Corticotropin-releasing hormone receptor antagonists

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Abstract
Corticotropin-releasing hormone (CRH), CRH-related peptides, and CRH receptors play major roles in coordinating the behavioral, endocrine, autonomic, and immune responses to stress. The wide influence of the CRH system on physiological processes in both brain and periphery implicates the respective peptides in the pathophysiology of numerous disorders characterized by dysregulated stress responses. The potential use of CRH antagonists is presently under intense investigation. Selective antagonists have been used experimentally to elucidate the role of CRH-related peptides in disease processes, such as anxiety and depression, sleep disorders, addictive behavior, inflammatory disorders, acute and chronic neurodegeneration, and preterm labor.

European Journal of Endocrinology 155 S85–S91

Introduction

CRH peptide family
Corticotropin-releasing hormone (CRH) has been identified as the hypothalamic factor controlling the hypothalamic–pituitary–adrenal (HPA) axis in response to stress (1). CRH activates the secretion of pituitary adrenocorticotropic hormone (ACTH), leading to adrenal glucocorticoid production (2, 3). This 41-amino acid (aa) peptide also acts as a neuromodulator, affecting various centers of the brain within the central nervous system (CNS). It is now well established that CRH plays major roles in coordinating the behavioral, endocrine, autonomic, and immune responses to stress.

Three additional endogenous neuropeptides that share significant homology with CRH have been identified, urocortins (Ucns) 1, 2, and 3, each encoded by a different gene (4–8). Non-mammalian peptides, including fish urotensin I and frog sauvagine are also members of CRH-related family of peptides. Ucn 1 is 40 aa long, shares 45% of its sequence with CRH, and is expressed in cell bodies of the Edinger Westphal nucleus (EW), lateral superior olive and supraoptic nucleus in the brain (4), as well as in the gastrointestinal (GI) tract, adipocytes, heart, testis, kidneys, adrenals, pancreases, cardiac myocytes, skin, immune tissues, placenta, and fetal membranes. Ucn possesses hypotensive and appetite-suppressive actions (5), and participates in the inflammation processes (9) and the regulation of cardiac contractility (10). Ucn 2, or stresscopin-related peptide, is a 38 aa peptide expressed in the hypothalamus, brainstem, and spinal cord in the CNS, and human heart, lung, skeletal muscle, stomach, adrenal, and peripheral blood cells (6, 8). Ucn 3, or stresscopin is present in the hypothalamus and medial amygdala in the CNS, and in adipose tissue, heart, muscle, adrenal gland, skin, thyroid, adrenals, β-cells of the pancreas, spleen, and the muscularis mucosa of the GI tract, in the periphery (6–8, 11).

CRH-binding sites
CRH and CRH-related peptides transduce signals across cells via activation of two types of CRH receptors (Rs), R1 and R2. In addition, the actions of CRH-like peptides are modulated by a soluble high-affinity CRH-binding protein (CRH-BP) (12). CRH-BP is circulating in humans and its physiological role is to control the availability of the free ligand.

CRH-R1α (18) is a 415 aa protein that contains seven hydrophobic α helices. Several splice variants have been identified which may encode different isoforms, R1α, R1β, R1c, R1d, R1e, R1f, R1g, and R1h (13–21). CRH-R2 is encoded by a distinct gene and also has three splice mRNA variants encoding R2α, R2β, and R2γ receptor subtypes with

This paper was presented at the 4th Ferring Pharmaceuticals International Paediatric Endocrinology Symposium, Paris (2006). Ferring Pharmaceuticals has supported the publication of these proceedings.

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DOI: 10.1530/eje.1.02259

Online version via www.eje-online.org
unique tissue distributions (20). The two CRH receptor families share 70% homology at the aa level. Recently, a third CRH-R type (CRH-R3) was identified in catfish brain and pituitary. CRH-R1 binds CRH, as well as CRH-related peptides (Ucn, urotensin, and sauvagine, but not Ucn 2 and 3) with equivalent high affinity. CRH-R2 exhibits ligand selectivity and binds Ucn, Ucn2 and Ucn3 with significantly higher binding affinity than the other CRH-like peptides, suggesting that these peptides may be the natural ligands.

**CRH receptor antagonists**

The first synthetic ligands for CRH receptors were peptide based. As they do not penetrate the blood–brain barrier, no clinical applications have been pursued as yet (22–24). Over 100 patent claims have been made for non-peptidic, selective CRH-R1 receptor antagonists with clinical potential (25, 26). Interest has been particularly focused on the following compounds: CP-154,526 and its methyl homolog antalarmin, R-121919, R-278995, DMP-696, and DMP-904, SSR-125543A, and NBI-35 965. The majority of these antagonists fit into a general pharmacophore that has the following features (27): a central ring core with a sp² basic nitrogen that seems to modulate the confirmation of an agonist-binding site, a small alkyl group region, a larger lipophilic side-chain region and an orthogonal aromatic pocket.

**Clinical implications of CRH-R antagonists**

The availability of multiple CRH-receptor antagonists has led to extensive research focusing on the stress axis and the diseases that may be associated with stress (Table 1). Major depression and anxiety, conditions that are related to HPA hyperactivity are good targets for non-peptidic CRH antagonists. Such compounds could be effective in reversing pathological CRH-mediated stress responses, without causing metabolic and other side effects by suppressing the HPA axis (28, 29). Molecules are presently being tested for their anti-anxiety and anti-depression efficacy in phases I and II trials (Table 1).

Experimental and preclinical data also indicate a therapeutic potential of non-peptidic CRH-R1 antagonists for conditions related to neurodegeneration, such as stroke or infantile seizures. Under pathologic conditions, the sites of degeneration have elevated levels of CRH expression (30). In addition, selective CRH-R1 antagonists have been demonstrated to exert anti-ischemic effects in rat models of permanent focal cerebral ischemia and to reverse limbic seizures in neonatal rats (31, 32). Stress is associated with the activation of intracranial mast cells through the sequential action of CRH and sensory neuropeptides (33). This finding could have implications in the treatment of neuroinflammatory disorders, such as migraines and cyclic vomiting syndrome.

The behavioral and physiological manifestations of drug withdrawal and the relapse to drug-taking behavior induced by environmental stressors, seems to be strongly related to the activity of extrahypothalamic brain CRH systems (34). CP-154,526 attenuates stress-induced relapse to drug seeking in rats induced by footshock (35). These results extend previous reports on the role of CRH in reinstatement of drug seeking induced by stressors. Antalarmin reversed the place aversion produced by precipitated opiate withdrawal similar to buprenorphine (36), suggesting a therapeutic potential in opiate dependence.

<table>
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<th>Disorder</th>
<th>CRH-R1 antagonists</th>
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<td>Anxiety</td>
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PTSD, post traumatic stress disorder; ACTH, adrenocorticotropic hormone.
Dysregulation of the CRH system may contribute to the motivational basis of continued alcohol-seeking behavior during alcohol dependence (37), suggesting a possible effectiveness of CRH-R1 antagonists. Increased CRH activity is a possible mechanism underlying the state of anxiety that develops in alcoholics and leads to mood disturbances and negative affect observed weeks after withdrawal.

Augmented HPA axis function is involved in all aspects of cocaine self-administration. CRH regulates this activation and may be involved in the incentive motivation for the drug and in cue-induced reinstatement of extinguished cocaine-seeking behavior (38). These preclinical data provide evidence for a CRH-based strategy for the treatment of compulsive drug use. CRH-R1 were shown to control the expression of cocaine hyperactivation and sensitization as well as the cocaine-induced relapse behavior, whereas they did not seem to play any role in cocaine discrimination and self-administration (39). These findings suggest that CRH-R1 antagonists should be considered as possible medications in the treatment of cocaine addiction in humans.

The role of the CRH family of neuropeptides in inflammation, as well as experimental data supporting potential therapeutic applications of CRH compounds in various immune-related disorders have recently been reviewed (40). The idea of a functional crosstalk between the neuroendocrine and the immune system was initially conceived by findings showing regulation of CRH secretion in the hypothalamus by the cytokine interleukin (IL)-1β (41–43). In parallel with the indirect influences of the CRH system on immune function through neuroendocrine activation of the HPA axis, a direct pathway exists through immune tissue-derived local inflammatory actions (44). Application of specific anti-CRH serum or CRH antagonists attenuates the inflammatory reaction in several in vivo models (45, 46). Moreover, CRH endogenous neuropeptides are expressed in different immune cells, including macrophages, lymphocytes, and mast cells in proximity to their receptors and thought to act as autocrine and/or paracrine modulators of inflammatory activity via mutual regulation with cytokines and other mechanisms.

In the mouse spleen, CRH-R1 expression is upregulated by lipopolysaccharide (LPS) stimulation (47) and CRH appears to be necessary for the development of local inflammation induced by carrageenin or turpentine, for basal and inflammation induced IL-6 expression and normal leucocyte function in vitro (45, 48, 49). Synthetic CRH-R1 antagonists attenuate inflammation, indicating a CRH-R1-mediated pro-inflammatory action. Indeed, chronic blockade of CRH-R1 with systemically administered antalarmin, significantly ameliorated adjuvant-induced arthritis in Lewis (LEW/N) rats, reducing the severity of inflammation in peripheral joints (50). In addition, antalarmin prolonged survival of LPS-induced endotoxin shock and suppressed LPS-induced elevation of the macrophage-derived cytokines tumor necrosis factor (TNF)-α, IL-1β, and IL-6 (51), whereas it inhibited stress-induced gastric ulcerogenesis in rats (52).

Increased levels of CRH-related neuropeptides have been found in several conditions presenting with chronic inflammation, including rheumatoid arthritis (53–55), ulcerative colitis (56, 57), Helicobacter pylori-related gastritis (58), endometriosis (59), and Hashimoto’s thyroiditis (60), making them potential indications for specific treatment through CRH receptor blockade.

Recently, CRH-deficient mice were shown to be resistant to experimental autoimmune encephalomyelitis (EAE) in a Th1-specific manner, whereas wild-type EAE mice treated with CRH antagonists showed a decrease in IFN-production by primed T cells in vitro, an effect independent of corticosterone production. These results indicate a proinflammatory role of peripheral CRH in EAE and have implications for the treatment of Th1-mediated diseases such as multiple sclerosis (61).

Based on in vitro and in vivo data, CRH receptor antagonists have been suggested as adjunct agents to retinol and flavonoids for the inhibition of mast cell activation in chronic cutaneous inflammatory skin diseases exacerbated by stress, such as psoriasis and atopic dermatitis (62). CRH induced mast cell degranulation and vascular permeability in the skin (63) and antalarmin inhibited CRH-induced degranulation of mast cells and the secretion of vascular endothelial growth factor (VEGF), which is elevated in psoriasis (64).

Hypothalamic CRH has a direct inhibitory effect on the female reproductive axis by suppression of gonadotropin-releasing hormone secretion (65). Indirectly, glucocorticoids inhibit gonadal axis function at the hypothalamic, pituitary, and uterine levels. CRH and its receptors have been identified in most female reproductive tissues (66, 67), including the ovary, uterus, and placenta. Endometrial implantation sites of the early pregnant rat uterus contain high concentrations of CRH (68). CRH has also been identified in human stromal endometrial cells exhibiting a decidual reaction, and this neuropeptide potentiates the decidualizing effect of progesterone on endometrial stromal cells in vitro (69). In addition, invasive trophoblasts induce apoptosis of activated Fas-expressing human T lymphocytes and this is a CRH-potentiated effect that can be inhibited by antalarmin.

Subcutaneous administration of antalarmin in female rats in the first 6 days of gestation led to a dose-dependent decrease of endometrial implantation sites and live embryos, and markedly diminished endometrial FasL expression (70). It is suggested that CRH participates in the process of both implantation and early pregnancy tolerance in a paradoxic combination of in tandem pro-inflammatory and anti-rejection
activities. Therefore, antalarmin or its analogs might represent a novel class of non-steroidal inhibitors of pregnancy at its very early stages. Furthermore, CRH was shown to inhibit interstitial trophoblast invasion in vitro, by decreasing the expression of CEACAM1 on extravillous trophoblasts. This effect was mediated by CRH-R1, since the addition of antalarmin completely reversed it. It was, therefore, suggested that a defective CRH/CRH-R1 system might be involved in the pathophysiology of clinical conditions characterized by impaired trophoblast invasion, such as preeclampsia and placenta accreta (71). Given the promising future of CRH antagonists in the therapy of depression and anxiety disorders, their effects on reproductive physiology should be thoroughly examined.

In humans, placental CRH is secreted mostly during the latter half of pregnancy and is responsible for the physiologic hypercortisolism observed during this period (72). Placental CRH expression increases during the last week of pregnancy. Placental CRH drives the pituitary–adrenal axis to produce increased amounts of cortisol during the latter half of pregnancy. CRH may be the placental clock determining the onset of parturition (73). Interestingly, maternal CRH levels are significantly elevated in the plasma of women with preterm labor (74). This is further supported by findings showing that CRH infused into preterm fetal sheep precipitated early parturition (75). CRH-R1 antagonism in the late gestation of fetal sheep, using antalarmin, delays the onset of parturition. Finally, in rats, administration of antalarmin after the fifth day of gestation and until the end of pregnancy has no abortifacient or fetotoxic effect, suggesting that CRH antagonists could be used to protect the fetus from maternal stress and/or to prevent premature labor and delivery (70).

GI diseases with high incidence, such as functional bowel disorders, irritable bowel syndrome (IBS), and peptic ulcer are related to stressful stimuli. Acute stressors inhibit gastric motility and emptying, whereas they exert stimulating effects in the lower GI tract, manifested by increased colonic motility, decreased colonic transit time, defecation, and watery diarrhea. CRH-R1 seems to be the major receptor type mediating the colonic stress-related phenomena. Indeed, CRH-induced colonic effects were reversed by non-selective CRH-R1/CRH-R2 peptidic antagonists and not by selective peptidic CRH-R2 antagonists (76–78). Moreover, the specific CRH-R2 ligands Ucn 2 and Ucn 3 had no effect on colonic function (77, 79, 80). Both central and peripheral pathways seem to contribute to these effects (81, 82).

Interestingly, non-peptidic selective CRH-R1 receptor antagonists, such as CP-154,526, CRA-1000, NBI-35 965, NBI-27 914, and antalarmin, injected peripherally, i.c.v., or given orally, blunted the colonic-stimulating effects induced by restraint or social stress, water avoidance or morphine withdrawal (83–89). These results, combined with in vivo data that confirm that selective non-peptidic CRH-R1 antagonists can inhibit stress or CRH-induced hyperalgesia to colorectal distention (90, 91), suggest a therapeutic potential of such compounds in IBS. Given the pro-inflammatory actions mediated by CRH receptors, CRH antagonists could benefit patients with ulcerative colitis, a common disorder characterized by chronic inflammation of the bowel that is related to increased levels of CRH and Ucn 2.

CRH-R2 seems to be the major receptor type mediating stress and CRH-related gastric motor alterations; however, antalarmin can inhibit stress-induced gastric ulcerogenesis in rats, possibly by blockade of brain CRH-R1 and vagal pathways (52). A prophylactic potential of CRH-R1 antagonists against ulcer is anticipated.

Conditions such as chronic stress-induced metabolic syndrome and congenital adrenal hyperplasia, associated with elevations of CRH secretion, might benefit from therapy with CRH antagonists. Also, pituitary ACTH-secreting adenomas that are dependent upon CRH stimulation might be controlled by CRH antagonists.

CRH-related peptides acting on CRH-R2 could, in theory, reduce stress-induced suppression of appetite, stimulate hypothalamic CRH secretion by interrupting putative CRH-R2-mediated auto-inhibition of CRH, and block CRH-R2-mediated peripheral vasodilatation (92–94). However, the potent cardiac inotropic actions of Ucn in rodents and sheep, its coronary vasodilatory actions in sheep, cardioprotective properties in mice and rats, and antivascularization effects in mice, suggest that any clinical experimentation with antagonists for CRH-R2 should be done cautiously. Such antagonists might be useful in the treatment of cancer- or AIDS-related anorexia and cachexia, atypical depression, chronic pain and fatigue syndromes, syndromes of excessive daytime sleepiness and cytokine-induced hypotension (95–98).

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