like peptides, the 40-amino-acid amphibian peptide sauvagine and the 41-amino-acid fish peptide urotensin I share about 50% sequence identity with human CRF. CRF 1 receptor (CRF_1) and CRF 2 receptor (CRF_2) differ in their ligand affinities for CRF, Ucn 1, Ucn 2 and Ucn 3 (CRF_1: Ucn 1 > CRF; CRF_2: Ucn 1 > Ucn 2 > Ucn 3 $\gg$ CRF) (Chalmers et al., 1996; Donaldson et al., 1996; Lovenberg et al., 1995). Compared to CRF, Ucn 1 has an approximately 100-fold higher affinity for the CRF_2 and a roughly 6-fold higher affinity for CRF_1 (Dautzenberg et al., 2001; Vaughan et al., 1995). Ucn 2 and Ucn 3 display specific affinity for the

CRF_1 at very high local concentrations only. Of known agonists, Ucn 3 displays the highest degree of selectivity in binding to the CRF_2. It is, therefore, likely that CRF and Ucn 1 represent the natural agonists for the CRF_1, whereas Ucn 1, Ucn 2 and Ucn 3 are likely to be the natural ligands for the CRF_2 with CRF remaining a candidate natural ligand for the CRF_2 at sufficiently high local concentrations. It is to emphasize, that it cannot be ruled out that yet undiscovered receptors exist.

In brief, the central distribution of Ucn 1, Ucn 2, and CRF_2 expressing neurons suggests that Ucn 1 may serve as the major CRF_2 ligand in the hindbrain whereas Ucn 3 may serve as the major CRF_2 ligand in the forebrain. Ucn 2 or a novel endogenous ligand may signal at CRF_2 expressed in brain regions lacking Ucn 1 or Ucn 3 innervation, e.g. the hippocampus (Hauger et al., 2003).

CRF receptors

Specific, high-affinity, G protein-coupled seven-transmembrane membrane receptors mediate the biological actions of CRF, Ucn 1, Ucn 2 and Ucn 3. To date, two distinct mammalian receptor subtypes have been characterized: CRF_1 and CRF_2 display a markedly different tissue distribution and pharmacological specificity (Chalmers et al., 1995; Steckler and Holsboer, 1999). In general, CRF_1 has been proposed to mediate the effects of CRF on HPA system function and anxiety-related behaviour (Liebsch et al., 1999, 1995; Skutella et al., 1998), whereas CRF_2 might be predominantly involved in the regulation of feeding behaviour (Spina et al., 1996), cardiovascular function and the recovery phase of the HPA response (Coste et al., 2000). A complex role for CRF_2 in modulating anxiety-related behaviour, however, is also very likely. Highest densities of CRF_1 mRNA have been described in the anterior pituitary, cerebral cortex, cerebellum, amygdala, hippocampus and olfactory bulb (Chalmers et al., 1995; Sanchez et al., 1999). Although there is some controversy, in non-human primates CRF_1 mRNA could also be localized in the locus coeruleus (Sanchez et al., 1999). Basal expression of CRF_1 mRNA within the PVN is very low. However, in the rat, but not in the mouse, PVN CRF_1 mRNA could be induced via stress exposure or intra-PVN CRF microinfusion (Imaki et al., 2003, 2001; Konishi et al., 2003). In the periphery, low levels of CRF_1 mRNA occur in the testis, ovary, retina and adrenal gland. The CRF_2 receptor family has additional diversity in that splice variants have been described: $CRF_{2(a)}$ and $CRF_{2(b)}$ (Chalmers et al., 1995; Lovenberg et al., 1995). The $CRF_{2(a)}$ receptor is expressed primarily in rat subcortical neuronal populations (lateral septum, amygdala, hippocampus, paraventricular nucleus of the hypothalamus), whereas the $CRF_{2(b)}$ is expressed in non-neuronal cells in the central nervous system (e.g., cerebral arterioles and choroid plexus). Peripherally, $CRF_{2(b)}$ mRNA is found in rodent cardiac myocytes, lung, ovary and skeletal muscle (Chalmers et al., 1995; Lovenberg et al., 1995). In contrast, in humans, $CRF_{2(a)}$ is the major peripheral splice variant (Dautzenberg et al., 2001). Taken together, with respect to species-specific differences, it is of importance that in the monkey brain both CRF_1 and CRF_2 were found in the pituitary and throughout the neocortex (i.e., in prefrontal, cingulate, striate, and insular cortices), amygdala, and hippocampal formation (Sanchez et al., 1999). In the rat and mouse brain only the CRF_1 is found in the anterior pituitary and the CRF_2 is present only at low expression levels in the neocortex. These results suggest that, in primates, both CRF_1 and CRF_2 may be involved in mediating the effects of CRF on cognition, behavior, and HPA system function. The presence of CRF_1 (but not CRF_2) within the amygdala, cerebellar cortex, nucleus of the solitary tract, thalamus, and striatum and of CRF_2 (but not CRF_1) receptors in the choroid plexus, certain hypothalamic nuclei, the nucleus prepositus, and the nucleus of the stria terminalis suggests that each receptor subtype also may have distinct functional roles within the primate central nervous system.

In humans only, a third functional splice variant, $CRF_{2(c)}$, has been identified which is expressed in selected brain areas, such as the septum and hippocampus and at lower levels in the amygdala, nucleus accumbens, midbrain and frontal cortex (Kostich et al., 1998).

As there still is some controversy whether or not CRF_1 or CRF_2 have been conclusively identified in some important stress-sensitive brain structures such as the locus coeruleus and the central nucleus of the amygdala (but see Plotsky et al., 2005; Sauvage and Steckler, 2001) it is possible that a novel CRF receptor is waiting to be cloned (Hauger et al., 2003).

The role of CRF receptors in anxiety

CRF_1 antagonistic approaches have anxiolytic-like properties in most, but not all anxiety paradigms (Griebel et al., 1998; Keck et al., 2001; Liebsch et al., 1995). The effectiveness of CRF_1 blockade to reduce anxiety is likely to depend on the animal's stress level and/or innate trait anxiety (Keck et al., 2001). Beyond CRF_1, recent pharmacological data point towards a complex involvement of

the CRF_2 in anxiety and stress-related behaviors and there is some controversy in the current literature about it's precise functional role. The role of CRF_2 in anxiety is likely complicated, and the site of action appears to be critical. Central administration of Ucn 1, an endogenous ligand for CRF_2, has been shown to induce a variety of effects, including behavioral consequences such as increased anxiety (Moreau et al., 1997; Slawecki et al., 1999). However, as Ucn 1 can bind and activate both CRF receptor subtypes, i.c.v. administered Ucn 1 might activate receptors non-selectively in areas where endogenous Ucn 1 may not exist. Interestingly, activation of the CRF_2 can result in either anxiolysis or anxiogenesis depending on when the animal is tested and, possibly, where the receptor is localized (Reul and Holsboer, 2002; Takahashi, 2001). Acute antagonism of $CRF_{2(a)}$ in the rat lateral septum, which abundantly expresses $CRF_{2(a)}$ but not CRF_1, produced a behaviorally, anatomically and pharmacologically specific reduction in stress-induced defensive behavior as measured by shock-induced freezing (Bakshi et al., 2002). CRF_2 in the dorsal raphé nucleus, where serotonergic neurons innervating the hippocampus emerge, has been shown to mediate the behavioral consequences, i.e. learned helplessness and increased anxiety, of uncontrollable stress (Hammack et al., 2003).

To date, combining molecular genetics with behavioral pharmacology studies with antisense probes in rats that selectively reduce CRF receptor subtype levels and genetically engineered mouse models (see below) have indicated that CRF_1 might be the primary target of interest at which selective compounds should be directed to treat pathological anxiety (Keck and Holsboer, 2001; Liebsch et al., 1995, 1998; Overstreet et al., 2005; Skutella et al., 1998; Timpl et al., 1998). It has to be kept in mind that, in addition to CRF_1 hyperfunction, CRF_2 dysfunction might play an important role in both causality and treatment of mood and anxiety disorders and that an altered CRF_2-mediated ''anxiolysis'' might result in an extended state of anxiety and arousal. Moreover, the animal studies mentioned account for the rodent situation whereas in humans and other primates the role of CRF_2 is likely more important due to its higher expression levels and wider distribution pattern (Dautzenberg and Hauger, 2002).

Vasopressin (AVP): growing importance

Beyond hyperactivity of central CRH neuropeptidergic circuits, AVP neuronal systems are thought to play a causal role in the aetiology and symptomatology of anxiety disorders (Hökfelt et al., 2003; Keck and Holsboer, 2001; Keck et al., 2002, 2003; Landgraf and Holsboer, 2005). AVP release occurs from dendrites, somata, and axons of neurosecretory neurons. Upon demand, both diffuse spread into the extracellular fluid following dendritic release and focal release from axon terminals contributes to regionally and temporally varying combinations of neuromodulator and neurotransmitter actions (Landgraf and Neumann, 2004). AVP has been shown to exert both behavioural effects such as, e.g., increased anxiety following intracerebroventricular administration, and to increase CRH-induced ACTH secretion from pituitary corticotrope cells (Antoni, 1993; Bhattacharya et al., 1998; Landgraf et al., 1995). After prolonged stress, AVP is increasingly expressed and released from hypothalamic neurons in both humans and rodents (Antoni, 1993). Recently, the involvement of hypothalamic AVP in benzodiazepine-induced HPA-system attenuation could be demonstrated (Welt et al., 2006). In clinical studies, plasma AVP concentrations were found to be significantly correlated with anxiety-related symptoms in healthy volunteers in response to an anxiogenic drug challenge (Abelson et al., 2001) and were demonstrated to be elevated in depressed patients (van Londen et al., 1997). Accordingly, administration of the non-peptide AVP 1b (V_{1b}) receptor antagonist SSR149415 was shown to display anxiolytic and antidepressant-like effects in rodents (Griebel et al., 2002, 2003). Similarly, the AVP 1a (V_{1a}) receptor, which is highly expressed in the rat lateral septum, thalamic nuclei and the amygdalostriatal transition area (Barberis and Tribollet, 1996), is well known to play a role in a variety of behaviours such as the modulation of emotionality and stress coping (Landgraf et al., 1998). Specifically, septal AVP has been shown to increase anxiety-related behaviour in rats (Ebner et al., 1999; Landgraf et al., 1995). Accordingly, in rats displaying an innately increased trait anxiety, V_{1a}-binding sites were higher in the lateral septum when compared to low-anxiety rats (Keck et al., 2003). In these high-anxiety rats also elevated levels of intra-PVN AVP were found and chronic administration of paroxetine, a clinically well established antidepressant, normalized aberrant behavioural and neuroendocrine patterns in this psychopathological animal model (Keck et al., 2003). As it could be demonstrated that a hypothalamic vasopressinergic hyperdrive (secondary to an impaired repression at an AVP promoter polymorphism) accounts for the disturbance in HPA system regulation prevalent in these rats (Keck et al., 2002; Murgatroyd et al., 2004), the paroxetine-induced reduction of vasopressinergic overexpression indicates that this neuropeptidergic system may be critically involved in the

action of antidepressant drugs known to be effective in the treatment of anxiety disorders (Keck et al., 2003).

Testing for anxiety when investigating for depression: a conundrum?

Anxiety is a common core symptom in depressed patients and there is a high comorbidity rate between anxiety and depressive disorders (Hettema et al., 2001; Roy-Burne et al., 2000). Moreover, anxiety disorders seem to precede the development of depression suggesting that there might be a continuum between these disorders with common pathophysiological features. Because both disorders imply inappropriate adaptation to stressors they are viewed as stress-related disorders suggesting a causal role of HPA system dysregulation (for review see de Kloet et al., 2005). Although anxiety disorders and depression have been classified as separate types of disorders for decades, there is a longstanding debate about whether anxiety and depression constitute different aspects of the same disorder or distinct, yet overlapping, conditions (Kendler, 2001; Nemeroff, 2002; Persons et al., 2003). The absence of a clear therapeutic demarcation is also of considerable interest: a variety of classic antidepressants such as serotonin reuptake inhibitors and tricyclics, are successfully used to treat anxiety disorders since many years. Animal models conceptualized to elucidate mechanisms underlying depression, therefore, often show altered anxiety-related behavior. With respect to CRF neuronal circuits, the possibility exists, however, that CRF might be mostly related to the anxiety symptomatology seen in many depressed patients. This could explain the wide variability observed in depressed patients in terms of HPA system function, with some patients having anxiety symptoms more than others (Holsboer, 1999a, b, 2000, 2003a, b).

Genetic targeting of CRF receptors – recent highlights

Conditional inactivation of limbic CRF_1

Studies on conventional knockout mice deficient for CRF_1 firmly established the requirement of pituitary CRF_1 for endocrine responses to stress (Smith et al., 1998; Timpl et al., 1998): in CRF_1 null mutants, both basal and stress-induced HPA system activity are markedly impaired (Müller et al., 2000; Preil et al., 2001; Timpl et al., 1998). Moreover, conventional knock-out of CRF_1 results in reduced anxiety-related behavior (Smith et al., 1998; Timpl et al., 1998). The behavioral analyses of CRF_1 null mutants, however, are hampered by the fact that CRF_1 knockout mice display severe glucocorticoid deficiency (Smith et al., 1998; Timpl et al., 1998). As glucocorticoids play important roles in modulating fear and anxiety-related behavior (Korte, 2001; Korte et al., 1996), the anxiolytic effect observed in conventional CRF_1 knockout mice may, therefore, result from either CRF_1 deficiency itself or be influenced by a marked reduction in circulating glucocorticoid hormone levels in these animals. The lack of circulating glucocorticoids may also result in developmental deficiencies what could easily influence behavioral characteristics.

To address this question and to dissect CRF/CRF_1 central nervous system pathways modulating behavior from those regulating neuroendocrine function, a conditional CRF_1 knockout using the *Cre/loxP* system (Lewandoski, 2001) driving *Cre* recombinase expression by a Calcium Calmodulin-kinase IIα (*CaMKIIα*) promotor (Minichiello et al., 1999) was generated recently (Müller et al., 2003). The *CaMKIIα* gene is expressed with tissue-specificity predominantly in the mouse anterior forebrain during postnatal development with high expression levels in hippocampal neurons (pyramidal and granule cell layer), cortical layers and the amygdala (Solà et al., 1999). Selective disruption of CRF/CRF_1 signaling pathways in behaviorally relevant limbic neuronal circuitries significantly reduces anxiety-related behavior (Müller et al., 2003). The robust anxiety-reduced phenotype of $Crf_1^{loxP/loxP}$ *CaMKIIαCre* conditional mutants could be confirmed in two different behavioral paradigms based on the natural avoidance behavior of mice, the dark-light box paradigm and the elevated plus-maze test. In contrast to CRF_1 null mutants, basal plasma ACTH and corticosterone levels are similar to wildtype levels in $Crf_1^{loxP/loxP}$ *CaMKIIαCre* conditional mutants. The behavioral phenotype of conditional CRF_1 mutants, therefore, is not likely to be influenced by central nervous system effects of circulating stress hormones.

Plasma ACTH and corticosterone levels are virtually identical between wildtype mice and $Crf_1^{loxP/loxP}$ *CaMKIIαCre* conditional mutants under basal conditions and immediately following 2, 5 or 10 min of acute immobilization stress. However, hormone levels remain significantly elevated in $Crf_1^{loxP/loxP}$ *CaMKIIαCre* conditional mutants 30 and 90 min, following a 5-min period of restraint stress. These data provide the first evidence that limbic CRF_1 is required for central control of HPA system feedback and hormonal adaptation to stress.

Taken together, the data from $Crf_1^{loxP/loxP}$ *CaMKIIαCre* conditional mutants underline the importance of limbic

CRF_1 in modulating anxiety-related behavior. Furthermore, the findings underline the clinical assumption that central CRF/CRF_1 neuropeptidergic circuits, other than those driving the peripherally accessible HPA system, may well be overactive and could be therapeutic targets (for review see Holsboer, 2003a, b).

Conventional CRF_2 knockout: increased anxiety-related behavior?

Behavioral and endocrine analysis of CRF_2 knockout mice has provided a less clear picture as compared to CRF_1 mutants and, consequently, the physiological role of CRF_2 in mediating anxiety-like behavior has been the subject of controversy. Differences in aspects of both the endocrine and behavioral phenotype were described between three independently created knockout mouse lines deficient for the CRF_2 (Bale et al., 2000; Coste et al., 2000; Kishimoto et al., 2000). This phenomenon points towards the fact that most likely the genetic background of genetically engineered mice plays a crucial role, especially when dealing with subtle behavioral alterations (Crusio, 2004; Lathe, 1996). Moreover, it is possible that beyond environmental interactions in different laboratories/housing facilities differences in compensatory gene expression are involved (Bale et al., 2000, 2002; Crabbe et al., 1999).

The behavioral performance reveals significant differences between the three independently created CRF_2 deficient mouse lines: whereas Coste et al. (2000) found no differences in anxiety-related behavior, Bale et al. (2000) and Kishimoto et al. (2000) detected a significant increase in anxiety-like behavior in their CRF_2 mutants. Interestingly, the latter behavioral phenotype could be observed only in male, but not in female CRF_2 deficient mice. Recently, the CRF_2 knockouts developed by Bale and coworkers were tested in the forced swim test where they displayed increased immobility as an indicator of depression-like behavior. When treated with the CRF_1 antagonist antalarmin the time spent immobile was found to be decreased while swimming and climbing, i.e. active stress coping behaviors, increased (Bale and Vale, 2003). Although there were no controls to indicate whether antalarmin reduces depression-like behavior in CRF_2 wildtype mice, the effectiveness of CRF_1 antagonism might be explained by the previous finding that CRF_2 deficient mice show increased CRF levels in the central nucleus of the amygdala and increased Ucn 1 levels in the Edinger Westphal nucleus (Bale et al., 2000). The specific interaction, however, between CRF_2 deletion effects and CRF_1 on depression-like behavior remains unproven.

V_{1b} receptor knockout

In the rat, extrahypothalamic AVP-containing neurons have been characterized mainly in the medial amygdala and the BNST, which innervate behaviourally relevant limbic structures such as the lateral septum and the ventral hippocampus. In these brain regions AVP acts as a neurotransmitter, exerting its action by binding to specific G protein-coupled receptors, i.e. V_{1a} and V_{1b} (Barberis and Tribollet, 1996; Hernando et al., 2001). V_{1b} receptor-null mutant mice show significantly reduced aggression when tested in a resident-intruder paradigm (Wersinger et al., 2002). This finding might also be indicative of a decreased anxiety-related behaviour. Anxiety-related behaviour on the elevated plus-maze, however, was found to be indistinguishable between knockouts and wildtype mice. This observation is in line with the finding that infusion of an V_{1a} antisense oligodeoxynucleotide into the rat lateral septum has been shown to exert anxiolytic effects (Landgraf et al., 1995) pointing towards the fact that in the context of anxiety-related behaviour the V_{1a} receptor subtype might be more important. With respect to HPA system regulation, basal and stress-induced plasma corticosterone concentrations showed no difference between V_{1b} receptor-null mutant mice and their wildtype littermates. Concerning the important role of AVP in learning and memory, V_{1b} knockouts displayed a slight impairment in the social recognition test (olfactory-cued memory) but not in the Morris water maze task (spatial memory) although this observation might be secondary to a reduced social motivation (Wersinger et al., 2002, 2004).

Conclusion

Targeted gene mutation has become an established tool for increasing our knowledge about neuropeptide functions in the central nervous system (Keck et al., 2005). As currently available treatments for affective disorders, which are amongst the most pervasive and costly brain diseases (Michaud et al., 2001), are far from being fully satisfactory in many patients there is a need for novel treatment strategies based on innovative neurobiological concepts (Berton and Nestler, 2006). The integrated information especially from the newer conditional mutant mice together with the increasing knowledge on the role of single nucleotide polymorphisms in psychiatric disorders

prophesies an enormous progress (e.g., Binder et al., 2004; Holsboer, 2001a, b; Leonardo and Hen, 2006). There is reason to believe that efficient strategies to characterize the deleterious effects of CRF hypersecretion in psychiatric diseases should include antagonism of CRF effects through CRF_1 blockade. This may ultimately open a new lead in the treatment of stress-related disorders such as depression and anxiety disorders. Among others (review: Heinrichs and Koob, 2004; Keck et al., 2005; Li et al., 2005; Müller and Wurst, 2004; Saunders and Williams, 2003; Takahashi, 2001), one compound recently examined is the high-affinity non-peptide CRF_1 antagonist NBI 30775 (R121919). In an open-label trial (phase IIa) in patients suffering from major depression, treatment with NBI 30775/R121919 led to a significant reduction in both anxiety and depression scores (Chen and Grigoriadis, 2005; Keck and Holsboer, 2001; Künzel et al., 2003; Zobel et al., 2000). Taken together, CRF_1 antagonists represent a promising new treatment modality for both depression and pathological anxiety that emerged from closely interrelated clinical and preclinical research.

References

Abelson JL, Le Melledo JM, Bichet DG (2001) Dose response of arginine vasopressin to the CCK-B agonist pentagastrin. Neuropsychopharmacology 24: 161–169

Antoni FA (1993) Vasopressinergic control of pituitary-adrenocorticotropin secretion comes of age. Frontiers Neuroendocrinol 14: 76–122

Bakshi VP, Smith-Roe S, Newman SM, Grigoriadis D, Kalin NH (2002) Reduction of stress-induced behavior by antagonism of corticotropin-releasing hormone 2 receptors in lateral septum or corticotropin-releasing hormone 1 receptors in amygdala. J Neurosci 22: 2926–2935

Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, Koob GF, Vale WW, Lee KF (2000) Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nature Genet 24: 410–414

Bale TL, Picetti R, Contarino A, Koob GF, Vale W, Lee KF (2002) Mice deficient for both corticotropin-releasing factor receptor 1 (CRFR1) and CRFR 2 have an impaired stress response and display sexually dichotomous anxiety-like behavior. J Neurosci 22: 193–199

Bale TL, Vale WW (2003) Increased depression-like behaviors in corticotropin-releasing factor receptor-2-deficient mice: sexually dichotomous responses. J Neurosci 23: 5295–5301

Barberis C, Tribollet E (1996) Vasopressin and oxytocin receptors in the central nervous system. Crit Rev Neurobiol 10: 119–154

Barden N, Reul JM, Holsboer F (1995) Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system? [Review] [53 refs]. Trends Neurosci 18: 6–11

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nature Rev Neurosci 7: 137–151

Bhattacharya SK, Bhattacharya A, Chakrabarti A (1998) Anxiogenic activity of intraventricularly administered arginine vasopressin in the rat. Biogenic Amines 14: 367–385

Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Pütz B, Papiol S, DSeaman S, Lucae S, Kohli MA, Nickel T, Künzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Köhnlein O, Dabitz H, Brückl T, Mülller N, Pfister H, Lieb R, Mueller JC, Lohmussar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B (2004) Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nature Genet 36: 1319–1325

Bissette G, Klimek V, Pan J, Stockmeier C, Ordway G (2003) Elevated concentrations of cortocotropin-releasing factoF in the locus coeruleus of depressed subjects. Neuropsychopharmacology 28: 1328–1335

Buckingham JC (2006) Glucocorticoids: exemplars of multi-tasking. Br J Pharmacol 147: S258–S268

Chalmers DT, Lovenberg TW, De Souza EB (1995) Localization of novel corticotropic-releasing factor receptor 2 (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. J Neurosci 15: 6340–6350

Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB (1996) Corticotropin-releasing factor receptors: from molecular biology to drug design. Trends Pharmacol Sci 17: 166–172

Chen C, Grigoriadis DE (2005) NBI 30775 (R121919), an orally active antagonist of the corticotropin-releasing factor (CRF) type-1 receptor for the treatment of anxiety and depression. Drug Dev Res 65: 216–226

Coste SC, Kesterson RA, Heldwein KA, Stevens SL, Heard AD, Hollis JH, Murray SE, Hill JK, Pantely GA, Hohimer AR, Hatton DC, Phillips T, Finn DA, Low MJ, Rittenberg MB, Stenzel P, Stenzel-Poore MP (2000) Abnormal adaptations to stress and impaired cardiovascular function in mice lacking CRH receptor-2. Nature Genet 24: 403–409

Crabbe JC, Wahlsten D, Dudek BC (1999) Genetics of mouse behavior: interactions with laboratory environment. Science 284: 1670–1672

Crusio WE (2004) Flanking gene and genetic background problems in genetically manipulated mice. Biol Psychiatry 56: 381–385

Dautzenberg FM, Hauger RL (2002) The corticotropin-releasing factor peptide family and their receptors: yet more partners discovered. Trends Pharmacol Sci 23: 71–77

Dautzenberg FM, Kilpatrick GJ, Hauger RL, Moreau JL (2001) Molecular biology of the corticotropin-releasing hormone receptors – in the mood. Peptides 22: 753–760

De Bellis MD, Gold PW, Geracioti TDJ, Listwak SJ, Kling MA (1993) Association of Fluoxetin treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. Am J Psychiatry 150: 656–657

de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nature Rev Neurosci 6: 463–475

Donaldson CJ, Sutton SW, Perrin MH, Corrigan AZ, Lewis KA, Rivier JE (1996) Cloning and characterization of human urocortin. Endocrinology 137: 2167–2170

Ebner K, Wotjak CT, Holsboer F, Landgraf R, Engelmann M (1999) Vasopressin released within the septal brain area during swim stress modulates the behavioural stress response in rats. Eur J Neurosci 11: 997–1002

Gold PW, Chrousos GP, Kellner C, Post RM, Roy A, Augerinas P, Schulte H, Oldgield EH, Loriaux DL (1984) Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. Am J Psychiatry 141: 619–627

Griebel G, Perrault G, Sanger DJ (1998) Characterization of the behavioral profile of the non-peptide corticotropin-releasing factor receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. Psychopharmacology 138: 55–66

Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, Geslin M, Scatton B, Maffrand JP, Soubrie P (2002) 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)-ethyl]5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor (1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. J Pharmacol Exp Ther 301: 333–345

Griebel G, Simiand J, Stemmelin J, Serradeil-Le Gal C, Steinberg R (2003) The vasopressin V1b receptor as a therapeutic target in stress-related disorders. Curr Drug Targ CNS Neurol Disorders 2: 191–200

Hammack SE, Schmid MJ, LoPresti ML, Der-Avakian A, Pellymounter MA, Foster AC, Watkins LR, Maier SF (2003) Corticotropin-releasing hormone type 2 receptors in the dorsal raphe nucleus mediate the behavioral consequences of uncontrollable stress. J Neurosci 23: 1019–1025

Hauger RL, Grigoriadis DE, Dallman MF, Plotsky PM, Vale WW, Dautzenberg FM (2003) International Union of Pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. Pharmacol Rev 55: 21–26

Heinrichs SC, Koob GF (2004) Corticotropin-releasing factor in brain: a role in activation, arousal, and affect regulation. J Pharmacol Exp Ther 311: 427–440

Hernando F, Schoots O, Lolait SJ, Burbach JPH (2001) Immunohistochemical localization of the vasopressin V1b receptor in the rat brain and pituitary gland: anatomical support for its involvement in the central effects of vasopressin. Endocrinology 142: 1659–1668

Hettema JM, Prescott CA, Kendler KS (2001) A population-based twin study of generalized anxiety disorder in men and women. J Nerv Mental Dis 189: 413–420

Heuser I, Yassouridis A, Holsboer F (1994) The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. J Psych Res 28: 341–356

Heuser IJE, Schweiger U, Gotthardt U, Schmider J, Lammers CH, Dettling M, Yassouridis A, Holsboer F (1996) Pituitary-adrenal system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and in normal control subjects. Am J Psychiatry 153: 93–99

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

Hökfelt T, Broberger C, Xu ZQD, Sergeyev V, Ubink R, Diez M (2000) Neuropeptides – an overview. Neuropharmacology 39: 1337–1356

Holsboer F (1999a) Animal models of mood disorders. In: Charney DS, Nestler EJ, Bunney BS (eds) Neurobiology of mental illness. Oxford University Press, New York, pp 317–332

Holsboer F (1999b) The rationale for the corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. J Psych Res 33: 181–214

Holsboer F (2000) The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23: 477–501

Holsboer F (2001a) Antidepressant drug discovery in the postgenomic area. World J Biol Psychiatry 2: 165–177

Holsboer F (2001b) Prospects for antidepressant drug discovery. Biol Psychol 57: 47–65

Holsboer F (2003a) Corticotropin-releasing hormone modulators and depression. Curr Opin Invest Drugs 4: 46–50

Holsboer F (2003b) The role of peptides in treatment of psychiatric disorders. J Neural Transm 64: 17–34

Hsu S, Hsueh A (2001) Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat Med 7: 605–611

Imaki T, Katsumata H, Konishi SI, Kasagi Y, Minami S (2003) Corticotropin-releasing factor type-1 receptor mRNA is not induced in mouse hypothalamus by either stress or osmotic stimulation. J Neuroendocrinol 15: 916–924

Imaki T, Katsumata H, Miyata M, Naruse M, Imaki I, Inami S (2001) Expression of corticotropin-releasing factor, urocortin and corticotropin-releasing factor type 1 receptors in hypothalamic-hypophyseal systems under osmotic stimulation. J Neuroendocrinol 13: 328–338

Ising M, Kunzel HE, Binder EB, Nickel T, Modell S, Holsboer F (2005) The combined dexamethasone/CRH test as a potential surrogate marker in depression. Progr Neuro-Psychopharmacol Biol Psychiatry 29: 1085–1093

Keck ME, Hatzinger M, Wotjak C, Landgraf R, Holsboer F, Neumann ID (2000) Ageing alters intrahypothalamic release patterns of vasopressin and oxytocin in rats. Eur J Neurosci 12: 1487–1494

Keck ME, Holsboer F (2001) Hyperactivity of corticotropin-releasing hormone neuronal circuits as a target for therapeutic interventions in affective disorders. Peptides 22: 835–844

Keck ME, Ohl F, Holsboer F, Müller MB (2005) Listening to mutant mice: a spotlight on the role of CRF/CRF receptor systems in affective disorders. Neurosci Biobehav Rev 29: 867–889

Keck ME, Welt T, Müller MB, Uhr M, Ohl F, Wigger A, Toschi N, Holsboer F, Landgraf R (2003) Reduction of hypothalamic vasopressinergic hyperdrive contributes to clinically relevant behavioral and neuroendocrine effects of chronic paroxetine treatment in a psychopathological rat model. Neuropsychopharmacology 28: 235–243

Keck ME, Welt T, Wigger A, Renner U, Engelmann M, Holsboer F, Landgraf R (2001) The anxiolytic effect of the corticotropin-releasing hormone 1 receptor antagonist R121919 depends on innate emotionality in rats. Eur J Neurosci 13: 373–380

Keck ME, Wigger A, Welt T, Müller MB, Gesing A, Reul JMHM, Holsboer F, Landgraf R, Neumann I (2002) Vasopressin mediates the response of the combined dexamethasone/CRH test in hyperanxious rats: Implications for pathogenesis of affective disorders. Neuropsychopharmacology 26: 94–105

Kendler KS (2001) Twin studies of psychiatric illness. Arch Gen Psychiatry 58: 1005–1014

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen H-U, Kendler KS (1994) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: results from the National Comorbidity Survey. Arch Gen Psychiatry 51: 8–19

Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, Hooshmand F, Hermanson O, Rosenfeld MG, Spiess J (2000) Deletion of corticotropin-releasing hormone receptor-2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nature Genet 24: 415–419

Kling MA, Geracioti TD, Licinio J, Michelson D, Oldfield EH, Gold PW (1994) Effects of electroconvulsive therapy on the CRH-ACTH-cortisol system in melancholic depression: preliminary findings. Psychopharmacol Bull 30: 489–494

Konishi S, Katsumata H, Kasagi Y, Minami S, Imaki T (2003) Regulation of corticotropin-releasing factor (CRF) type-1 receptor gene expression by CRF in the hypothalamus. Endocrine J 50: 21–36

Korte SM (2001) Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci Biobehav Rev 25: 117–142

Korte SM, Korte-Bouws GA, Koob GF, De Kloet ER, Bohus B (1996) Mineralocorticoid and glucocorticoid receptor antagonists in animal models of anxiety. Pharmacol Biochem Behav 54: 261–267

Kostich WA, Chen A, Sperle K, Largent BL (1998) Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF2gamma receptor. Mol Endocrinol 12: 1077–1085

Künzel HE, Zobel AW, Nickel T, Ackl N, Uhr M, Sonntag A, Ising M, Holsboer F (2003) Treatment of depression with the corticotropin-releasing hormone-1-receptor antagonist R121919: endocrine changes and side effects. J Psych Res 37: 525–533

Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F, Engelmann M (1995) Release of vasopressin and oxytocin within the brain and into blood: microdialysis and antisense targeting. In: Saito T, Kurokawa K, Yoshida S (eds) Neurohypophysis: recent progress of vasopressin and oxytocin research. Elsevier, Amsterdam, pp 243–256

Landgraf R, Gerstenberger R, Montkowski A, Probst JC, Wotjak C, Holsboer F (1995) V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discriminiation abilities and anxiety-related behavior in rats. J Neurosci 15: 4250–4258

Landgraf R, Holsboer F (2005) The involvement of neuropeptides in evolution, signaling, behavioral regulation and psychopathology: Focus on vasopressin. Drug Dev Res 65: 185–190

Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Frontiers Neuroendocrinol 25: 150–176

Landgraf R, Wotjak CT, Neumann ID, Engelmann M (1998) Release of vasopressin within the brain contributes to neuroendocrine and behavioral regulation. Progr Brain Res 119: 201–220

Lathe R (1996) Mice, gene targeting and behaviour: more than just genetic background. Trends Neurosci 19: 183–186

Leonardo ED, Hen R (2006) Genetics of affective and anxiety disorders. Annu Rev Psychol 57: 117–137

Lewandoski M (2001) Conditional control of gene expression in the mouse. Nature Rev Genet 2: 743–755

Lewis K, Li C, Perrin M, Blount A, Kunitake K, Donaldson C, Vaughan J, Reyes T, Gulyas J, Fischer W, Bilezikjian L, Rivier J, Sawchenko P, Vale W (2001) Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc Natl Acad Sci USA 98: 7570–7575

Li YW, Fitzgerald L, Wong H, Lelas S, Zhang G, Lindner MD, Wallace T, McElroy J, Lodge NJ, Gilligan P, Zaczek R (2005) The pharmacology of DMP696 and DMP904, non-peptidergic CRF1 receptor antagonists. CNS Drug Rev 11: 21–52

Liebsch G, Landgraf R, Engelmann M, Lorscher P, Holsboer F (1999) Differential behavioural effects of chronic infusion of CRH 1 and CRH 2 receptor antisense oligonucleotides into the rat brain. J Psych Res 33: 153–163

Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A (1995) Chronic infusion of a corticotropin-releasing hormone 1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-releated behavior in socially defeated rats. Regul Peptides 59: 229–239

Liebsch G, Linthorst ACE, Neumann ID, Reul JMHM, Holsboer F, Landgraf R (1998) Behavioral, physiological, and neuroendocrine stress responses and differential sensitivity to diazepam in two wistar rat lines selectively bred for high- and low-anxiety-related behavior. Neuropsychopharmacology 19: 381–396

Lovenberg TW, Chalmers DT, Liu C, De Souza EB (1995) Corticotropin-releasing factor 2 alpha and corticotropin-releasing factor 2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology 136: 4139–4142

Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T (1995) Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype. Proc Natl Acad Sci USA 92: 836–840

Michaud CM, Murray CJ, Bloom BR (2001) Burden of disease: implications for future research. J Am Med Assoc 285: 535–539

Minichiello L, Korte M, Wolfer DP, Kühn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R (1999) Essential role of trkB receptors in hippocampus-mediated learning. Neuron 24: 401–414

Moreau J, Kilpatrick G, Jenck F (1997) Urocortin, a novel neuropeptide with anxiogenic-like properties. Neuroreport 8: 1697–1701

Müller MB, Landgraf R, Sillaber I, Kresse AE, Keck ME, Zimmermann S, Holsboer F, Wurst W (2000) Selective activation of the hypothalamic vasopressinergic system in mice deficient for the corticotropin-releasing hormone receptor 1 is dependent on glucocorticoids. Endocrinology 141: 4262–4269

Müller MB, Wurst W (2004) Getting closer to affective disorders: the role of CRH receptor systems. Trends Mol Med 10: 409–415

Müller MB, Zimmermann S, Sillaber I, Hagemeyer TP, Deussing JM, Timpl P, Kormann MSD, Droste S, Kühn R, Reul JMHM, Holsboer F, Wurst W (2003) Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behaviour and is required for hormonal adaptation to stress. Nature Neurosci 6: 1100–1107

Murgatroyd C, Wigger A, Frank E, Singewald N, Bunck M, Holsboer F, Landggraf R, Spengler D (2004) Impaired repression at a vasopressin promotor polymorphism underlies overexpression of vasopressin in a rat model of trait anxiety. J Neurosci 24: 7762–7770

Nemeroff CB (1996) The corticotropin-releasing factor (CRF) hypothesis of depression: new finding and new directions. Mol Psychiatry 1: 336–342

Nemeroff CB (2002) Comorbidity of mood and anxiety disorders: The rule, not the exception? Am J Psychiatry 159: 3–4

Nemeroff CB, Bissette G, Akil H, Fink M (1991) Neuropeptide concentrations in the cerebrospinal fluid of depressed patients treated with electroconvulsive therapy: corticotropin-releasing factor, b-endorphin, and somatostatin. Br J Psychiatry 158: 59–63

Nemeroff CB, Owens MJ (2002) Treatment of mood disorders. Nature Neurosci 5: 1068–1070

Overstreet DH, Knapp DJ, Breese GR (2005) Can CRF1 receptor antagonists become antidepressant and/or anxiolytic agents? Drug Dev Res 65: 191–204

Persons JB, Roberts NA, Zalecki CA (2003) Anxiety and depression change together during treatment. Behav Ther 34: 149–163

Plotsky PM, Thrivikraman KV, Nemeroff CB, Caldji C, Sharma S, Meaney MJ (2005) Long-term consequences of neonatal rearing on central corticotropin-releasing factor systems in adult male rat offspring. Neuropsychopharmacology 30: 2192–2204

Preil J, Müller MB, Gesing A, Reul JMHM, Sillaber I, van Gaalen M, Landgrebe J, Stenzel-Poore M, Holsboer F, Wurst W (2001) Regulation of the hypothalamic-pituitary-adrenocortical system in mice deficient for CRH receptors 1 and 2. Endocrinology 142: 4946–4955

Purba JS, Hoogendijk WJG, Hofman MA, Swaab DF (1996) Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. Arch Gen Psychiatry 53: 137–143

Raadsheer FC, Hoogendijk WJG, Stam FC, Tilders FJH, Swaab DF (1994) Increased number of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. Neuroendocrinology 60: 436–444

Reul JMHM, Holsboer F (2002) Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. Curr Opin Pharmacol 2: 23–33

Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, Arias CA, Hogenesch JB, Gulyas J, Rivier J, Vale WW, Sawchenko PE (2001) Urocortin II: A member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc Natl Acad Sci USA 98: 2843–2848

Roy-Burne PP, Stang PE, Wittchen H-U, Ustun TB, Walters EE, Kessler RC (2000) Lifetime panic- depression comorbidity in the national comorbidity survey. Association with symptoms, impairment, course and help-seeking. Br J Psychiatry 176: 229–235

Sanchez MM, Young LJ, Plotsky PM, Insel TR (1999) Autoradiographic and in situ hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. J Comp Neurol 408: 365–377

Saunders J, Williams J (2003) Antagonists of the corticotropin releasing hormone receptor. Progr Med Chem 41: 195–247

Sauvage M, Steckler T (2001) Detection of corticotropin-releasing hormone receptor 1 immunoreactivity in cholinergic, dopaminergic and noradrenergic neurons of the murine basal forebrain and brainstem nuclei – Potential implication for arousal and attention. Neuroscience 104: 643–652

Schreiber W, Lauer CJ, Krumrey K, Holsboer F, Krieg JC (1996) Dysregulation of the hypothalamic-pituitary-adrenocortical system in panic disorder. Neuropsychopharmacology 15: 7–15

Skutella T, Probst JC, Renner U, Holsboer F, Behl C (1998) Corticotropin-releasing hormone receptor (type 1) antisense targeting reduces anxiety. Neuroscience 85: 795–805

Slawecki CJ, Somes C, Rivier JE, Ehlers CL (1999) Neurophysiological effects of intracerebroventricular administration of urocortin. Peptides 20: 211–218

Smith GW, Aubry J-M, Dellu F, Contarino A, Bilezikijan LM, Gold LH, Chen R, Marchuk Y, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee K-F (1998) Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20: 1093–1102

Solà C, Tusell JM, Serratosa J (1999) Comparative study of the distribution of calmodulin kinase II and calcineurin in the mouse brain. J Neurosci Res 57: 651–662

Spina M, Merlo-Pich E, Chan RKW, Basso AM, Rivier J, Vale W, Koob GF (1996) Appetite-suppressing effects of Urocortin, a corticotropin-releasing factor-related neuropeptide. Science 273: 1561–1564

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

Takahashi LK (2001) Role of cortocotropin-releasing factor 1 and corticotropin-releasing factor 2 receptors in fear and anxiety. Neurosci Biobehav Rev 25: 627–636

Tichomirowa MA, Keck ME, Schneider HJ, Paez-Pereda M, Renner U, Holsboer F, Stalla GK (2005) Endocrine disturbances in depression. J Endocrinol Invest 28: 89–99

Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JMHM, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W (1998) Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nature Genet 19: 162–166

Vale W, Spiess J, Rivier C, Rivier J (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213: 1394–1397

Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, Rivier J, Sawchenko PE, Vale W (1995) Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378: 287–292

Welt T, Engelmann M, Renner U, Erhardt A, Müller MB, Landgraf R, Holsboer F, Keck ME (2006) Temazepam triggers the release of vasopressin into the rat hypothalamic paraventricular nucleus: novel insights into benzodiazepine action on hypothalamic-pituitary-adrenocortical system regulation during stress. Neuropsychopharmacology (in press) (DOI: 10.1038/sj.npp.1301006)

Wersinger SR, Ginns E, Lolait SJ, Young Ill WS (2002) Vasopressin V1b receptor knockout reduces aggressive behavior in male mice. Mol Psychiatry 7: 975–984

Wersinger SR, Kelliher KR, Zufall F, Lolait SJ, O'Carroll AM, Young WS (2004) Social motivation is reduced in vasopressin 1b receptor null mice despite normal performance in an olfactory discrimination task. Hormones Behav 46: 638–645

Zobel FA, Nickel T, Künzel 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 Psych Res 34: 171–181

Zobel FA, Yassouridis A, Frieboes R-M, Holsboer F (1999) Prediction of medium-term outcome by cortisol response to the combined dexamethasone/CRH test in patients with remitted depression. Am J Psychiatry 156: 949–951

Author's address: Martin E. Keck, MD, PhD, Division of Psychiatry Research, University Hospital of Psychiatry Zurich, Minervastrasse 145, CH-8032 Zurich, Switzerland,
Fax: +41-44-3891414, E-mail: martin.keck@puk.zh.ch