Corticotropin-Releasing Hormone Family and Their Receptors in the Cardiovascular System

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The identification of corticotropin-releasing hormone (CRH) has led to the discovery of a growing family of ligands and receptors. CRH receptor 1 (CRHR1) and CRHR2 are mammalian G-protein coupled receptors (GPCRs) with high affinity for CRH and the CRH family of peptides. CRHR1 is predominantly expressed in the brain and plays a vital role in the hypothalamic-pituitary-adrenal (HPA) axis stress responses by secreting adrenal corticotropic hormone (ACTH). CRHR2 is predominantly expressed in the heart, and a CRHR2-specific ligand, urocortin 2 (UCN2), shows positive cardiac chronotropic and inotropic effects through 3',5'-cyclic adenosine monophosphate (cAMP) signaling in response to CRHR2-mediated Gαs activation in mice and humans. Central administration of the CRH family of peptides increases mean arterial pressure through CRHR1 activation, whereas peripheral administration of the peptides decreases mean arterial pressure through CRHR2 activation. These observations have led to further investigations of CRHR2 as an important and unique GPCR in the physiological and pathological functioning of the cardiovascular (CV) system. Moreover, recent clinical trials demonstrate CRHR2 as a potentially therapeutic target in the treatment of heart failure. We present recent reviews of the role of CRHRs in basic CV physiology and in the pathophysiology of CV diseases.

Key Words: Corticotropin-releasing hormone receptor 2; G-protein coupled receptors; Heart failure; Urocortin

G-protein coupled receptors (GPCRs) belong to the largest and most diverse superfamilly of cell surface receptors. They react to extracellular stimuli and regulate cardiovascular (CV) function through cellular G-protein-mediated signaling.1 GPCRs are a conserved family of 7 transmembrane receptors that have been targeted for drug therapy.2 GPCRs are involved in cardiac dysfunction and hypertension, and inhibitors of GPCRs are widely used to treat patients with CV diseases such as heart failure and hypertension.3 Several studies have investigated various aspects of 2 GPCR families, α-adrenergic receptors and angiotensin II receptors, in CV diseases. There are approximately 800 GPCRs in humans, but the role of most of the GPCRs in CV diseases remains unclear, suggesting that uncharacterized GPCRs have considerable potential in the development of novel therapeutics for CV diseases. This review focuses on corticotropin-releasing hormone receptors (CRHRs) as potential therapeutic GPCRs in CV diseases, as well as their agonists and antagonists.

Corticotropin-Releasing Hormone Family

CRH, also named corticotropin-releasing factor, is a peptide that was first characterized from the ovine hypothalamus.4 CRH is a 41-amino acid polypeptide (Figure) generated by the cleavage of the C-terminus of pre-proCRH, a 196-amino acid precursor.5 CRH plays a key role in regulating the basal and stress-induced pituitary-adrenal axis that increases glucocorticoid and androgen secretion.6 CRH release in response to acute stress is essential for the survival of the organism, but CRH released as a result of exposure to chronic stress may influence emotions and exert negative effects on the homeostasis of the organism’s physiological functions.7 Urocortin 1 (UCN1), -2, and -3 were identified as a 2nd mammalian CRH family of peptides. Vaughan et al identified UCN1 in the Edinger-Westphal nucleus and lateral superior olive regions of the rat brain as a mammalian member of the mammalian CRH family of peptides.8 The peptide was named UCN1 because of its homology with fish urotensin and CRH. UCN2 (38 amino acids) and UCN3 (38 amino acids) were identified as members of CRH-like peptides by searching human genome databases and cloning from human and mouse cDNA libraries.9 Urocortin 1 (UCN1), -2, and -3 were identified as a 2nd mammalian CRH family of peptides. Vaughan et al identified UCN1 in the Edinger-Westphal nucleus and lateral superior olive regions of the rat brain as a mammalian member of the mammalian CRH family of peptides.9 The peptide was named UCN1 because of its homology with fish urotensin and CRH. UCN2 (38 amino acids) and UCN3 (38 amino acids) were identified as members of CRH-like peptides by searching human genome databases and cloning from human and mouse cDNA libraries.10,11 Simultaneously, human stresscopin (N-terminally 2-amino acid extended UCN3) and stresscopin-related peptide (N-terminally 5-amino acid extended UCN2) were identified as members of the CRH family of peptides by Hsu et al.12 Although CRH mRNA is expressed widely throughout the brain, UCN mRNA expression has restricted expression levels in the mammalian brain.13 In peripheral tissue, UCN1 mRNA is broadly expressed in multiple organs, including the pituitary, gastrointestinal tract, testis, heart,
and thymus, UCN2 mRNA is highly expressed in skin and skeletal muscle, and UCN3 mRNA is expressed in the skin and small intestine. The CRH family of peptides regulates various physiological processes, including glucose metabolism, CV regulation, immune function, and behavior. The sites of mRNA expression of the CRH family of peptides have been well studied in various tissues; however, the organs or tissues from which the UCNs are released to mediate physiological and pathological processes remain largely unknown.

**CRHRs**

CRH and the UCNs bind to CRHR1 and CRHR2, which belong to the secretin-like class B family of GPCRs. There are 18 class B GPCRs, which are characterized by a large extracellular N-terminal domain of 120–160 residues, which is part of the binding site for agonists. Both CRHR1 and CRHR2 genes are found in humans and other mammals, and their receptors exhibit a 70% identity at the amino acid level. Although the intracellular and transmembrane amino acid sequences of CRHR1 and CRHR2 are highly homologous, a low degree of homology exists in their extracellular N termini (~47%), which are responsible for their agonist selectivity. CRHR1 has a high affinity for CRH as well as UCN1, but not for UCN2 or UCN3 (Table). UCN1, -2, and -3 bind with considerably higher affinity for CRHR2 than for CRHR1. Therefore, UCN1 is considered as an endogenous ligand for both CRHR1 and CRHR2, whereas UCN2 and UCN3 are generally considered as endogenous ligands exclusive to CRHR2.

CRHR1 mRNA is widely expressed in the mammalian brain with high levels of expression in the anterior pituitary; splice variants of CRHR1 mRNA have been identified in humans. CRHR1 appears to have biological activity, but the physiological significance of the remaining CRHR1 variants is uncertain. CRHR2 is expressed as 3 splice variants: CRHR2α, CRHR2β, and CRHR2γ. mRNA of both CRHR2α and CRHR2γ is expressed in the brain, whereas CRHR2β mRNA is widely expressed in peripheral tissues, particularly the heart and skeletal muscle. Pharmacological characterization of the CRHR2 splicing variants revealed no major differences among CRHR2α, -2β, and -2γ. The physiological and pathological roles of CRHR1 and CRHR2 may depend on their tissue distribution.
Vascular Effects of CRHRs

Essentially, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis involving CRH has been associated with elevated blood pressure and CV diseases.\textsuperscript{25,26} Central administration of CRH increases mean arterial pressure in the rat through the HPA axis,\textsuperscript{27} and treatment with antalarmin, a CRHR1 antagonist, decreases hypertension produced by intracerebroventricular injection of CRH in the rat.\textsuperscript{28} Intracerebroventricular injection of α-helical CRH\textsubscript{9-41}, a non-selective CRHR antagonist, blocks hypertension in rats.\textsuperscript{29} The mechanisms of HPA axis-induced hypertension probably involve several processes, such as microvascular dysfunction and irreversible reductions in nephron number by cortisol.\textsuperscript{30}

Although central administration of CRH increases mean arterial pressure, peripheral administration of CRH decreases mean arterial pressure in rats.\textsuperscript{31} The hypotensive effect of intravenous injection of CRH in rats was decreased by treatment with α-helical CRH\textsubscript{9-41}, but not with antalarmin.\textsuperscript{26} Conventional CRHR2-deficient mice (CRHR2\textsuperscript{−/−}) show elevated basal mean arterial pressure and diastolic pressure compared with wild-type mice, and suppressed intravenous UCN1-induced blood pressure reduction.\textsuperscript{32} Intravenous administration of UCN2, which binds with much higher affinity to CRHR2 than to CRHR1, decreased mean arterial pressure in rats, and treatment with astressin2-B (a CRHR2 antagonist) blocked UCN2-induced decreases in mean arterial pressure without influencing the basal mean arterial pressure.\textsuperscript{33} CRHRs have 2 interesting effects on blood pressure: CRHR1 in the brain elevates blood pressure and CRHR2 in peripheral tissues causes vasodilation.

Cardiac Effects of CRHRs

RT-PCR analysis using human heart tissue shows that CRHR2a is highly expressed in all 4 chambers of the heart, whereas CRHR2β is weakly expressed only in the left atrium, and the expression of CRHR1 in the human heart is unclear.\textsuperscript{34} A non-biased quantitative RT-PCR (qRT-PCR) analysis, which determined the gene copy numbers of 475 GPCRs in adult murine cardiomyocytes, revealed that CRHR2 was the 4th most abundantly expressed GPCR in GPCRs in adult murine cardiomyocytes, revealed that CRHR1 in the human heart is unclear.\textsuperscript{34} A non-biased quantitative RT-PCR (qRT-PCR) analysis, which determined the gene copy numbers of 475 GPCRs in adult murine cardiomyocytes, revealed that CRHR2 was the 4th most abundantly expressed GPCR in adult murine cardiomyocytes, revealed that CRHR1 in the human heart is unclear.\textsuperscript{34} A non-biased quantitative RT-PCR (qRT-PCR) analysis, which determined the gene copy numbers of 475 GPCRs in adult murine cardiomyocytes, revealed that CRHR2 was the 4th most abundantly expressed GPCR in adult murine cardiomyocytes.\textsuperscript{35} In vitro, UCN2 and UCN3 significantly increased myocyte contractility in a dose-dependent manner, as characterized by increased fractional shortening and peak systolic Ca\textsuperscript{2+} transients.\textsuperscript{36} In rabbit and mouse ventricular myocytes, UCN2 mediates inotropic and lusitropic effects via the cAMP- and Ca\textsuperscript{2+}/calmodulin-CaMKII signaling pathways, and also induces arrhythmogenic effects.\textsuperscript{36,37} Ex vivo experiments using Langendorff-perfused hearts isolated from Wistar rats showed that UCN1 induces positive inotropic effects.\textsuperscript{38}

In vivo, intravenous administration of UCN1 increased cardiac contractility analyzed by transthoracic echocardiography in wild-type mice to approximately twice the baseline, but had no effect on CRHR2\textsuperscript{−/−} mice.\textsuperscript{39} Intravenous injection of UCN1 in healthy sheep increased heart rate, cardiac output, and cardiac contractility.\textsuperscript{39} Experiments involving administration of UCNs have revealed the inotropic effects of CRHR2 in the heart, but in conventional CRHR2\textsuperscript{−/−} mice and in cardiomyocyte-specific CRHR2 deficiency, basal cardiac function remains unaffected.\textsuperscript{33,34} Thus, the physiological role of CRHR2 in basal cardiac function is still unclear.

Potential therapeutic effects of UCNs in CV diseases have also been investigated. When mice were treated with intraperitoneal injection of UCN2 prior to occlusion of the left anterior descending coronary artery, the cardiac infarct size was reduced.\textsuperscript{40} Single intravenous bolus administration of UCN2 to a mouse with heart failure (muscle-specific LIM protein-deficient mice, a model of dilated cardiomyopathy) produced a significant enhancement of inotropic and lusitropic effects on left ventricular function and improved cardiac output.\textsuperscript{41} UCN1 treatment prevented further deterioration of cardiac dysfunction induced by rapid left ventricular pacing in sheep. Infusion of UCNs into normal and failing hearts improved cardiac output. However, the cardiac effects of long-term activation of CRHR2 remain unclear. Continuous UCN2 overexpression in the liver (>15-fold) using an adenovirus system increased cardiac output in mice.\textsuperscript{42} Chronic treatment with UCN2 for 1 month in a mouse myocardial infarction model reduced infarct size and ameliorated cardiac remodeling with a decrease in mean blood pressure.\textsuperscript{43} Continuous UCN2 infusion (a 2-fold increase in plasma UCN2) without a significant effect on blood pressure resulted in cardiac dysfunction, whereas CRHR2 blockade suppressed pressure overload-induced chronic cardiac dysfunction, suggesting that chronic UCN2 activation without vasorelaxation may have cardiotoxic effects.\textsuperscript{44} The situation may be similar to that seen with cardiac β-adrenergic receptors, which are also coupled to Gas. Although adrenaline treatment is beneficial during the acute phase, chronic activation of β-adrenergic receptors are harmful and cardiotoxic in chronic heart failure. Beta-blockers have become a frontline drug for the treatment of chronic heart failure. It is still unclear whether UCNs mediate protective effects of cardiac contractility through a direct effect on cardiac myocytes or through reduction of blood pressure.\textsuperscript{44}

### Table. CRHR1- and CRHR2-Mediated Cardiovascular Effects

<table>
<thead>
<tr>
<th>Primary location</th>
<th>Ligand</th>
<th>Cardiovascular effects</th>
</tr>
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<tbody>
<tr>
<td>Brain</td>
<td>CRH</td>
<td>Intravascular volume ↑ (human)</td>
</tr>
<tr>
<td>Brain</td>
<td>UCN1</td>
<td>Cardiac contraction ↑ (mouse, human)</td>
</tr>
<tr>
<td>Heart</td>
<td>UCN2</td>
<td>Heart rate ↑ (sheep, human)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>UCN3</td>
<td>Cardiac remodeling ↑↑↓ (mouse)</td>
</tr>
<tr>
<td>HUVEC (cell line)</td>
<td>UCN1</td>
<td>Vasodilation ↑ (mouse, rat, human)</td>
</tr>
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CRHR, corticotropin-releasing hormone receptor; HUVEC, human umbilical vein endothelial cell; UCN, urocortin.
CRH2-mediated cAMP signaling in response to CRH activation activates adenyl cyclase and protein kinase A (PKA), which in turn phosphorylate several proteins involved in excitation-contraction coupling, including L-type Ca^{2+} channels, phospholamban, ryanodine receptor (RyR), and troponin I in cardiomyocytes (Figure). Gas-mediated cellular signaling controls the activity of well-known cardiac functions, stimulating positive chronotropic, lusitropic, and inotropic effects.

Although transgenic overexpression of Gas in mice resulted in an enhanced efficacy of heart rate and cardiac contraction in response to catecholamines, the mice developed cardiac hypertrophy and dilation as they aged. The CRH peptide family is known to activate the PKA, CaMKII and AKT signaling pathways to temporally increase cardiac function, but the effects of chronic activation of these pathways in the heart remains controversial. GPCR-activated β-arrestin facilitates recycling and degradation of GPCRs, and also mediates G-protein-independent signaling at GPCRs. The β-arrestin-dependent activation of the ERK signaling confers cardioprotection in mice exposed to catecholamines. UCN stimulation may activate β-arrestin-mediated signaling in cardiomyocytes.

Exchange protein directly activated by cAMP (EPAC) acts in parallel with, or independently of PKA to mediate cAMP-induced effects in cellular contexts. EPAC causes cardiac hypertrophy and remodeling in cardiomyocytes, but also induces the relaxation of vascular smooth muscle through Rap1 signaling and endothelial-dependent vascular relaxation by activating endothelial nitric oxide synthase and production of nitric oxide (NO). Furthermore, UCN1 induces accumulation of cAMP through CRHR2 in aortic smooth muscle cells. Removal of the endothelium decreases the relaxant effect of UCN1 in rat coronary arteries, indicating that endothelial-derived factors are involved in this process. UCN2 induces NO production through cAMP- and Ca^{2+}-mediated pathways in porcine aortic endothelial cells. These findings suggest that CRHR2 in the endothelium, as well as in aortic smooth muscle, causes vasodilation, but further studies are necessary to understand the mechanisms of the CRHR2-mediated vascular relaxation.

CRHR and Plasma UCNs in Humans
Gene expression levels of CRHR1, CRHR2, CRH, UCN1, UCN2, and UCN3 were examined by qPCR in 108 donors for heart transplantation (control) and in 110 patients with heart failure. The qPCR analysis showed that cardiac expression of CRHR1, CRH, and UCN3 was higher (P<0.001) and that of CRHR2 was lower (P=0.012) in the patients than in the controls.

Plasma UCN1 is elevated in heart failure, and UCN1 levels are related to clinical signs of heart failure and circulation levels of plasma natriuretic peptides (NPs). An increase in plasma UCN2 levels has been examined in patients with heart failure, and in patients with abdominal aortic aneurysm. Validated ELISA analysis showed that the plasma NT-proUCN2 concentration was significantly increased with heart failure. These findings suggest that UCNs are elevated in patients with heart failure; however, UCN assays have not been internationally standardized. More accurate methods are required to examine circulating levels of endogenous UCNs in healthy subjects and patients with heart failure.

UCN1 infusion significantly elevates the circulation levels of ACTH, cortisol, and ANP, but not BNP in healthy volunteers. Heart rate, cardiac output, blood pressure, and ejection fraction are unchanged in healthy volunteers treated with UCN1. In 8 males with stable heart failure, brief intravenous UCN1 infusion increased corticotropic and cortisol without increasing ANP levels and hemodynamic effects.

Brief intravenous infusions of UCN2 in 8 healthy humans increased plasma UCN2 concentrations by 15- to 60-fold and induced dose-related increases in cardiac output, heart rate, and left ventricular ejection fraction. The administration of UCN2 in healthy humans decreased diastolic blood pressure and mean arterial pressure, but not systolic blood pressure. UCN2 and UCN3 infusion caused arterial vasodilatation in 18 healthy volunteers, with the effects partly dependent on endothelial NO and cytochrome P450 metabolites of arachidonic acid. In 8 males with stable congestive heart failure, intravenous UCN2 administration increased cardiac output substantially, secondary to decreased afterload through vasodilation. In patients with acute decompensated heart failure, UCN2 infusion reduced blood pressure and increased cardiac output. These findings suggest that brief intravenous infusion of UCN2 may be a novel therapeutic approach to treating acute heart failure, but further studies are necessary to investigate the therapeutic effects of continuous infusion of UCN2 against heart failure.

Conclusions
CRH has been investigated as a primary hormone in stress response, together with its receptor CRHR1. In addition to CRH, the role of UCN2 and CRHR2 in the CV system is garnering attention. Injection of UCNs revealed the inotropic effects of CRHR2 in the heart and on vascular tone, but the physiological role of endogenous UCNs and CRHR2 and the identity of the organs and tissues secreting UCNs, remain unclear. Furthermore, whether excessive or insufficient activation of UCN2–CRHR2 is related to CV diseases needs to be determined. Further basic and clinical investigations of UCNs and CRHRs can advance the diagnosis of heart failure, as well as its treatment.

Grants
This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (M.T.). The authors declare that they have no competing interests.

Conflict of Interest Statement
The authors declare that they have no competing interests.

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Circulation Journal Vol.83, February 2019


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