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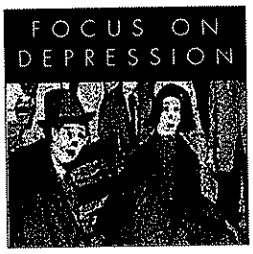

CRF Receptor Antagonists: A New Approach to the Treatment of Depression

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Corticotropin-Releasing Factor

Although its existence has been recognized since the 1950s, corticotropin-releasing factor (CRF) was first isolated and characterized by Vale and colleagues in 1981 [1]. In the 19 years since the elucidation of the sequence of this 41 amino acid peptide, a burgeoning database has accrued concerning the neural and endocrine roles of this peptide. CRF is a member of a family of peptides found in different species that act as agonists at CRF receptors (vide infra). These peptides include the frog skin peptide, sauvagine, the teleost fish urophysis peptide, urotensin I, and the mammalian peptide urocortin. It is now well established that CRF is the primary physiological secretagogue in mammals controlling hypothalamic-pituitary-adrenal (HPA) axis activity. CRF containing neurons in the paraventricular nucleus of the hypothalamus project nerve terminals to the median eminence where CRF is released into the hypothalamo-hypophysial portal venous system. These vessels transport CRF to anterior pituitary corticotrophs where the peptide stimulates the synthesis and secretion of adrenocorticotropin (ACTH), which, in turn, acts upon adreno-cortical cells to release glucocorticoids—principally, cortisol in primates and corticosterone in rodents [2]. This represents the classic neuroendocrine pathway by which mammals respond to stress.

Distribution and Pharmacology of the CRF System

Studies completed shortly after the initial characterization of CRF indicated a widespread, but heterogenous distribution throughout the brain. The presence of CRF neurons and processes in cortical, limbic, and autonomic CNS regions suggested other possible neurobiological roles for this peptide distinct from its classic function as a secretagogue for ACTH and other POMC-derived peptides in the adrenohypophysis. Studies that have been conducted since have revealed that CRF modulates not only the endocrine responses to stress, but the autonomic, immunologic, and behavioral responses as well [2].

Two CRF receptor subtypes, CRF_1 and CRF_2, with distinct anatomical localization and receptor pharmacology have been identified. The CRF_1 receptor was cloned from human and rat DNA; it is a protein containing 415 amino acids and comprises 7 putative transmembrane spanning regions similar to that of other members of the G-protein

coupled receptor family [3]. Several groups have now cloned, sequenced and expressed subtypes of the CRF_2 receptor from rat, mouse and human cDNA libraries. The rat CRF_{2A} receptor encodes a 411-amino acid G-protein coupled receptor with approximately 70% homology to the CRF_1 receptor [3]. In addition, a novel third receptor for CRF has been cloned in catfish [4], though a mammalian homolog has yet to be cloned.

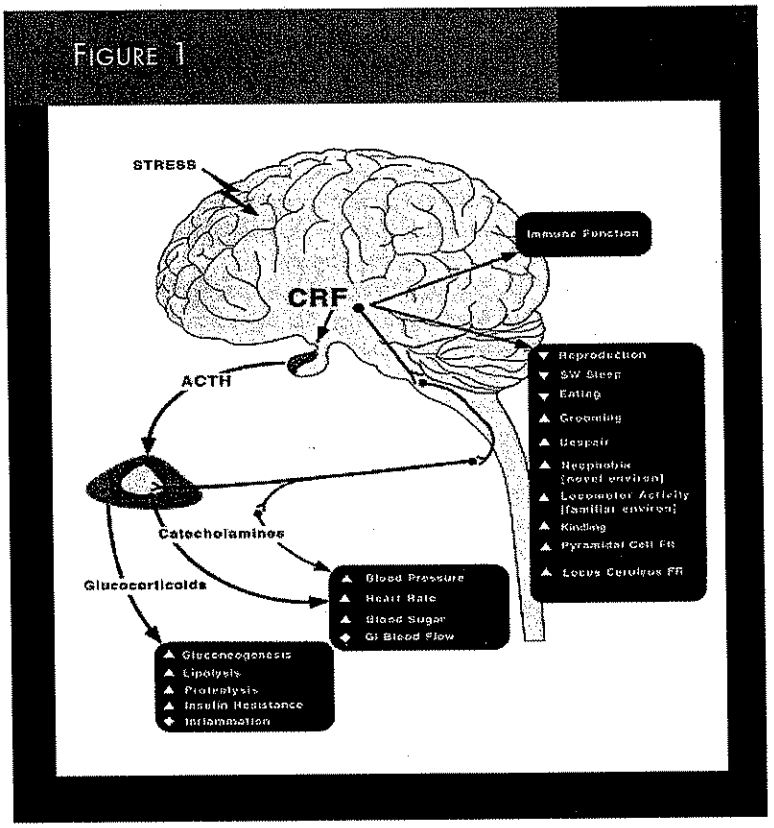
The pharmacological profile of the rat CRF_{2A} and CRF_{2B} receptors and the human CRF_2 receptor are similar and have been assessed in cells selectively expressing the various CRF receptors. Binding affinity and cyclic adenosine monophosphate (cAMP) responsiveness have also been examined. The most notable finding is the lower potency of CRF, compared with that of urocortin, for CRF_2 receptors. In addition, the newly discovered peptide urocortin II shows selective binding at CRF_2 receptors [5].

Additional findings can be summarized as follows (see [3] for review):

- CRF has high affinity for CRF_1 receptors and the CRF-binding protein (CRF-BP) [inhibition constant (K_i) <1 nmol/L].
- CRF has approximately 10-fold lower, but still relatively high, affinity for rat and human CRF_2 receptors compared with CRF_1 receptors.
- Urocortin has high affinity (K_i <1 nmol/L) for CRF_1 and CRF_2 receptors, and the CRF-BP.
- Urocortin II has high affinity (K_i < 1 nmol/L) for CRF_2 receptors, with very low affinity at CRF_1 receptors (K_i > 100 nmol/L).

In the rat, CRF_{2A} receptor mRNA is most prominently expressed in the lateral septum and ventromedial nucleus of the hypothalamus [6, 7]. Relatively high expression is also observed in the paraventricular and supraoptic nuclei of the hypothalamus, olfactory bulb, and amygdala. CRF_{2A} mRNA expressing cells are also observed in the bed nucleus of the stria terminalis, the hippocampal formation, anterior and lateral hypothalamic areas, and arterioles in the CNS. Amygdaloid CRF neurons project from the central nucleus of the amygdala through the bed nucleus of the stria terminalis to widespread regions of the basal forebrain and brain stem [8] including lateral hypothalamus, dorsal raphei nuclei, parabrachial region and the solitary tract. The presence of CRF in the amygdala is of particular interest due to the amygdala's proposed role in modulating affective behaviors. The extrahypothalmic distribution of CRF in these regions, taken in light of the known or suspected function of these regions, is consistent with a major role for CRF in mediating the stress response and mood [9]. The presence of CRF immunoreactivity in both serotonergic (dorsal raphe) and noradrenergic regions (locus coeruleus) also deserves further attention. These systems have long been implicated to play a role in the pathophysiology of depression and anxiety, and are believed to be the principal targets of a large number of the currently available psychotherapeutic drugs.

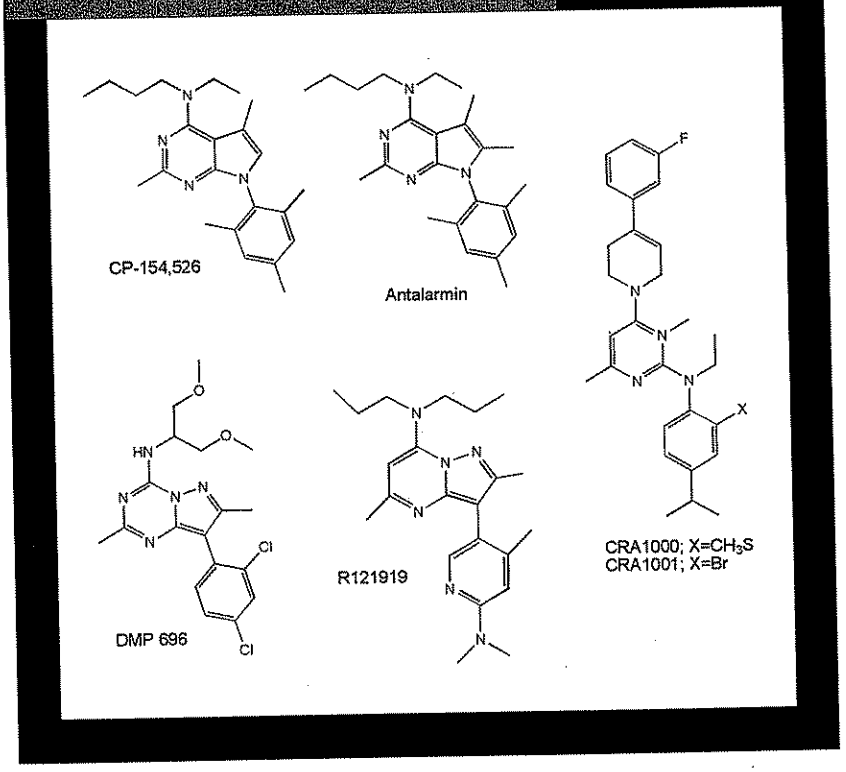
FIGURE 1



Overview of the hypothalamic-pituitary-adrenal axis and a schematic representation of the endocrine, behavioral, autonomic, and immunologic responses to stress mediated by central CRF neurons. AMY, amygdala; AP, anterior pituitary; E, epinephrine; HIP, hippocampus; LC, locus coeruleus; NE, norepinephrine; PVN, paraventricular nucleus; GI gastrointestinal; FR, firing rate; SW, slow wave. Reproduced by permission of the Society for Endocrinology [9].

While CRF_{2A} mRNA expression appears to be almost exclusively in the CNS, CRF_{2B} mRNA expression appears in both the brain and periphery, with greatest abundance in the heart, skeletal muscle, and choroid plexus. Indeed, it appears that CRF_{2A} mRNA is found predominantly on neurons and that CRF_{2B} mRNA is located predominantly on non-neuronal cells (e.g. choroid plexus, arterioles, and cardiac muscle). Lovenberg et al. [6] noted the conspicuous absence of either CRF_2 receptor subtype in the pituitary, cerebral cortex, or cerebellum, which are all prominent sites of CRF_1 receptor mRNA expression. In the rat, neither the distribution of CRF_{2A} or CRF_{2B} mRNA have significant overlap with each other or the previously described distribution of CRF_1 mRNA. These differences in CRF receptor subtype expression levels are particularly evident within the paraventricular nucleus where CRF_{2A} receptor expression is readily evident, while CRF_1 receptor mRNA is present only

FIGURE 2



Chemical structure of several CRF_1 receptor antagonists.

in scattered cells. The distribution of cells expressing CRF_{2A} receptor mRNA within the PVN coincides with the cellular distribution of CRF mRNA suggesting a possible autoreceptor role for CRF_{2A} receptors in this nucleus. In contrast to the above data from rats, preliminary evidence suggests that there is prominent CRF_2 receptor expression in human (Owens et al., unpublished observations) and nonhuman primate cortex [10]. The significant expression of CRF_2 receptors in primate cortex and limbic systems suggests that selective CRF_2 receptor agonists or antagonists may be of therapeutic interest.

Mice with targeted knockouts of the CRF_1 receptor have been generated, and demonstrate an impaired stress response [11]. The CRF_1 receptor knockout mice were less anxious than their wild type littermates when tested in the elevated plus maze, a commonly used behavioral paradigm. In addition, these transgenic animals demonstrated a significant reduction in stress-induced release of ACTH and corticosterone. Recently, CRF_2 receptor knockout mice have also been generated [12, 13]. In contrast to the CRF_1 receptor knockouts, these mice demonstrated increased anxiety-like behaviors and hypersensitivity to stress. This suggests the presence of two separate, but interrelated systems that can coordinately and inversely regulate the stress response.

CRF and Behavior

High affinity CRF binding sites, presumably CRF receptors, identified by autoradiography or 'grind and bind' methods are heterogeneously distributed in the CNS in a pattern not dissimilar to that of CRF itself. CRF binding site density and mRNA expression have shown alterations in both preclinical and clinical studies, presumably in response to changes in CRF availability. Our group has previously reported a marked (23%) reduction in the number of CRF binding sites in the frontal cortex of suicide victims compared with the controls [14]; we have now replicated this finding in a second study. In a seminal study, Raadsheer et al demonstrated an increase in CRF-mRNA expression in the paraventricular nucleus of depressed patients compared to controls [15]. This illustrates the well documented phenomena of receptor 'up and down' regulation suggesting that CRF is hypersecreted in a subset of depressed patients.

As noted above, CRF receptors are involved in modulating the behavioral responses to stress. A number of studies have shown that central administration of CRF produces behaviors that are very similar to that observed following exposure to stress and are not dissimilar from those observed in patients with major depression [2, 9, 16, 17].

Peptide based CRF antagonists (α-helical CRF_{9-41} or D-Phe CRF_{12-41}) were first developed over 15 years ago [2]. Most of the early work in the field used these compounds to elucidate the role of CRF in various behaviors. However due to the chemical nature of these compounds, they do not cross the blood-brain barrier and need to be infused directly to the site of action in the brain.

CRF receptor antagonists have shown activity in both animal models of anxiety and depression. CRF receptor antagonists have been tested in many different paradigms including elevated plus-maze, foot shock, restraint stress, and defensive withdrawal. The general outcome in these experiments has been that pretreatment with a CRF receptor antagonist decreases measures of anxiety induced by these stressors. There is also some evidence that CRF receptor antagonists may reduce the effects of drug withdrawal and stress-induced relapse to drug-seeking in rats [18-20]. In rats undergoing ethanol withdrawal, treatment with the CRF antagonist α-helical CRF_{9-41} reversed the 'anxiogenic' response to ethanol withdrawal as assessed in an elevated plus-maze [21]. Later studies in animals withdrawn from chronic cocaine administration indicated a CRF antagonist

might attenuate some of the 'anxiogenic-like' responses during acute drug withdrawal [22].

Although relatively little is known regarding the neurobiology of the CRF_2 receptor system, these receptors are located in regions known to be involved in integrative aspects of stress-related behaviours (e.g. hypothalamus, lateral septum, dorsal raphe and, at least in primates, cerebral cortex). Recently it has been shown that chronic administration of the triazalobenzodiazepine alprazolam decreases CRF mRNA expression in the central nucleus of the amygdala and decreases CRF_1 mRNA expression and binding in the basolateral amygdala, while in contrast, urocortin mRNA expression in the Edinger-Westphal nucleus and CRF_{2A} receptor binding in the lateral septum and ventromedial hypothalamus are *increased* [20]. This relationship suggests antiparallel regulation of CRF_1 and CRF_2 receptor expression and furthermore that CRF neuronal systems may contain two separate subdivisions that regulate and are regulated by stress, anxiety, and anxiolytic drugs. Because alprazolam has anxiolytic effects, the upregulation seen following chronic administration suggests activation of CRF_2 receptors may be in part responsible for anxiolysis. Studies with the new CRF_2 receptor specific peptide, urocortin II, may help to further tease apart the functions of these two receptor subtypes [5]. Initial experiments have shown that urocortin II attenuates nighttime feeding with a time course different from CRF itself, but does not increase spontaneous locomotor activity. Interestingly, although urocortin II binds specifically to the CRF_2 receptor, its pattern of distribution does not broadly mimic the expression pattern of the CRF_2 receptor.

CRF and Depression

Stressful live events are well documented to precipitate depression, and recent studies have convincingly revealed that adverse early life events produce persistent alterations in the CNS, thus predisposing such individuals to affective illness as adults. Moreover, hyperactivity of the HPA axis in a substantial subgroup of patients with major depression remains one of the most consistent findings in biological psychiatry. The reported HPA axis alterations include hypercortisolemia, enlarged adrenal and pituitary glands, resistance of cortisol secretion to suppression by the synthetic glucocorticoid dexamethasone, elevated ACTH responses to CRF following dexamethasone administration, elevated cerebrospinal fluid CRF concentrations, and increased CRF mRNA expression in the hypothalamus. Changes in CRF or CRF receptor mRNA expression in extrahypothalamic regions has not previously been reported though decreased CRF receptor binding has been observed in the prefrontal cortex of suicide victims [23]. Although we have hypothesized that this down regulation of CRF binding in frontal cortex is a compensatory response to chronic CRF hypersecretion, the exact pathological mechanism(s) underlying HPA axis dysregulation in major depression and other affective disorders remain to be elucidated [23]. Nevertheless, CRF hyperactivity is one likely candidate.

Small Molecule CRF antagonists

Certainly by the late 1980s, a number of research groups, including our own, had hypothesised that a lipophilic, small molecule CRF receptor antagonist that readily penetrates the blood-brain barrier and has oral bioavailability would represent a novel class of antidepressant and/or anxiolytic agents. As noted above, continuing preclinical and clinical research has unequivocally confirmed that CRF antagonists represent a novel class of pharmacotherapeutic agents in the treatment of anxiety and depression. Although detailed analyses of the critical moieties on CRF receptors for agonist and antagonist binding domains have only recently been conducted [24, 25], the first patent for a small molecule CRF antagonist was issued to the Nova Pharmaceutical Corporation in 1991. Following screening of chemical libraries by investigators from Nova, a series of compounds with micromolar affinity for CRF receptors was described. Since that time, Pfizer, Neurocrine Biosciences/Janssen Pharmaceutica, DuPont Pharmaceuticals, Taisho Pharmaceuticals, Neurogen Corporation, and Parke-Davis & Company have been issued patents for compounds with purported CRF_1 antagonist activity [26, 27]. The chemical structures of several CRF_1 receptor antagonists are illustrated in figure 2. It is of interest to note that working quite independently, scientists at these companies have designed CRF_1 receptor antagonists with remarkably similar structures including pyrrolo-, pyrazolo- and other substituted pyrimidines.

The first report on the pharmacology of a small molecule CRF antagonist (CP 154526) (Figure 2) in the peer-reviewed literature appeared 4 years ago from Pfizer [28]. Pfizer scientists reported that CP 154526, a pyrrololopyrimidine (K_i = 2.7 nmol/L), inhibited ^{125}I-oCRF binding to human CRF_1 receptors *in vitro*. The affinity of this compound for CRF_2 receptors is >10 µmol/L. As predicted for a CRF_1 receptor antagonist, CP 154526 blocked CRF-stimulated cAMP production *in vitro* and the ACTH response to exogenously administered CRF *in vivo* in rats. Intravenous administration of CP 154526 also attenuated the intracerebroventricularly administered CRF-induced increases in locus coeruleus firing rates. Finally, these authors reported that intraperitoneal administration of CP 154526 attenuated both CRF- and fear-induced startle amplitude [28].

Soon thereafter, Lundkvist et al. [29] also reported on *in vitro* inhibition of receptor binding and adenylate cyclase activity by CP 154526. These authors also reported that intraperitoneal administration of CP 154526 (1 mg/kg) produced anxiolytic activity in the elevated plus maze test, a standard anxiolytic screen. Interestingly, these authors were unable to observe anxiolytic activity at higher doses (3 and 10 mg/kg). More recently, Mansbach et al. [30] reported that CP 154526 possesses antidepressant-like activity in a learned helplessness model of depression. In this model, animals pre-exposed to 3 days of inescapable shock performed poorly in a shock-escape test when compared to control animals who did not receive inescapable shocks prior to testing. CP 154526 reversed this escape deficit if administered 60 min prior to the test session in animals previously exposed

to inescapable shock, but did not effect the performance of control rats not receiving prior exposure to inescapable shock. Shaham et al. [18] reported that CP 154526 attenuated the reinstatement of heroin- and cocaine-seeking behaviour, which is normally observed in response to stress, in laboratory animals no longer exhibiting this drug seeking behaviour. Most recently, Arborelius et al. reported CP-154,526 significantly decreased defensive withdrawal behavior after 9 days of chronic treatment (3.2 mg/kg/day). No changes in CRF_1 mRNA expression were observed in parietal cortex, basolateral amygdala, or cerebellum after chronic treatment, although a dose-dependent decrease in CRF mRNA expression was seen in the paraventricular nucleus (PVN) and Barrington's nucleus. Although treatment did produce anxiolytic-like effects, chronic administration in these experiments did not appear to lead to adrenal insufficiency [31]. Chen and colleagues [32] recently described both the synthesis of CP 154526 and the preliminary pharmacokinetic data of this agent in rats.

Antalarmin (Figure 2) differs from CP 154526 only by the addition of a methyl group to the 6 position of the fused pyrrolopyrimidine system. Like CP 154526, antalarmin is a potent CRF_1 receptor antagonist (K_i = 0.9 to 1.4 nmol/L in various rat tissues), and blocks CRF-stimulated ACTH release [33]. Long term treatment with antalarmin (20 mg/kg intraperitoneally for 11 days) significantly decreased basal plasma ACTH and corticosterone levels, but did not alter bodyweight, blood glucose or leptin levels [34]. Similarly, antalarmin treatment (20 mg/kg; ip; twice daily x 8 weeks) decreased basal plasma ACTH and corticosterone concentrations in rats [35]. In rats, antalarmin (20 mg/kg, intraperitoneally) impaired both the induction and expression of conditioned fear following inescapable foot shock. In addition, antalarmin blocked the enhancement of fear conditioning produced by prior exposure to inescapable shock [36]. Despite the marked behavioral effects, antalarmin did not alter inescapable shock or restraint stress-induced increases in plasma ACTH or corticosterone levels [35, 36]. This work was recently extended into primates where it was found that an oral dose of 20 mg/kg/day of antalarmin significantly blunted behavioral and endocrine effects in adult male rhesus macaques exposed to an intense social stressor. In this experiment, two unfamiliar males were placed in adjacent cages separated only by a transparent Plexiglass screen; antalarmin significantly reduced a variety of anxious behaviors including body tremors, teeth gnashing, urination and defecation [37].

The drug discovery group at DuPont Pharmaceuticals has recently published a series of manuscripts detailing the synthesis and design framework of their CRF_1 receptor antagonists [38-40]. The DuPont Pharmaceuticals compound, DMP 696 [27] (K_i = 1.6 nmol/L), has anxiolytic activity in rodent and monkey models of anxiety [41-43]. Hindman and colleagues [42] noted that, at anxiolytic doses, only minimal-to-moderate HPA axis inhibition was observed. Following 8 weeks of administration, CRF mRNA expression increased by 30% in the PVN of the hypothalamus, but basal plasma corticosterone levels were unchanged. This dosage regimen had no effect on CRF mRNA expression in rat cerebral cortex.

Taisho Pharmaceuticals has recently reported that their compounds CRA 1000 and CRA 1001 (Figure 2) are selective and moderately potent CRF_1 receptor antagonists *in vitro* and *in vivo* [44]. In mice, oral administration of both compounds reversed swim stress–induced anxiety in a light box/dark box exploration test. Both compounds were without effect in nonstressed mice. Oral or intravenous administration of either compound reversed the actions of intracerebroventricular CRF infusion on anxiety measured in the elevated plus maze task or excitation of locus coeruleus neuronal firing rate, respectively.

Janssen Pharmaceutica and Neurocrine Biosciences have developed R 121919, formerly NBI 30775. R 121919 is a moderately water soluble CRF_1 receptor antagonist and binds with high affinity (K_i ~3 nmol/L) to cloned human CRF_1 receptors. Using quantitative receptor autoradiography, central CRF_1 receptor occupancy was demonstrated *ex vivo* in brain slices of rats, gerbils and dogs. R 121919 inhibits CRF_1-mediated signal transduction in transfected cells (IC_{50} = 60 nmol/L) and CRF-stimulated ACTH release in cultured rat anterior pituitary cells (IC_{50} = 18 nmol/L). *In vivo*, using subcutaneous doses ≥2.5 mg/kg and oral doses of 10 to 40 mg/kg, R 121919 antagonised increases in locomotor activity induced by intracerebroventricular administration of CRF in rats. R 121919 also reduced stress-induced increases in plasma ACTH and corticosterone/cortisol levels in rats, gerbils and dogs, as well as the adrenalectomy-induced increases in ACTH level. Furthermore, R 121919 reduced the effects of acute and repeated stressors on behaviour in rodents, and some activity was present in classical models used to screen anxiolytics and antidepressants. In CRF-overexpressing transgenic mice, R 121919 normalized the diminished sexual receptivity in female mice, as well as the reduced exploratory activity in male animals. Preliminary cardiovascular experiments indicated that R 121919 had no major cardiohemodynamic effects at intravenous doses up to 1.25 mg/kg, nor any major electrophysiological effects at intravenous doses up to 5 mg/kg in anesthetised guinea pigs. In dogs, no major cardiohemodynamic or electrophysiological effects were observed at oral doses up to 40 mg/kg.

In vivo testing with R 121919 has demonstrated its potential as an anxiolytic agent. Pretreatment of R 121919 (3- 30 mg/kg; p.o.) 60 minutes before a CRF injection (0.3 nmol/kg) was able to blunt ACTH release. R 121919 (8 mg/kg, I.V.) was also able to block ACTH release following footshock, and decreased CRF-induced increases in locomotor activity following an intracerebroventricular injection of CRF (0.5 µg) [45]. R 121919 also reversed swim-stress induced anxiogenic-like behavior as determined by the elevated-plus maze model of anxiety [46]. Additionally, a single subcutaneous dose [0.33 mg/kg – 10.0 mg/kg] dose dependently decreased latency to exit the tube, and total time spent in the tube in the defensive withdrawal paradigm and decreased ACTH and corticosterone response to novelty in this paradigm by 82% and 97% respectively (10 mg/kg) [47]. This dose was associated with an 85% blockade of the CRF_1 receptor determined 75 minutes following

the s.c. injection. Furthermore, R 121919 attenuated the behavioral and neuroendocrine response to precipitated morphine and lorazepam withdrawal in rats [48], lending further evidence for the use of CRF-receptor antagonists to help treat drug withdrawal. Overall these recent studies indicate that R 121919 is able to cross the blood-brain barrier and act as a CRF_1 receptor antagonist in the brain *in vivo* and moreover it possesses anxiolytic properties.

Recently, the first open-label study examining the effects of R 121919 in 20 patients was completed. The doses used in the study (5-40 mg/day or 40 to 80 mg for 30 days) were well tolerated by the patients, and did not significantly affect ACTH or cortisol levels at baseline or following a CRH challenge. It is important that any potential CRF-antagonist does not lead to complete ACTH block and adrenal insufficiency. Hamilton Depression Rating Scale (HAMD) and Hamilton Scale for Anxiety (HAMA) were both significantly reduced following 30 day treatment with the drug. Although it must be noted that this small open-label study does not provide unequivocal proof, it does provide further evidence that a selective CRF-receptor antagonist have antidepressant and anti-anxiety applications in humans [49].

Conclusions and Future Directions

The evidence that has accumulated since the discovery of CRF nearly twenty years ago is a preeminent role for the peptide in the symptomatology of depression and anxiety. The recent introduction of small-molecule CRF receptor antagonists as a novel class of antidepressant and anxiolytic remains very promising. These compounds block the actions of exogenous and endogenous CRF in a variety of *in vivo* models supporting a putative role for these agents in the treatment of stress and/or anxiety and affective disorders. The promising clinical results in depressed patients in the completed open trial of R 121919 is of great interest. It is of interest to note that, at the doses utilized in these studies, plasma ACTH and/or corticosterone levels are not greatly affected. Therefore, any concerns regarding potential serious adverse endocrine effects such as adrenal insufficiency appear to be minimal at present.

Nevertheless, it is somewhat unexpected that antalarmin treatment does not attenuate ACTH responses to stress. This lack of effect in these laboratory animal studies may be related to pharmacokinetic aspects of antalarmin clearance (i.e., there was little drug present at the time of the stressors) or that the stressors were of such a severe nature that other systems (e.g. vasopressin) play a major role in pituitary ACTH release. Indeed, it is known that transgenic mice lacking CRF_1 receptors still mount a credible ACTH and corticosterone response to stress.

As we await the results of additional clinical trials examining the efficacy of CRF_1 receptor antagonists in anxiety and mood disorders, it should also be pointed out that these compounds may be of benefit in a broad array of neuropsychiatric disorders including eating disorders, neurodegenerative disorders such as stroke and trauma, child abuse, drug abuse, irritable bowel syndrome, inflammatory diseases, as well as preoperatively. Whether these drugs will be effective as monotherapy or whether they represent an important class of augmenting agents remains to be determined. Furthermore, the development of SPECT and PET ligands from these lead compounds for use in neuroimaging studies are certain to be useful in furthering our understanding the pathophysiology of these CNS disorders [50].

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