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Commentary

Urocortins in heart failure

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ABSTRACT

Despite modern advances in the treatment of the causes and consequences of cardiovascular illness, heart disease and heart failure remain a leading cause of death in the western world. Many novel peptides are emerging as biomarkers and potential therapeutic tools for this debilitating condition. Urocortins represent one such group of peptides whose role in normal cardiovascular physiology and disease states is now increasingly being recognized. The cardiovascular effects of the urocortins are mediated via corticotrophin-releasing hormone (CRH) receptors through a variety of intra-cellular signaling pathways. Studies to date have demonstrated a favourable effect of urocortins on hemodynamic and neurohumoral regulation. They cause relaxation of the vasculature as well as having positive inotropic, chronotropic and lusitropic effects on the heart. This makes the urocortins a potentially attractive target in the treatment of heart failure. Indeed, a number of studies have demonstrated increased urocortin activity in experimental and clinical heart failure, with apparent augmented responses in these states. This article provides a review of the role of urocortins in normal cardiovascular physiology and in the pathophysiology of heart failure.

1. Introduction

Despite modern advances in the treatment of the causes and consequences of cardiovascular illness, heart disease remains a leading cause of death in the western world. In particular, heart failure continues to carry a poor prognosis with a considerable burden on the health care system throughout the world: the estimated direct cost for heart failure in the United States was $30 billion in 2006 [1]. Many novel peptides are emerging as biomarkers and potential therapeutic tools for this debilitating condition. Urocortins represent one such group of peptides whose role in normal cardiovascular physiology and disease states is now being increasingly recognized. After their initial discovery in 1995 [2], subsequent research has furthered understanding of their mechanisms, predominantly in pre-clinical models, with expansion of this knowledge into potential therapeutic applications in humans. This article provides a review of the role of urocortins in normal cardiovascular physiology and in the pathophysiology of heart failure.

2. The urocortin–CRH system

Urocortins belong to the corticotrophin-releasing hormone (CRH) family which includes CRH, fish urotensin I, frog sauvagine, urocortin 1, urocortin 2 and urocortin 3 [3] (Fig. 1). CRH is produced in the brain in response to stress, has central effects upon behaviour, and exerts a variety of peripheral responses. However, CRH is unlikely to have major effects upon cardiac function as it is not expressed locally and its plasma concentrations are very low.

In 1995, Vaughan et al. [4] observed urotensin-like immunoreactivity in the Edinger Westphal nucleus and lateral superior olive regions of the adult rat brain. It was named urocortin (now known as urocortin 1) to reflect its similarities of structure and biological properties to urotensin (suckerfish urotensin) and rat CRH. It is believed to be the second endogenous mammalian ligand for CRH receptors [5]. Subsequently, two further paralogues of CRH were identified—urocortin 2 and urocortin 3. Human CRH and urocortin 1 genes have been localized to chromosomes 8 (8q13) and 2 (2p23-p21), respectively. Urocortin 2 and urocortin 3 have prominent cardiovascular roles and are expressed in the heart. In contrast to CRH, the urocortins do not increase corticosterone secretion and do not appear to have any physiologic role in the regulation of the hypothalamic–pituitary–adrenal axis [6,7].

2.1. CRH receptors

The effect of CRH and urocortins is mediated via CRH receptors (CRH-R). These seven transmembrane G-protein coupled receptors are members of the secretin family [8] and the human CRH-R gene has been localized to chromosomes 17 (17q12-qter) and 7 (7p21-p15) [9,10]. Two subtypes of CRH receptors have been identified in mammals and rodents—CRF-R1 and -R2. Structurally, the two

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
CRH	S	E	E	P	P	I	S	L	D	L	T	F	H	L	L	R	E	V	L	E	M
Urocortin		D	D	P	P	L	S	I	D	L	T	F	H	L	L	R	T	L	L	E	L
Urocortin 2				V	I	L	S	L	D	V	P	I	G	L	L	R	I	L	L	E	Q
Urocortin 3				F	T	L	S	L	D	V	P	T	N	I	M	N	I	L	F	N	I
Sauvagine		E	G	P	P	I	S	L	D	L	S	L	E	L	L	R	K	M	I	E	I
Urotensin 1	N	D	D	P	P	I	S	L	D	L	T	F	H	L	L	R	N	M	I	E	M

	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
CRH	A	R	A	E	Q	L	A	Q	Q	A	H	S	N	R	K	L	M	E	I	I
Urocortin	A	R	T	Q	S	Q	R	E	R	A	E	Q	N	R	I	I	F	D	S	V
Urocortin 2	A	R	Y	K	A	A	R	N	Q	A	A	T	N	A	Q	I	L	A	H	V
Urocortin 3	D	K	A	K	N	L	R	A	K	A	A	A	N	A	Q	L	M	A	Q	I
Sauvagine	E	K	Q	E	K	E	K	Q	Q	A	A	N	N	R	L	L	L	D	T	I
Urotensin 1	A	R	I	E	N	E	R	E	Q	A	G	L	N	R	K	Y	L	D	E	V

Fig. 1. Amino acid sequences of CRH and its analogue peptides. Sequences shown are that of mammalian CRH and urocortins, amphibian sauvagine and teleost urotensin 1. Highlighted sequences represent similarity to CRH.

subtypes exhibit considerable divergence at the N terminus, consistent with their distinct pharmacological properties. Furthermore, three splice variants of CRH-R2 have been identified. These variants differ in the structure of their N-terminal extra-cellular domain. R2α and R2β have been observed in rodents and in man, whilst R2γ is specific to humans (isolated in the limbic regions of the human brain) [8]. It is, however, unclear whether the γ splice variant has any specific physiological role. Low homology of the extra-cellular domains of CRH-R1 and -R2 accounts for differences in their ligand specificity [8]. Urocortin 1 and CRH both act at CRH-R1 but the affinity of urocortin 1 for CRH-R2 is more than 10-fold higher than that of CRH [5]. Whilst urocortin 1 can activate both receptors, urocortins 2 and 3 are potent and specific agonists at CRH-R2 [4,11] with little effect at CRH-R1.

3. Biology of urocortins

3.1. Anatomy (tissue distribution of urocortins and CRH receptors)

Immunoreactivity to the urocortins and their receptors has been demonstrated in the central nervous, digestive, reproductive, cardiovascular, immune and endocrine systems, suggesting important roles throughout the body [12]. In the brain, urocortin 1 is most prominent in the Edinger Westphal nucleus and lateral superior olive. Urocortin 1 mRNA or immunoreactivity has also been reported in other regions of the brain, such as the cerebellum and hypothalamus [8], and it appears to be co-localized with dopamine in the basal ganglia and hypothalamus. Urocortin 1 mRNA is also expressed in vascular smooth muscle cells and in cardiac myocytes. Urocortin 2 has a similar distribution in the central nervous system in mouse and rats, but is also seen in high concentrations in the peripheral tissues including the heart, adrenals, placenta, stomach, ovary, skin, gastrointestinal tract, uterine smooth muscle, skeletal muscle and peripheral blood vessels [8].

The distribution of urocortin 3 is distinct. In the central nervous system, it is demonstrable in regions of high CRH-R2 expression, supporting the notion that it is an endogenous ligand [8]. In humans, urocortin 3 is also seen in peripheral tissues such as adrenals, heart and kidney—particularly in the distal tubules [13].

CRH-R1 is predominantly found in the central nervous system. In addition to its central nervous system expression, CRH-R2 is found in peripheral tissues such as the gut, heart, lymphocytes and adrenals. In humans, urocortin 1 and CRH-R2α has been identified in all four chambers of the heart, suggesting that urocortin acts in an autocrine or paracrine fashion through CRH-Rs [14]. In contrast to rats where CRH-R2β is the predominant splice variant in the heart and vascular smooth muscle cells, humans appear to predominantly express CRH-R2α in these tissues. CRH-R2 has also been characterized in the human left ventricle and intramyocardial blood vessels [15]. In humans, both CRH-R1 and -R2 are found in the periphery, although their specific role remains to be fully characterized in human physiology and pathophysiology.

3.2. Biochemistry

3.2.1. Molecular structure

Urocortin is a 40 amino acid-containing neuropeptide, related to urotensin (63% sequence identity) and CRH (45% sequence identity) [4]. Rat and human urocortin bear 95% homology to each other. The precursor protein contains 122 amino acid residues with an N-terminal methionine and consensus signal peptide sequence, whilst the carboxy terminus of the precursor contains the C terminally amidated peptide of urocortin. The CRH analogue peptides possess an α helical conformation with varying degrees of amphipathicity. The amphipathic N-terminal helices could play a crucial role in selectivity of the analogues to CRH-R1, whereas it may not be as important for CRH-R2 binding [16]. The parent protein is half the length of urotensin and CRH precursors with little sequence similarity to either [8]. Urocortin 2 shows moderate homology with human and rat CRH (34%), urocortin 1 (43%) and urocortin 3 (37–40%). The half-life of urocortin 1 in healthy humans and those with stable heart failure is approximately 50 min [17,18]. Urocortin 2 has a shorter half-life of 10 min in

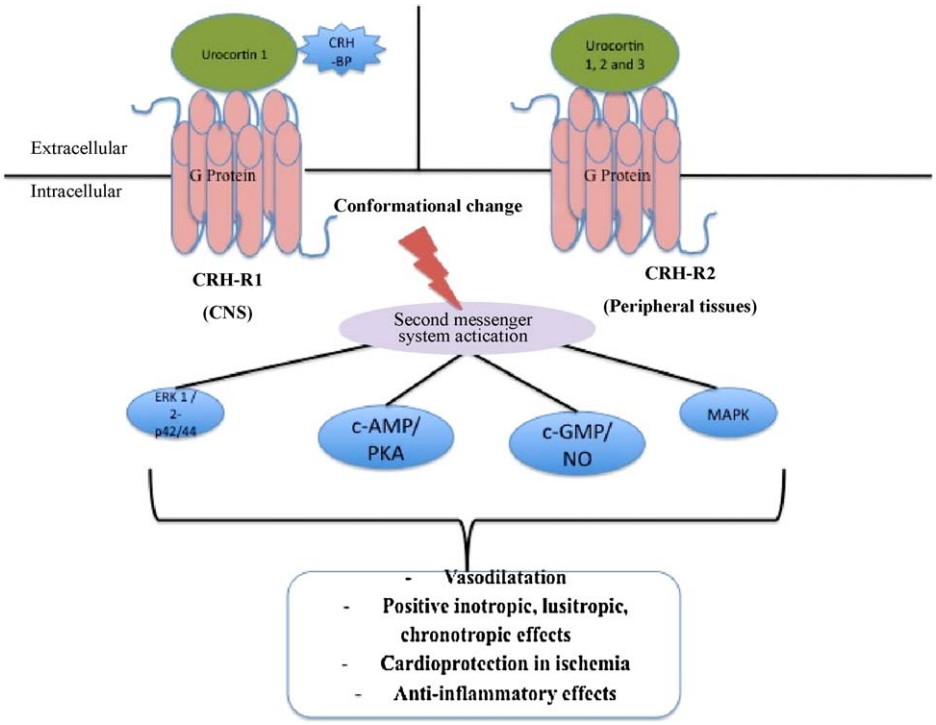

Fig. 2. Schematic of Urocortin intra-cellular signaling pathway. Urocortins bind to CRH receptors to induce conformational changes in the G protein receptors and activate the second messenger systems. CRH-BP has a greater affinity to Urocortin 1 and may play a role in its metabolism. Urocortin 1 binds to both CRH-R1 and -R2, whereas Urocortins 2 and 3 are potent, specific CRH-R2 agonists. CRH-BP: corticotrophin releasing hormone-binding protein; CRH-R: corticotrophin releasing hormone receptor; CNS: central nervous system; ERK 1/2-p42/44: extra-cellular signal-related kinases 1/2-p42/44; c-AMP: cyclic adenosine monophosphate; PKA: protein kinase A; cGMP: cyclic guanosine monophosphate; NO: nitric oxide; MAPK: mitogen-activated protein kinase.

healthy humans [19]. The exact half-life of urocortin 3 is not yet known, but it appears to have a more rapid onset and shorter duration of action [20].

3.2.2. CRH binding protein

Corticotrophin releasing hormone-binding protein (CRH-BP) is a 37 kDa protein that was first isolated in human plasma in 1989 [21] and binds to both CRH and urocortin 1. Given that the expression of CRF-BP overlaps that of both CRH and urocortin 1 in the central nervous system, it has been proposed that the CRH-BP plays a role in the modulation of the action of urocortin and CRH at these sites. In humans, CRH-BP has been detected in the brain, pituitary, liver and placenta [22–24]. In ovine models of heart failure [25], the half-life of urocortin 1 was markedly prolonged compared to human models. This has been attributed to the possible role of CRF-BPs in clearance of urocortin 1 in humans. However, the exact role of these binding proteins remains to be fully elucidated in health and disease states.

3.2.3. Intra-cellular signaling pathways

The urocortins bind to G-protein coupled CRH (R1 and R2) receptors to induce conformational changes in the receptor that activate intra-cellular signaling pathways (Fig. 2). In most cells, this involves adenylyl cyclase and cyclic adenosine monophosphate (cAMP). Indeed, Kageyama et al. demonstrated that urocortin 2 induces vasodilatation in vascular smooth muscle cells via CRH-R2 in association with increased cAMP accumulation via activation of adenylate cyclase [26,27]. Mitogen-activated protein kinases (MAPK) are also implicated in urocortin-mediated vasodilatation [26] as well as in the cardioprotective role of urocortin in response to ischemic or hypoxic injury [28]. However, in some studies, inhibition of the c-AMP or protein kinase A (PKA) pathway has failed to inhibit the effects of CRH and its related peptides, suggesting the involvement of other signaling mechanisms.

CRH and related agonists can evoke the endothelial release of nitric oxide via nitric oxide synthase with subsequent accumulation of cyclic guanosine monophosphate (cGMP). This endothelium-dependent mechanism is important in CRH or urocortin-induced relaxation in placental vasculature [29] and peripheral arteries, and has been studied in isolated arterial segments such as the human internal mammary artery graft [30] and in rat coronary artery [31]. The nitric oxide and cGMP-dependent component of this vasodilator effect is mediated via activation of calcium-activated potassium channels in underlying vascular smooth muscle. Indeed, the vasorelaxant effect of urocortin is blunted in the presence of L-NAME (N^G-nitro-L-arginine methyl ester; a NOS inhibitor) and ODQ (1H-[1,2,4] oxadiazolo [4,2-a] quinoxalin-1-one; inhibitor of guanylyl cyclase) [30].

There is increasing evidence that urocortin and CRH-related peptides play an important role in cell survival mechanisms in a number of systems. Similar to CRH, urocortin 1 activates the MAPK extra-cellular signal-related kinases (ERK) 1/2-p42/44 signaling cascade in *in vitro* cultures of isolated rat cardiac myocytes, which is inhibited by blockade of MEK 1/2. This signaling cascade mediates the cardioprotective function of urocortin in stimulated hypoxia or ischemia [28]. Urocortins also possess anti-inflammatory properties that appear to be mediated via pro-apoptotic effects on macrophages via a direct effect on pro-apoptotic Bcl-2 related proteins [32].

3.3. Physiology of urocortins

3.3.1. Cardiovascular actions

The important roles of urocortins in the regulation of normal cardiovascular physiology are being increasingly recognized.

Genetically engineered mice lacking CRH-R2 are resistant to otherwise marked urocortin-evoked changes in cardiac performance and blood pressure [33]. Studies, which are largely preclinical, have so far demonstrated a favourable effect of urocortins on hemodynamic and neurohumoral regulation. Urocortins 1, 2 and 3 produce positive inotropic and lusitropic effects, reduction in the mean arterial pressure due to decreased peripheral vascular resistance, and increased coronary perfusion in rodent and ovine studies.

3.3.1.1. Vascular effects. Central administration of CRH in rats produces a pressor response, which appears to be mediated through CRH-R1 receptors [34]. Peripheral administration of CRH produces a depressor response, which is blocked by α helical CRH (a non-selective CRH antagonist), and not by antalarmin (selective CRH-R1 antagonist). As a peptide, CRH has limited access to the central nervous system. This suggests that the hypotensive effect is mediated via peripheral CRH-R2 receptors. CRH-R2 may have a more prominent role in changes in arterial pressure in comparison to CRH-R1. CRH-R2 may contribute to the maintenance of basal vascular tone in mice. Indeed, mice deficient in CRH-R2 receptors are hypertensive with no fall in the mean arterial pressure in response to exogenously administered urocortin [35]. It remains unknown whether urocortins and CRH-R have a similar role in the maintenance of basal vascular tone in humans.

Intravenous administration of CRH produces vasodilatation in rats and a consequent fall in blood pressure with a reflex increase in heart rate, although this is not observed in sheep and is seen only with relatively high doses in higher primates like monkeys and humans. In anesthetized rats, intravenous injection of human urocortin 2 reduced basal systemic blood pressure in a dose-dependent fashion [36]. As demonstrated by Vaughan et al. [4], urocortin 1 also possesses a potent and long lasting hypotensive action. Decrease in mean arterial pressure of 18.3 ± 0.7 mm Hg was observed with urocortin 1 in rats and lasted for almost 2 h. Dieterle et al. [37] studied the effects of urocortin 2 injection in control and hypertensive rats. They showed an immediate and sustained lowering of blood pressure in hypertensive rats with no rise in heart rate. This effect on the blood pressure was seen for up to 12 h after intraperitoneal injection of urocortin 2.

Several mechanisms have been postulated for the blood pressure lowering effects of urocortins. These may include a direct smooth muscle relaxant effect in combination with an associated reduction in plasma concentrations of vasoconstrictor hormones, such as endothelin 1, angiotensin II and arginine vasopressin (AVP), as seen in animal models of heart failure (see Section 4.3 on Neurohormonal effects).

Ex vivo studies in the human internal mammary artery [30] and in the rat coronary artery [31] suggest both endothelium-dependent and independent components for vasorelaxation. In isolated rat coronary artery [31], potent vasorelaxant effect of urocortin 1 was observed with an IC_{50} of 2.24 nM, in the presence of an intact endothelium. The endothelium-dependent component appears to be, at least in part, mediated by nitric oxide via cGMP, as outlined above. Huang et al. [31] have also shown the role of activation of barium chloride ($BaCl_2$) sensitive potassium channels in arterial smooth muscle cells, mediating the endothelial component of urocortin-induced coronary relaxation. Indeed, these studies also demonstrate a blunted, but not abolished, vasorelaxant response to urocortin in endothelium-denuded arterial segments, further suggesting the role of additional endothelium-independent mediators. Endothelium-independent regulation of vascular tone appears to involve calcium (Ca^{2+}) independent phospholipase A_1 and store-operated Ca^{2+} entry

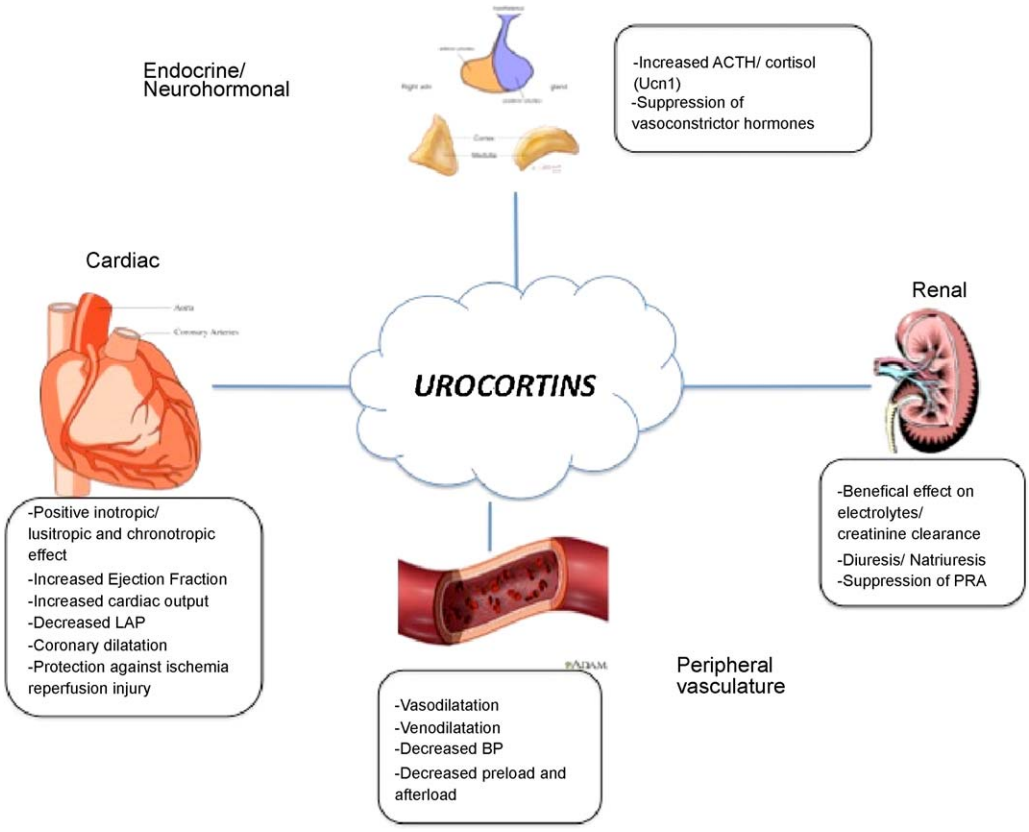

Fig. 3. Beneficial effects of urocortins in heart failure. The beneficial effects of urocortins on various systems as seen in animal and human models of heart failure. ACTH: adrenocorticotrophic hormone; Ucn 1: urocortin 1; LAP: left atrial pressure; BP: blood pressure; PRA: plasma renin activity.

modulation [38]. Other mechanisms demonstrated in mediating urocortin-induced vasorelaxation include the MAPK and PKA pathway [26].

3.3.1.2. Cardiac effects. It is not entirely clear whether urocortins mediate their protective effects upon cardiac contractility via a direct effect on cardiac myocytes or via sympathetic stimulation in response to reduced peripheral resistance. Brar et al. [28] have demonstrated the presence of a 22 kDa urocortin 1 precursor protein in neonatal rat cardiac myocytes and release of urocortin into the supernatant of cardiac myocytes exposed to stimulated ischemia or hypoxia, suggesting endogenous release of urocortin from ischemic cardiac myocytes. This may suggest potential direct and local action of urocortins on cardiac myocytes, mediated via CRH -R.

To determine whether urocortin induced direct effects on contractility of cardiac myocytes, Yang et al. [39] observed the effects of application of 100 nmol/L of urocortin 2 to isolated adult rabbit ventricular cardiomyocytes. They showed a progressive enhancement in myocyte contractility with reduction in diastolic length and concluded that urocortins exert direct positive inotropic as well as lusitropic effects. These effects were mediated via activation of CRH-R2 and subsequent stimulation of PKA activity, leading to augmentation of L-type calcium channel (I_{Ca}), and sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase (SERCA)-mediated Ca^{2+} uptake into the sarcoplasmic reticulum.

Immediate improvement of left ventricular fractional shortening and circumferential fibre shortening velocity were noted following acute injection of urocortin 2 in rats [37]. These beneficial effects were preserved even at 5 weeks after treatment. In ovine models, intravenous injection of urocortin 1 causes a marked dose-dependent increase in cardiac output and contractility, which is sustained at 24 h post injection [40].

3.3.1.3. Effects in humans. A small number of clinical studies have carried forward data obtained from animal studies, to look at the effects of urocortins on cardiovascular and neurohormonal responses.

In contrast to the favourable hemodynamic responses seen in normal sheep, infusion of urocortin 1 in healthy humans caused no change in hemodynamic variables, nor did it affect the plasma concentrations of humoral factors, such as aldosterone or arginine vasopressin. The pharmacokinetics of urocortin 1 were similar in normal humans and in subjects with heart failure: plasma urocortin 1 half-life of 52 ± 3 min in healthy volunteers and 54 ± 3 min in those with heart failure [18]. Pemberton and colleagues did not observe any increase in urinary urocortin 1 following its infusion suggesting that urocortin 1 is not excreted in the kidneys. Consistent with its predicted cardiovascular profile, urocortin 2 infusion caused a dose-dependent increase in cardiac output with a decrease in mean arterial and diastolic blood pressure, and systemic vascular resistance [19]. The effects of systemic urocortin 3 in humans are yet to be explored. Wiley and Davenport [15] have demonstrated that urocortin 3, like urocortin 2, causes vasodilatation in isolated human internal mammary artery segments and that this effect is mediated via the direct effect of urocortin 3 on the vascular smooth muscle cells. Although not yet assessed in man, urocortin 3 is likely to exert cardiovascular effects similar to those evoked by urocortin 2.

3.3.2. Cardioprotective effects—role in ischemia reperfusion injury

Urocortins appear to have a cardioprotective role and indeed, urocortin expression and peptide release is increased by ischemia [41].

Urocortin 1 reduces myocyte cell death caused by ischemia reperfusion injury. This appears to be mediated via several mechanisms including up-regulation of cardiotrophin 1 expression [42], stimulation of heat shock protein [43] and natriuretic peptides, and attenuation of calcium-insensitive phospholipase A2 gene expression [44]. *In vitro* secretion of urocortin is also enhanced by inflammatory cytokines such as interleukin-6, interleukin-1 and tumour necrosis factor α: factors that are elevated in patients with heart failure and acute coronary syndromes [64].

Urocortins 2 and 3 protect neonatal rat cardiac myocytes *in vitro* when administered before hypoxia or at the point of reoxygenation. Urocortins 2 and 3 also protect the adult rat heart *ex vivo* and acts via the MAPK pathway to reduce the infarct size of a perfused intact rat heart exposed to regional ischemia [45]. Brar et al. [45] have demonstrated that urocortin induces ERK 1/2-p42/44 phosphorylation in neonatal rat cardiac myocytes and that inhibition of MEK 1/2 inhibits its cardioprotective effects.

Nitric oxide is recognized as a key determinant of vascular health. It acts as a potent vasodilator, inhibits expression of several pro-inflammatory cytokines and chemokines, and plays a key role in vascular smooth muscle proliferation, platelet aggregation and endogenous fibrinolysis [46–48]. Whilst urocortin may act via nitric oxide to mediate cardiovascular protective effects [46], studies to date have not yet completely elucidated the effect of urocortins on the endothelium or nitric oxide.

4. Urocortins and heart failure

Given the potent vasorelaxant and inotropic effects of the urocortins, interest has grown in their role in the pathophysiology and potential therapeutic utility in the treatment of heart failure. A number of studies have demonstrated increased concentrations of urocortin 1 in cardiac tissue [49,50] and plasma [25,51,52] in experimental and clinical heart failure. Nishkimi et al. [49] demonstrated up-regulation of expression of urocortin 1 mRNA in left ventricular hypertrophy. They also demonstrated increased urocortin 1 immunoreactivity in the failing heart. This has been cited as possible evidence that urocortins play a role in the pathophysiology of cardiac hypertrophy and heart failure (Fig. 3).

4.1. Animal models

Pre-clinical studies of heart failure have previously examined the roles of urocortins 1 and 2 whilst urocortin 3 remains less well characterized. The beneficial cardiovascular and neurohumoral responses of urocortins are preserved, and may be augmented in the presence of heart failure.

Intravenous infusion of urocortin 1 in an ovine model of heart failure attenuates the hemodynamic deterioration and harmful neurohormonal activation associated with heart failure [53]. These hormones include renin, angiotensin II, aldosterone, endothelin-1, vasopressin and catecholamines that, along with sympathetic nervous system activation, combine to exert the hemodynamic and endocrine hallmarks of heart failure. Furthermore, in this model, urocortin 1 also protected renal function. The preservation of cardiac output with urocortin 1 infusion may be partly due to its inotropic actions, in addition to its coronary arterial vasodilator effects and improved cardiac bioenergetics. When infused at the onset of left ventricular pacing, urocortin 1 infusion restricts the increase in left atrial pressure and attenuates the reduction in cardiac output. It also has lusitropic and venodilating effects [54]. These results highlight that treatment with urocortin 1 may be beneficial in the treatment of heart failure initiated early in the disease. Of note, these favourable hemodynamics persisted during prolonged (4 day) infusion of urocortin 1.

In keeping with a favourable hemodynamic profile of urocortin 1 in ovine heart failure, urocortin 2 infusion in MLP (muscle specific LIM protein) deficient mice, a model of dilated cardiomy-

opathy, caused a dramatic improvement in cardiac output and left ventricular function, enhanced cardiac contractility and reduced systolic load [55]. The enhancement in ejection fraction by urocortin 2 is partly attributed to reduction in arterial load: this may prove more notable in the failing heart where there is an afterload mismatch.

Patients with heart failure are likely to receive any new treatment in conjunction with conventional treatment. Hence it is important to ensure that any potential new treatment does not interact with drugs such as ACE inhibitors and β blockers. Rademaker et al. [56] assessed the combined effects of captopril with urocortin 2 in sheep with pacing-induced heart failure. Combined treatment of urocortin 2 with captopril augmented the decrease in total peripheral resistance by an additional 20% compared with either agent alone. One of the potential drawbacks of treatment with ACE inhibitors is its profound hypotensive effect, which in the presence of heart failure can compromise blood flow to vital organs, such as the kidneys. When used in combination with an ACE inhibitor, urocortin 2 evoked an additional decrease in peripheral resistance without further reduction in the systolic blood pressure. This makes the combination of these agents an attractive tool in the management of heart failure. In addition, combination of urocortin 2 with captopril improved cardiac performance, decreased peripheral resistance and ventricular filling pressures in association with reduction in plasma aldosterone and endothelin-1 concentrations. In a murine acute heart failure model, pre-treatment with a β-adrenergic receptor (AR) antagonist, did not affect the inotropic or lusitropic actions of urocortin 2 *in vivo* indicating that its actions are independent of β-adrenergic receptors [55].

The effects of combined treatment with urocortin 2 and furosemide has been studied recently [57]. When this combination was administered to sheep with pacing-induced heart failure, it caused increased diuresis, natriuresis and sustained increase in creatinine excretion and clearance without additional potassium elimination. Urocortin 2 alone or in combination increased cardiac output and contractility whilst furosemide had no effect. Importantly, the combination of the two drugs, produced reversal of furosemide-induced increase in plasma renin activity (PRA) and a greater decrease in plasma aldosterone and vasopressin concentrations. These beneficial effects of Ucn 2 may be mediated by an increase in cardiac output and consequently improved renal perfusion. Indeed, improvements in glomerular filtration rate, urine volume and sodium excretion have been demonstrated in the ovine model of heart failure [58]. In addition to increased cardiac output and renal vasodilatation, other postulated mechanisms include a direct tubular action of Ucn 2 [11] as well as attenuation of anti-natriuretic and anti-diuretic factors resulting in increased diuresis and natriuresis [57].

As already noted, the hemodynamic and humoral responses of urocortin 3 in experimental heart failure are less well studied than urocortin 1 or 2. In a study of sheep with pacing-induced heart failure [20], the hemodynamic responses produced by urocortin 3 were similar to that produced by equivalent doses of urocortin 1 or 2. Urocortin 3 caused a marked dose-dependent improvement in cardiac output and reduction in peripheral resistance and left atrial pressure. This was associated with a reduction in mean arterial pressure, beneficial effect on hormonal responses (attenuation of vasoconstrictor peptide systems) and improved renal function (dose-dependent increases in urine volume, sodium and creatinine excretion). The onset and duration of action was much shorter than that of urocortin 1 or 2.

4.2. Patients with heart failure

Plasma concentrations of urocortins are elevated in patients with heart failure. Ng et al. [51] found higher plasma urocortin 1 concentrations in men with heart failure as well as in elderly patients. There appeared to be an inverse relationship between plasma urocortin concentrations and New York Heart Association (NYHA) class. In more severe heart failure, as reflected by NYHA class III or IV and low left ventricular ejection fraction, plasma urocortin concentrations appeared to be suppressed, suggesting that up-regulation of the urocortin system in early heart failure may be cardioprotective. In keeping with this report, more recent work by Wright et al. [59] has demonstrated elevated concentrations of plasma urocortin 1 in patients with heart failure with positive relationships to other circulating neurohormones such as

Table 1
Neurohormonal effects of Urocortins in human and ovine models.

	Humans				Sheep					
	Healthy		Heart failure		Healthy			Heart failure		
	Ucn 1 [17]	Ucn 2 [19]	Ucn 1 [18]	Ucn 2 [62]	Ucn2 [58]	Ucn 3 [20]	Ucn1	Ucn 1 [53,63]	Ucn 2 [58]	Ucn 3 [20]
cAMP	=	↑	=	=	=	=		↓[a]↑[b]	↓	↓↓
cGMP	↑	↑		=						
ACTH	↑↑	=	↑	↑	↑	↑↑		↑	↑	↑↑
Cortisol	↑↑	=	↑	=	↑	↑↑		↑	↑	↑↑
GH	=									
Ghrelin	↓	=	=	=						
LH/FSH/Prolactin	=									
TSH	=									
AVP	↑	=	=		↑	↑↑		↓↓	↑	↓↓
ANP	=		=		↓	↓		↓	↓↓	↓↓
BNP	=		=		↑	↑		↓↓	↓↓	↓↓
NT–BNP	=	=	=	↑				↓		
PRA	=	↑↑	=	=	=	=		↓	↓↓	↓↓
Aldosterone	=	↑	=		↓	=		↓	↓↓	↓↓
Adrenaline	=	↓	=	=	↓	↓		↓	↓↓	↓↓
Nor adrenaline	=	↑↑	=	=	=	=		↓	↓	↓
Endothelin	=	=	=	=	=	=		↓↓	↓↓	↓↓
Adrenomedullin	=	=	=	↑						
Insulin		=	=	=						
Angiotensin II		↑↑								
Glucose			=					=		

Fields left blank, no available data; (↑) increase, (↓) decrease, (=) no change.
[a] Suppression of cAMP by Ucn 1 in LV pacing-induced heart failure in sheep—as an acute effect.
[b] On prolonged exposure to Ucn 1 cAMP levels were substantially increased with a lag between the onset of hemodynamic and hormonal effects.

brain natriuretic peptide, adrenomedullin and endothelin-1. However, in contradiction to findings from Ng et al., Wright's group noted an inverse relationship of the level of plasma urocortin 1 to left ventricular ejection fraction, with a linear increase in plasma concentration of urocortin 1 with increasing NYHA class. It is possible that this difference in results is attributable to differences in the immunoassay used or potential effect of CRH-BP on the assay performance. Further research is, however, required to establish the relationship between urocortin concentrations and NYHA class.

Systemic intravenous infusions of urocortins 1 and 2 have been administered to a small number of patients with heart failure. Urocortin 1 infusion increased corticotrophin and cortisol, but produced no changes in hemodynamic, renal or neurohormonal parameters [18]. Infusion of urocortin 2 [19] evoked an increase in cardiac output with peripheral vasodilatation and a small increase in heart rate. Consistent with findings from the ovine model, systolic blood pressure fell in patients with congestive cardiac failure but not in control subjects [60]. It has been hypothesised that, in the presence of heart failure, the urocortin-induced rise in cardiac output is insufficient to compensate for the pronounced decrease in systemic vascular resistance [60]. However, this phenomenon may otherwise be explained by heightened peripheral sensitivity to the vasodilator effects of urocortin in the presence of heart failure. In agreement with this suggestion, administration of a CRH-R2 antagonist increases mean arterial blood pressure in sheep with heart failure but not in those without [61].

Apart from the positive influences on cardiovascular parameters when used in treatment in heart failure, urocortins may serve as potential biomarkers in identification of early heart failure, in combination with other biomarkers such as brain natriuretic peptide [59].

4.3. Neurohormonal effects of urocortins in heart failure

As noted above, urocortins cause a pronounced suppression of vasoconstrictor hormones in animal models of heart failure, which further supports its potential therapeutic role. However, studies of urocortins 1 and 2 in humans have only shown modest changes in neurohormonal activity. It is important to note that in man, infusion of urocortin 2 in healthy volunteers and in patients with heart failure does not alter plasma adrenocorticotropic hormone or cortisol concentrations. Table 1 summarizes the effects of urocortins on neurohormonal activity in ovine and human experiments.

5. Future of urocortins

There is increasing evidence that urocortins have several potential uses in management of cardiovascular conditions such as hypertension, ischemic heart disease and heart failure. The immediate and sustained blood pressure lowering effects by urocortin 2 [37] appears to pose a novel and attractive approach for antihypertensive treatment. The favourable effects on hemodynamics, renal and neurohumoral mechanisms have generated much interest in the use of urocortins in heart failure. In particular, the positive inotropic effect, combined with its ability to reduce peripheral arterial resistance, favours use in this group of patients. In humans, studies to date have largely looked at combined systemic effects of urocortins. Direct arterial and venous effects of urocortins have not yet been described in man. It has not been possible to tease out the relative contribution of urocortin-induced changes in hemodynamic variables on the augmentation of cardiac output. Further studies are required to look at potential effects of long-term administration of these peptides.

In the search for novel treatments for heart failure, the focus is on urocortins 2 and 3. Indeed, urocortin 1 has no hemodynamic effects in man and also bears the potential to induce unwanted side effects by activating CRH-R1 and stimulation of the hypothalamus–pituitary axis. However, it may have a role as an early biomarker of heart failure.

The potential application of urocortins in protection from ischemia reperfusion injury remains of major interest. Evidence available from pre-clinical models suggests that urocortins may have a role in protecting against ischemic reperfusion injury and in limiting infarct size. This has yet to be evaluated in man.

In conclusion, urocortins are emerging as an important group of peptidic mediators with important roles in human physiology and pathophysiology. This review provides an overview of their effects on the cardiovascular system alone. There are several other potential applications of this group of peptides, including roles in appetite suppression, in muscle wasting and central nervous system disorders to name a few. However, their major effects in the cardiovascular system implicate them as potential therapeutic targets in a range of processes, particularly heart failure.

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