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Commentary

The powerful cardioprotective effects of urocortin and the corticotropin releasing hormone (CRH) family

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ABSTRACT

The urocortins are members of the corticotropin releasing hormone (CRH) family of peptide hormones. The archetypal member of this family, CRH, plays an important role in regulating thermogenesis and homeostasis by acting centrally and systemically in target organs via its two receptors CRH-R1 and CRH-R2. However, by virtue of their much greater relative affinity for CRH-R2, the physiological effects of the urocortin peptides are largely restricted to peripheral organs such as the heart. A powerful cytoprotective effect of urocortin peptide administration against ischemia and reperfusion injury has been demonstrated in isolated cardiomyocyte models, as well as in the intact heart both *in vitro* and *in vivo*. Extremely promising data has shown the beneficial effect of treating pacing-induced heart failure in sheep with urocortin molecules. Though the efficacy and specificity of these molecules in humans is not yet established, molecular dissection of the cytoprotective pathways activated by urocortin peptides suggests that the beneficial effects may be separable from potentially deleterious effects.

1. Introduction

Urocortin (Ucn 1) is a 40-amino acid member of the corticotropin releasing hormone (CRH) family of peptide hormones [1] (see Fig. 1 for the urocortin peptide structures). The archetypical member, CRH itself, is produced in the brain in response to stress, and can affect behaviour by acting locally on the brain, as well as by affecting autonomic responses in peripheral organs. The various peptides of the CRH family appear to have overlapping roles in various tissues including the placenta, immune system, digestive system, central nervous system and cardiovascular system, with their relative importance in each system depending on their site of production, plasma distribution and specific receptor affinity.

This review is intended as a synthesis of current knowledge on the role of CRH-family proteins in the heart, particularly their therapeutic potential in terms of cardioprotection. We will also cover their important centrally mediated physiological effects, which must be taken into account if Ucn 1 or one of its homologs is to ever be developed as pharmaceutical agent. The reader is referred elsewhere for reviews on the role of the CRH family in other tissues [2].

2. The CRH-peptide family and their receptors

There are two mammalian genes encoding CRH receptors, both of which are expressed in the brain, and both of which

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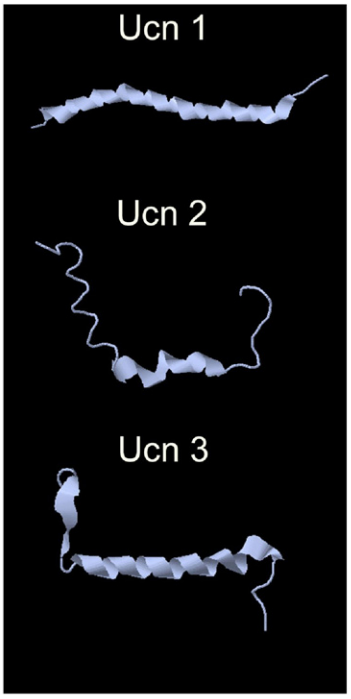
(A)



(B)

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CRH     SEEPPISLDLTFHLLREVLEMARAEQLAQQAHSNRKLMEII
Ucn1    -DNPSLSIDLTFHLLRTLLELARTQSQRERAEQNRIIFDSV
Ucn2    ---IVLSLDVPIGLLQILLEQARARAAREQATTNARILARV
Ucn3    --TKFLSLDVPTNIMNLLFNIAKAKNLRAQAAANAHLMAQI
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(C)

	HGNC	mRNA	PID	PDB
CRH	2355	NM_000756	P06850	-
Ucn 1	12516	NM_003353	P55089	2RMF
Ucn 2	18414	NM_033199	Q96RP3	2RMG
Ucn 3	17781	NM_053049	Q969E3	2RMH

Fig. 1 – (A) Peptide structures obtained from PDB file co-ordinates [12]. (B) Sequence alignments of human CRH-family peptides created using ClustalW2, where amino acids coloured red are nonpolar or hydrophobic; blue are acidic; magenta are basic; green are polar or non-charged. (C) Database references for human Ucn 1, Ucn 2, and Ucn 3. HGNC: the unique reference identity given to the human gene by the HUGO gene committee; mRNA: the Genbank reference id; PID: the EBI UniProt identifier; PDB: the 3D structural data identifier. (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

can bind all members of the CRH family albeit with different affinities. CRH-R1 is not detected in the heart [3], but CRH-R2 is highly expressed in cardiomyocytes, as well as in the gastrointestinal tract and epididymis [3–6]. There are two splicing variants of CRH-R2 in the heart—while CRH-R2α has been detected in all chambers of the human heart, CRH-R2β seems to be restricted to the left atrium [3]. CRH-R2β is also found in endothelial and smooth muscle cells of the systemic vasculature [5].

Expression of Ucn 1 has been detected in all four chambers of the human heart, although CRH is absent [3]. Since Ucn 1 is about 10-fold more potent than CRH at binding and activating CRH-R2 [1], Ucn 1 was anticipated to have effects on the heart—as was later borne out experimentally (see below). However, since it also binds with strong affinity to CRH-R1 it has concurrent effects in the brain.

Urocortin 2 (Ucn 2, also called stresscopin-related protein or SRP [7], a 38-aa peptide originally detected in mouse brain, binds CRH-R2 selectively and with high affinity [8]. Ucn 2 can be detected by RT-PCR in various tissues including heart and muscle [7]. Apparently because of its low affinity for CRH-R1, administration of the peptide to mice does not increase gross motor activity, but it does still have effects on central autonomic and appetite control [8].

Urocortin 3 (Ucn 3, also called stresscopin or SCP [7]) was identified on the basis of its sequence homology to other CRH-family members, and encodes a 38-aa mature peptide that is 90% homologous in mouse and humans. It is widely expressed, particularly in areas of the brain, the digestive system, and cardiac and skeletal muscle [7,9]. Ucn 3 is also strongly expressed in pancreatic β-cells, where it appears to act locally to augment insulin production—consequently mice lacking Ucn 3 are relatively protected from the harmful metabolic consequences of a high-fat diet, maintaining better glucose tolerance [10]. Additionally, it can be detected in normal human plasma [11]. Like Ucn 2, Ucn 3 is selective for CRH-R2 [9].

The NMR structures of human Ucn 1, Ucn 2 and Ucn 3 in solution have been determined—they are primarily alpha-

helical, with a small kink or turn at residues 25–27, resulting in a helix-turn-helix motif believed to be important for their binding to and activation of CRH receptors [12]. An important tool in examining the role of CRH-family peptides is the artificially synthesized antagonist, alpha-helical CRH-(9–41), which potently inhibits the effects of CRH-family peptides [13].

3. The role of the CRH-peptide family in homeostasis

CRH has an interesting and very important role in regulating thermogenesis and energy homeostasis. Mice deficient for CRH-R2 have significantly elevated basal levels of thermogenesis in brown adipose tissue, and exhibit behavioural changes indicating a loss of body heat [14]. CRH can act directly on skeletal muscle to stimulate thermogenesis, possibly via substrate cycling between *de novo* lipogenesis and lipid oxidation. This effect can be blocked by inhibitors of phosphatidylinositol 3-kinase or AMP-activated protein kinase [15]. Ucn 2 is proposed to function as a local negative regulator of glucose uptake in skeletal muscle, since mice deficient in Ucn 2 or administered with a CRH-R2 antagonist exhibit increased insulin sensitivity and are protected against fat-induced insulin resistance [16]. Other homeostatic effects of Ucn may be centrally mediated, for example intracerebrovascular injection of an alpha-helical CRH-(9–41) into rats blocks the pyrogenic and thermogenic effects of interleukin 1β [17].

The effects of CRH-family peptides on anxiety and activity seem to be mediated by CRH-R1 whereas effects on appetite are mediated via CRH-R2 [18], therefore the different effects of Ucn [19] and CRH may be explained by different receptor binding profiles [20]. Other centrally mediated effects of Ucn include a role in fluid homeostasis [21] and in osmoregulation, reflecting the role of orthologous proteins in insects [22] Table 1A.

4. The CRH-peptide family and disease

Due to the wide range of expression of the urocortins and their receptors, and their powerful effects on homeostasis, their potential involvement in various diseases has been investigated. It has been found that, unlike CRH, the urocortins do not increase corticosterone secretion and seem not to have a physiological role in regulating the hypothalamic–pituitary–adrenal (HPA) axis [18].

CRH-family peptides also exhibit an anti-inflammatory effect that appears to involve a direct effect on macrophages [23] but may also be stress-related. Consequently, the potential for CRH-1 receptors as a therapeutic target for inflammatory diseases such as irritable bowel syndrome has been investigated [24]. An increase in Ucn expression is detected in lung tissue of rats with allergic asthma [25], although whether it is playing a pro- or anti-inflammatory role in this case is not known.

5. Cardiovascular effects of the CRH-peptide family

CRH is unlikely to have major direct effects on the heart, since plasma concentrations are very low and it is not highly expressed locally. In contrast, as mentioned above, Ucn 1, Ucn 2 and Ucn 3 are expressed in the heart. Expression and secretion of Ucn 1 increases in isolated rat cardiomyocytes exposed to simulated ischemia [26]. Furthermore, cardiac release of Ucn 1 occurs in the absence of tissue necrosis when rats are exposed to sublethal ischemia and reperfusion injury, reaching ~8 pM in the plasma, compared to undetectable levels in control rats [27]. Arterial plasma Ucn 1 levels measured 15.2 ± 0.5 pM in normal sheep and increased significantly following development of heart failure to

Table 1A – General physiological effects of urocortins

Species	Work performed	Relative functional effects	References
Rat	Food and water intake suppression Gastric motility inhibition Autonomic control of gastric emptying	Regulation of caloric intake	[19,85]
Rat	Contribution to an increase in lung vascular permeability through activation of CRH receptor	May act as an autocrine and paracrine proinflammatory factor in lungs	[86]
HF sheep (experimental heart failure)	Urine volume increase	Effects on renal hemodynamics	[35]
Human (synovial tissue of patients with rheumatoid arthritis RA)	Possible inhibitor of extravesation	Possible suppression of the inflammation in RA	[87]
Human (normal cycling human ovaries)	Most likely inhibition of ovarian steroidogenesis in the process of luteal degeneration	May act as autocrine and/or paracrine regulator in the ovarian corpus luteum	[88]
Mouse and rat (mesangial cells)	Suppresses production of ROS in endothelial cells and sustains endothelium-dependent relaxations of coronary artery	Could significantly ameliorate diabetic nephropathy	[89]
Mouse	Growth inhibition of hepatocellular carcinoma (HCC) and reduction of tumor microvessel density	Reported to be a tonic suppressor of vascularization, implying its role in tumor angiogenesis	[90]

Table 1B – Cardiovascular effects of urocortins

Species	Model	Effects observed	References
Mouse	*in vitro*	→ Protection of cardiac myocytes from cell death induced by hypoxia	[47]
	in vivo	↓ inotropic, lusitropic and systemic arterial load on the LV myocardium ↑ cardiac output	[38]
	in vivo (dilated cardiomyopathy)	Significant improvement in HF	
	in vivo (conscious rats)	↓ blood pressure ↑ heart rate	[1]
Rat	*in vivo* (anesthetized rats)	↓ arterial pressure ↓ cardiac output ↑ heart rate ↑↑ cardiac contractility	[85]
	in vitro	Protection of cardiac myocytes from cell death induced by hypoxia	[45,46,49,55–57,59,60,66]
	Langendorff	Protection against ischemia and reperfusion injury	
Sheep	*in vivo*	↑ arterial pressure ↑ cardiac output ↑ heart rate ↑ coronary blood flow ↑↑ cardiac contractility	[32]
	in vivo (experimental heart failure)	↓ arterial pressure ↑ cardiac output ↓ blood pressure	[35–37]
	in vivo (heart failure)	↑ cardiac output ↑ left ventricular ejection fraction ↓ arterial pressure	[43]
Human	*in vivo* (healthy individuals)	↑ cardiac output ↑ heart rate ↑ left ventricular ejection fraction	[44]

19.1 ± 1.6 pM [28]. Similarly, Ucn 1 levels are increased from ~19 pM in normal human males to ~50 pM in those with systolic heart failure [29]. An immunohistochemical study also demonstrated increased immunoreactivity for Ucn 1 in the left ventricular myocytes of 9 dilated cardiomyopathic hearts compared to 5 normal human hearts [5]. Circulating Ucn 3 in normal human plasma has been reported at a level of ~50 pM [11] (Table 1B).

Ucn 1 has direct effects on the heart, increasing inotropy and coronary vasodilation in the isolated rat heart after administration of a bolus dose estimated to have attained a plasma concentration of >200 nM [30], and these effects appear to be mediated by CRH-R2 since the response is absent in mice lacking this receptor [31]. Administration of a 100 μg Ucn 1 but not CRH potently elevates cardiac contractility in sheep for up to 24 h [32]. Furthermore, intravenous administration of lower doses of Ucn 1 (2.5–10 μg) was recently shown to induce potent inhibition of sympathetic nerve activity to the heart despite being below the threshold for direct actions of Ucn1 on the myocardium [33], suggesting Ucn 1 has a role in normal cardiac homeostasis, and may have further beneficial effects in heart failure via indirect mechanisms. Indeed, endogenous urocortins have been shown to have a protective role in pacing-induced heart failure in experiments in which a CRH-R2 antagonist was administered to sheep [34].

The group of Richards in Christchurch, New Zealand, have made great inroads into exploring the putative benefits of Ucn and related peptides in treating heart failure. Their initial studies of intravenous administration of Ucn 1, Ucn 2 and Ucn 3 in sheep with pacing-induced heart failure demonstrated dramatic improvements in cardiac output, peripheral resistance, left atrial pressure, mean arterial pressure, renal function and suppression of detrimental neurohormonal circulation [35–37], though the relative involvement of direct and indirect effects of Ucn is not known. Thirty minutes after bolus administration of 10 μg, 50 μg, or 100 μg, they measured increases from the control plasma concentration of Ucn 1 of 12.3 ± 1.3 pM up to 219 ± 19 pM, 1777 ± 91 pM and 4142 ± 175 pM, respectively [35], with most significant cardiac effects occurring after 50 μg or 100 μg. In mice treated with Ucn 2, these effects have been shown to be independent of β-adrenergic receptors [38]. The vasodilatory but not other cardiac effects of Ucn 1 and Ucn 2 involve nitric oxide [39,40]. The more rapid onset of action of Ucn 3 and its shorter duration of action may be a reflection of different pharmacokinetic profiles [37].

Importantly, there does not seem to be a desensitization effect to prolonged Ucn administration, at least over 4 days of intravenous infusion of Ucn 1 (0.3 μg/(kg h), attaining up to 8000 pM plasma Ucn 1) in sheep with pacing-induced heart failure, with sustained beneficial effects observed at the end of the experiment [41]. Interestingly, potential "side-effects" such as food intake suppression and stress hormone production appeared to be transient and diminished after 2 days [41]. Furthermore, Ucn 1 treatment commencing from the onset of cardiac overload was able to repress progression to overt heart failure, supporting its therapeutic use early in the disease [42].

Recently, the effect of Ucn 2 administration to human heart failure patients has been examined. After 1 h intravenous infusion of 25 μg or 100 μg Ucn 2 (attaining plasma concentrations of ~114 pM and ~440 pM vs. 19 pM in those receiving placebo), there was a significant increase in cardiac output and left ventricular ejection fraction and decreased mean arterial pressure, systemic vascular resistance, and cardiac work, with no effect on hormone levels observed [43]. This is similar to the effects observed by the same investigators in healthy humans, although in that case subtle renal effects and activation of plasma renin, angiotensin II, and norepinephrine (at high-dose only) were also observed [44].

The CRH family is highly cardioprotective, with an ED_{50} in the nanomolar range in various experimental systems. Treatment with any of the urocortin peptides *in vitro* protects isolated neonatal and adult rat cardiomyocytes from ischemia and reperfusion injury [45–47]. In addition, they have been shown to be protective in the isolated perfused rat heart [27,45,47,48], even when present only during reperfusion [49]. An extensive variety of peptides are known to activate common cardioprotective pathways after binding their respective G-protein-coupled receptors, and these same pathways are believed to be involved in other mechanisms of cardioprotection such as ischemic preconditioning and postconditioning (reviewed in [50–52]). In order to understand the powerful cardioprotective mechanism of the CRH-family peptides, their signal transduction pathways have been extensively investigated.

6. Signal transduction mechanisms of the CRH-peptide family

The cardioprotective effects of urocortins against ischemia and reperfusion injury involve the activation of several signalling pathways that have both cytoplasmic and mitochondrial targets and culminate in the alteration of cellular metabolism and the modulation of apoptosis (see Fig. 2). Although most of this signalling pathway has been investigated using Ucn 1, the other members of the family are believed to act similarly by virtue of their interaction with the same cell surface receptors. By initially binding to G protein-coupled receptors, Ucn 1 initiates a complex signalling cascade involving activation of phosphoinositide-3-kinase (PI3K), protein kinase A (PKA), protein kinase B/Akt (Akt), protein kinase C (PKC) and mitogen-activated protein kinases (MAPKs) amongst other signalling pathways [53]. Activation of these kinase pathways alters the activity of various channels

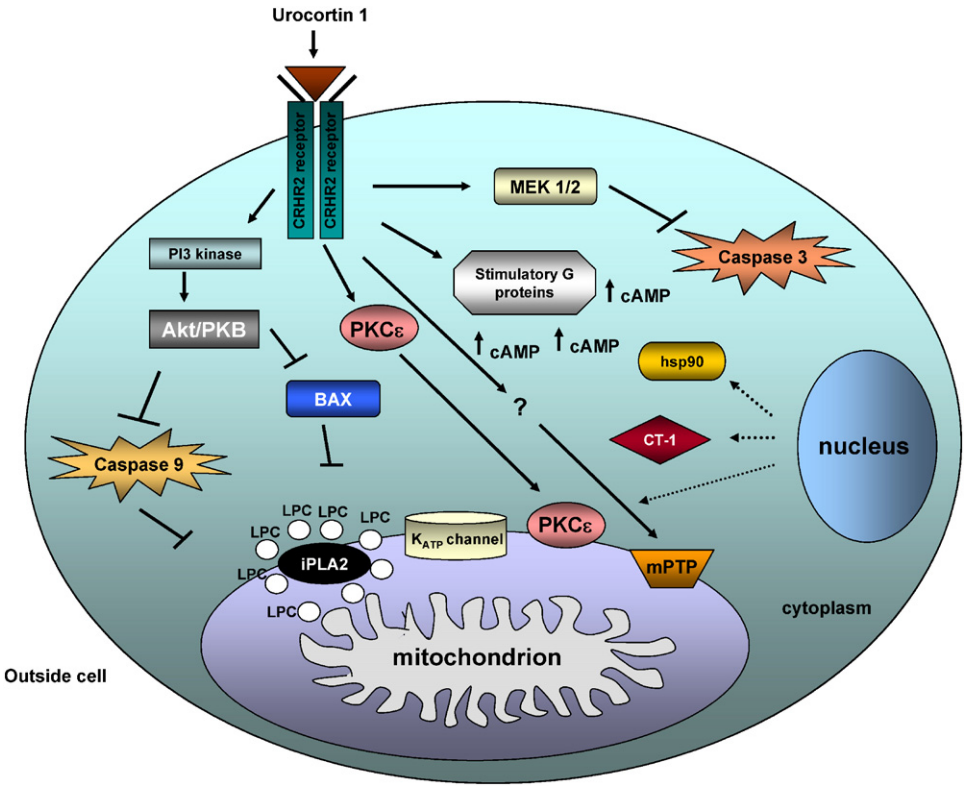

Fig. 2 – Schematic of signalling pathways activated by urocortin in cardiomyocytes. Urocortin 1 binds to CRH-R2 receptor, which causes the activation of stimulatory G proteins and results in activation of adenylyl cyclase and increased levels of cyclic adenosine monophosphate (cAMP). In parallel, mitogen-activated kinase p42/p44 (MAPK), phosphoinositide-3 kinase (PI3K) signalling pathways and protein kinase C epsilon (PKCε) are activated. All three pathways have been reported to play a crucial role in cardioprotection. Downstream, late effects following urocortin exposure include increases in the expression of heat shock protein 90 (HSP90), cardiotrophin-1 (CT-1), K_{ATP} channel components, and PKCε, in addition to a decrease in expression of the calcium insensitive phospholipase A_2 ($iPLA_2$). Opening of the mitochondrial permeability transition pore (MPTP) may be inhibited via PKCε, or indirectly by decreasing levels of oxidative stress (see text).

including the mitochondrial permeability transition pore (MPTP), which is centrally involved in the induction of cell death [45,54]. In addition to acute effects on different kinase pathways urocortin peptides have more chronic, modulatory activity as shown by transcriptional and translational effects on mitochondrial ATP-sensitive potassium channel (K_{ATP}), calcium insensitive phospholipase A_2 ($iPLA_2$) and protein kinase C epsilon (PKCε), all three intimately involved in cardioprotection [55–58].

Considerable data suggests that Ucn 1 acts via a p42/p44 MAPK (ERK1/2)-dependent signalling pathway in protecting both *in vitro* primary cell models and *ex vivo* and *in vivo* rodent heart from reperfusion injury. The extracellular signal-related kinases (ERK) belong to one sub-family of MAP kinases and consist of 42- and 44-kDa kinases. Their phosphorylation and activation is mediated by the MAP kinase MEK1/2 following Ucn 1 treatment [59,60]. Pharmacological ''proof of concept'' studies using the MEK1/2 inhibitors PD98059 and U0126 have demonstrated that Ucn 1-mediated cardioprotection requires activation of this signalling pathway. Specifically, in primary isolated cardiac myocytes, treatment with PD98059 abolished cytoprotection (in both ischemia and reperfusion) produced by Ucn 1 as assayed by trypan blue exclusion, annexin V staining and TUNEL labeling [48,59,60]. Moreover, inhibition of the MEK1/2 pathway by PD98059 decreased the ability of Ucn 1 to reduce infarct size during ischemia/reperfusion in an *ex vivo* perfused rat heart model as well as *in vivo* [59]. Importantly, similar findings were also reported for Ucn 2 and Ucn 3 [47]. Clearly, the p42/p44 MAPK pathway, which is activated by MEK1/2, seems to play a significant role in cardioprotective effect of Ucn.

Equally important, however, is phosphatidylinositol 3-OH kinase (PI3K) and one of its downstream effectors, protein kinase B (Akt), both of which are crucial for Ucn-stimulated increase in survival of cardiomyocytes [61]. Phosphorylation on both Thr-308 and Ser-473 as well as direct interaction with the PI3K lipid product (phosphatidylinositol-3 and 4-biphosphate) is required for Akt activation. Akt mediates cell survival in turn by phosphorylating the serine residues of a number of proteins including the pro-apoptotic protein BAD. This results in it binding to the 14-3-3 protein, thereby preventing it from interacting with mitochondria, and thus conferring resistance against apoptosis [62]. Both MEK1/2 and PI3K are responsible for preventing procaspases 9 and 3 from being cleaved into their active forms [63,64]. Furthermore, chemical inhibitors of the PI3K pathway, such as wortmannin and LY294002, have been reported to block Ucn 1-mediated cardioprotection in both neonatal and adult cardiomyocytes [61]. In this study, forced overexpression of active PI3K prevented the death of cardiomyocytes in response to simulated hypoxia and reoxygenation. Both Ucn 2 and Ucn 3 have also been demonstrated to act through the PI3K pathway [47].

In addition to its protective effects, urocortin treatment of both neonatal and adult rat cardiomyocytes has been shown to stimulate the secretion of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP)—both of which are commonly used markers for hypertrophy [65–67]. Importantly, there is a potential divergence between the signalling pathways involved in the cardioprotective response and the hypertrophic response to urocortins. Namely, the p42/p44 MAPK pathway, though required for cardioprotection, does not appear to be involved in the hypertrophic effect of Ucn 1. Conversely, the PI3K/Akt pathway does appear to be required for it to induce hypertrophy [65]. In terms of practical application, it would be extremely helpful if a method is found to synthesize analogues of urocortins that are capable of activating selected protective pathways without any concurrent hypertrophic response.

A number of elegant studies have revealed a role for PKCε in cardioprotection during cardiac ischemia/reperfusion injury (reviewed in [52]). The PKC family of kinases phosphorylates serine and threonine residues on target proteins. Eleven isozymes of PKC have been recognized so far and are classified on the basis of calcium dependency and regulation by lipid modulators [68]. However, the specificity of the PKC response to Ucn 1 exposure has been gleaned from microarray analyses that show elevated levels of PKCε in rat primary cardiomyocytes following 24 h exposure to Ucn 1, with no differences detected for other PKC isozymes; thus suggesting urocortins selectively activate PKCε [45,55,57,58]. When activated, PKCs translocate from the cytosol to the plasma membranes. This translocation occurs when cytosolic PKC binds to an isozyme-specific membrane-bound anchor protein or RACK (receptor for activated C kinase) which in turn activates PKC isozyme transported to its target protein [58]. Small peptides of six to eight amino acids have been used to inhibit specific isozymes of PKC from binding to the specific RACK, as well as, pseudo-RACK peptides to enhance the function of specific PKCs. Pseudo RACKS specific for PKCε have shown that it acts as a cardioprotective agent [69–71]. Furthermore, evidence that PKCε is the downstream modulator of Ucn 1 was provided by the use of specific PKCε inhibitor peptides, that when introduced into cardiomyocytes, prior to simulated ischemia, resulted in the loss of Ucn 1 cardioprotective effect [58].

Interestingly, the PI3K/Akt and ERK1/2 kinase cardioprotective signalling pathways, in addition to PKCε appear to be common to most cardioprotective peptide agents [50–52,72]. However, this does not exclude the possible involvement of other kinase pathways such as STAT-3, recently shown to be required for cardioprotection by the canonical Akt activator, insulin [73]. Furthermore, the involvement of other kinases known to be activated by urocortins, such as PKA, remains to be tested.

7. Other cardioprotective mechanisms of the CRH-peptide family

In addition to the activation of protein kinase signalling pathways, a significant contribution of Ucn 1-mediated cardioprotection involves *de novo* protein synthesis. Expression of the cardioprotective heat shock protein 90 (HSP90) has been reported to be induced by Ucn 1 and this effect is blocked by the MEK1/2 inhibitor PD98059 [74]. Moreover, the expression of cardiotrophin-1 (CT-1), an additional cardioprotective peptide, is enhanced upon exposure of cells to Ucn 1 [75].

Microarray profiling has helped dramatically to unravel the gene expression profile effects of Ucn 1 [55]. In this study the protein identified was the K_{ATP} channel. This channel opens in

response to a drop in the cytosolic concentration of ATP, and therefore it is regarded as a sensor of the metabolic state of a cell. These channels, when open during stressful stimuli such as ischemia/reperfusion are ''cardioprotective'' [55,57]. There are two known subtypes of K_{ATP} channel, Kir 6.1 and Kir 6.2, small transmembrane proteins that form the pore of the channel. For a K_{ATP} channel to be fully functional requires additional protein subunits – namely the sulphonylurea receptors (SUR). These SUR receptors are responsible for sensing and binding ATP/ADP and ultimately gating the channel pore [53,76]. Ucn 1 induces the expression of the Kir 6.1 potassium channel subunit in both primary cardiomyocytes and the whole heart [55]. Using an antagonist to the mitochondrial K_{ATP} channel, 5-hydroxydecanoate (5-HD) and dominant negative versions of Kir 6.1, the complete loss of the cardioprotective properties of Ucn 1 was demonstrated when Kir 6.1 was inhibited. Furthermore, openers of K_{ATP} channel, including cromakalim, are accordingly cardioprotective during simulated ischemia/reperfusion injury *in vitro* [55].

Ucn 1 also regulates $iPLA_2$ which belongs to a superfamily of phosphoplipases. $iPLA_2$ catalyses the breakdown of membrane phospholipids into arachidonic acid (AA), a precursor of prostaglandins, leukotrienes and a minor metabolite lysophosphatidylcholine (LPC). $iPLA_2$ catalytic activity is enhanced during ischemia/reperfusion, with a corresponding increase in its metabolites AA and LPC; the latter being highly cardiotoxic, and as such are implicated in cell death during ischemia/reperfusion injury [77,78]. Ucn 1, as well as the $iPLA_2$ inhibitor, bromoenol lactone (BEL), was found to lower the expression levels of LPC in cardiomyocytes and therefore protect cardiomyocytes against injury caused by LPC and ischemia/reperfusion to a similar degree [56,57].

In recent years the crucial role of the MPTP in reperfusion injury has become increasingly apparent. This inner mitochondrial channel opens in response to elevated mitochondrial calcium, oxidative stress and adenine nucleotide depletion—the exact conditions that occur early during reperfusion following a period of ischemia [79]. A recent study, using the Langendorff perfused heart model, has shown that Ucn 1 can decrease MPTP opening during reperfusion in the intact heart [45,54]. The role of the mitochondria was demonstrated using the 'hot dog' technique in which 2-[^{3}H]deoxyglucose (''hot DOG'') becomes entrapped within mitochondria that undergo mitochondria permeability transition (MPT). An acute, 30 min pre-treatment with Ucn 1 significantly reduced the loss of 2-[^{3}H]deoxyglucose with an accompanying reduction in the degree of ischemia/reperfusion-induced MPTP and associated tissue damage in rat hearts [45]. How Ucn 1 blocks the opening of MPTP is still not fully understood although it seems that, via activation of PKCε, it decreases the burst of reactive oxygen species (ROS) upon reperfusion, and therefore greatly reduces one of the prime activators of MPTP. Both by using a redox-sensitive dye in isolated cells, and by quantifying protein carbonylation as an index of oxidatively damaged proteins in the intact heart, Ucn 1 was shown to decrease levels of oxidative stress during reperfusion, and this could be blocked by the PKC inhibitor chelerythrine [45]. However, further detailed studies are needed to explain the role of PKCε on the inhibition of MPTP and its precise mechanism of action.

Further research and a better understanding of the signalling mechanism of the urocortins in cardioprotection and their role in the reduction of cell death is of immense importance and will enhance our ability to focus on exogenous modulation of cardioprotection, a useful clinical strategy for cardiac damage.

8. Urocortin as a therapeutic agent

The therapeutic potential of the urocortin peptides has been recognized for some time now, both in regard to its powerful cardioprotective properties, and other favourable cardiovascular effects mentioned above. The pharmacokinetics have been investigated, and the plasma half-life of 50 μg Ucn 1 in humans was found to be 52 min [80]. One particularly interesting aspect of urocortin treatment of heart failure is the apparent lack of desensitization after prolonged treatment [41]. This would be worthy of further investigation in relation to cardioprotection, since most forms of cardioprotection currently being investigated – including the canonical form, ischemic preconditioning – are transient.

As mentioned previously, urocortins have various effects on the heart, some of which would be undesirable for a human therapeutic agent. Some divergent aspects of the signalling pathways by which urocortins lead to hypertrophy and/or cardioprotection have been identified [65,81], raising the possibility of dissecting out these separate actions. A further important consideration is the non-cardiac effects of CRH administration. For this reason Ucn 2 and Ucn 3 are generally regarded as having greater potential, since they have much less affinity for the CRH-R1 receptor responsible for most of the centrally mediated effects. In sheep, activation of the pituitary–adrenal axis was observed in response to Ucn 2 or Ucn 3, though only minimal effects were observed in rats, mice and humans, possibly because the peptides have less affinity to CRH-R1 in these species.

In addition to being effective in experimental systems, therapeutic agents must be beneficial in heart failure patients who are often already receiving multiple pharmaceuticals. Using their sheep model of heart failure, Rademaker et al. showed that co-treatment with Ucn 2 and an ACE inhibitor, captopril, in heart failure produced significantly greater improvements in hemodynamics, hormonal profile and renal function than achieved by captopril alone [82].

9. What does the future hold for the urocortins?

The wide range of biological effects mediated by the urocortins are far from being completely understood. For example, although it has been known for some years that mice deficient for CRH-R2 become hypervascularized postnatally [83], only recently has it been shown that inhibition of neo-vascularization by Ucn 2 overexpression can contribute to tumor growth inhibition *in vivo* [84]. Although an exciting finding in terms of cancer research, this suggests that the administration of Ucn 2 or its homologues may have detrimental effects in patients with heart failure or post-myocardial infarction, in which

neovascularization is desirable. Similarly, Ucn 2 has recently been shown to function as a local negative regulator of glucose uptake in skeletal muscle, suggesting that prolonged Ucn 2 treatment might alter blood glucose levels [16]. Long-term studies of urocortin administration in animal studies still seem warranted.

On the other hand, the strong preference that some members of the family exhibit for binding to CRH-R2, and the highly beneficial effects observed even after several days administration in sheep models of heart failure, does seem extremely promising. Of course, its effect in humans must be determined, and indeed, one company (Neurocrine Biosciences Inc.), has now completed a phase I clinical trial using Ucn 2, and plans to initiate phase II studies in patients with mild to moderate congestive heart disease in 2008. Clearly, the precise underlying molecular mechanisms by which they act still need to be fully described yet the *in vitro*, *in vivo* and *ex vivo* data presented demonstrate a bright future for the use of the urocortins as a therapeutic small molecule.

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