Urocortins (Ucn) 1, 2 and 3 are a group of endogenous peptide hormones belonging to the corticotropin-releasing hormone (CRH) family of peptides. The presence of urocortins has been detected in the central nervous system as well as in peripheral tissues. They play an important role in a stress response (with respect to its duration, intensity and restoration of homeostasis). They also act as regulatory factors of the cardiovascular, gastrointestinal, reproductive and immune systems. Urocortins act by binding to G-protein-coupled receptors (GPCR). The "central" effects of urocortins are mediated mainly by activation of CRH receptor 1 (CRH-R1), and the "peripheral" effects by activation of CRH-R2. Ucn2 and Ucn3 are selective CRH-R2 agonists and have much higher binding affinity to this receptor than CRH and Ucn1. Recent studies have shown that urocortins exert various biological effects in the cardiovascular system, such as vasodilation, positive inotropic and lusitropic effects, as well as cardioprotection against ischemia-reperfusion injury. They also suppress the renin-angiotensin system and may have an impact on the sympathetic nervous system. Urocortins and CRH-R2 may be a potential therapeutic target in coronary heart disease, congestive heart failure and hypertension. This review summarizes the data published to date on the role of urocortins in the cardiovascular system.

Key words: urocortin 1, urocortin 2, urocortin 3, corticotropin-releasing hormone receptor 1, corticotropin-releasing hormone receptor 2, cardiovascular diseases, cardioprotection, vasodilation
Ucn3 share a sequence homology of up to 40% (10). Human genes encoding Ucn2 and Ucn3 are located on chromosomes 3 and 10, respectively (15, 16). Transcription of the Ucn2 gene is upregulated by glucocorticoids, which is most probably due to the involvement of Ucn2 in the stress response pathway (17). Nuclear magnetic resonance (NMR) structures of the three human urocortins have been solved and it was found that all of them have alpha-helical structure. Ucn1 forms almost continuous alpha-helix. Ucn2 comprises of two alpha helices, short N-terminal and long C-terminal, thus resulting in a helix-loop-helix structure. Ucn3 contains a small N-terminal 3/10 helix and a long C-terminal alpha helix containing 20 residues. This type of helix-loop-helix structure has critical importance for interactions with specific receptors (18). The N-terminal helices are believed to play a seminal role in the selective binding of the urocortins to CRH-R1. The kinks between the N- and C-terminal helices are thought to be crucial to ligand-receptor interaction (18).

The amino acid sequence of urocortins is of great importance in determining their binding specificity. For instance, a proline residue at position 11 is characteristic for CRH-R2 selective ligands Ucn2 and Ucn3. Furthermore, CRH-R2-selective peptides contain alanine residues at positions 35 and 39. Introduction of proline at position 11 and alanine at position 35 and 39 increases CRH-R2 selectivity of CRH-R non-selective peptides, mainly through the loss of CRH-R1 binding potency (19). Another element of the urocortin system and related receptors is CRH-BP, a protein synthesized in the liver, brain and placenta, recognized for its ability to bind to CRH and modulate its biological activity (20).

**DISTRIBUTION OF UROCORTINS**

Presence of urocortins in central nervous system and in peripheral tissues has been documented. The location of Ucn1 is mainly subcoirtical (it is primarily synthesized in the Edinger-Westphal nucleus) (21), but its expression has also been observed in the pituitary gland (22), where it is has a variety of functions (23-30). In the peripheral tissues of the human body, Ucn1 has been found in: adipose tissue (31), heart (32), adrenal glands (33), placenta and fetal membranes (34, 35), cells of the immune system including lymphocytes (36), macrophages and fibroblasts (37), and mastocytes (38). It has also been found in all four chambers of the human heart with the greatest concentration in the left ventricle, followed by the right ventricle, the left atrium and eventually the right atrium, in which the concentration was lowest (10). Moreover, experiments on animal models have confirmed the presence of Ucn1 in the intestinal nervous system (39), stomach (parietal cells) (40), testicles, kidneys, the spleen and the thymus (41).

Ucn2 is also present in the brain, but its role there has yet to be clearly defined. It is expressed in the supraoptic nucleus, paraventricular nucleus of the hypothalamus, motor neurons of the brainstem, spinal cord, the arcuate nucleus and the locus ceruleus in the brainstem (15). In human body, the expression of the Ucn2 gene has been observed in the majority of the peripheral tissues, with the highest concentrations in the heart, lungs, muscles, stomach, adrenal glands and blood cells (14) and lower concentrations in the skin (42), the placenta and fetal membranes (43). In rats, Ucn2 has also been isolated from the hypothalamus, the pituitary gland the adrenal medulla (44).

Ucn3 is found in the hypothalamus, amygdala and the brainstem (16). Its transcripts have been identified in various peripheral tissues, with the highest expression in the heart, adrenal glands and blood vessels (14). Expression of the Ucn3 gene has been documented in adipose tissue (31), the skin (16), the thyroid gland, the pituitary gland, the spleen, ovaries, kidneys (45), pancreatic beta cells (46), the placenta and fetal membranes (43), and the muscularis mucosa of the digestive tract (stomach, small intestine, colon, rectum) (14, 16, 47). Furthermore, it has been identified in plasma, and in the heart, including both ventricles and atria (10).

**METABOLISM**

Pharmacodynamics and pharmacokinetics of all three urocortins have been evaluated in experimental studies. The clearance was lowest in Ucn1 (0.486 L/h) and highest in Ucn3 (220 L/h) (48). Consequently, the half-life of Ucn1 is longest (2.9 h in the alpha phase and 8.3 h in the beta phase), while those of Ucn2 and Ucn3 are significantly shorter (15.7 min and 4.4 min, respectively) (48). The onset of action and clinical effects of Ucn2 and Ucn3 were observed much earlier than in the case of Ucn1. Interestingly, EC50 values (concentrations producing a half-maximal response) of Ucn2 and Ucn3 were lower than that of Ucn1, thus suggesting their lower potency (48).

**CORTICOTROPIN RELEASING HORMONE AND UROCORTIN RECEPTORS**

Two CRH receptors are known: CRH-R1 and CRH-R2. Despite their similar structure they have different locations and functions. At the amino acid sequence level, they are 70% homologous, as they both belong to class B1 of G-protein-coupled receptor (GPCR) family consisting of seven transmembrane helices with an N-terminal extracellular ligand-binding domain (ECD) (11). Due to the different structure of this domain of the CRH-R2, they are divided into the alpha, beta and gamma subtypes. These minor structural differences influence their affinity to specific peptides of the CRH family and hence, their induction of certain intracellular pathways. The “central” effects of urocortins are mediated mainly by activation of CRH-R1, whereas the “peripheral” effects are mediated mostly by activation of CRH-R2.

CRH-R1 is expressed mainly in the brain (forebrain, hypothalamus, subcortical limbic structures), the pituitary gland, the cerebellum and the brainstem (49). Activation of CRH-R1 leads to synthesis and secretion of ACTH (6). In rats, no expression of CRH-R1 has been found in cardiomyocytes or other cardiac cells (50). Nevertheless, in humans weak expression of CRH-R1 mRNA has been documented in the left ventricle and left atrium (32), suggesting its minor role in the functioning of the cardiovascular system. CRH-R1 is activated by CRH as well as by Ucn1, but Ucn1 has a threefold higher binding affinity to the receptor and causes more efficient stimulation of cAMP (6). Activation of CRH-R1 modulates numerous intracellular signaling pathways, including activation of adenylyl cyclase and cAMP production, activation of protein kinase C (PKC) and mitogen activated kinase (MAPK) pathways, production of nitric oxygen (NO) and activation of calcium channels (6).

CRH-R2 is activated by both urocortins and CRH. However, Ucn2 and Ucn3 are selective CRH-R2 agonists and have much higher binding affinity to CRH-R2 than CRH or Ucn1 (51). Unlike CRH-R1, only small quantities of CRH-R2 have been found in the central nervous system, mainly in areas responsible for modulation of the stress response (the olfactory bulb, the hippocampus, amygdala, septal nuclei, the dorsal and median raphe nuclei, hypothalamic nuclei) (52). However, in the peripheral organs, including the cardiovascular system, CRH-R2 is much more abundant (51). It has been found in the vascular endothelium, vascular smooth muscle and the heart. As mentioned above, on the basis of the structure of the ligand-
binding domain, CRH-R2 can be classified into three isoforms, alpha, beta and gamma (53). In humans CRH-R2α has been identified in all four chambers of the heart, whereas CRH-R2β has been isolated mainly from the left atrium and (in one case) from the right atrium (32). In rats no CRH-R2α expression has been observed in cardiac cells, contrary to CRH-R2β, whose expression has been observed predominantly in myocytes, but also in other cells (50). It has been shown that the beneficial effects of urocortins on the cardiovascular system (vasodilation, increase in cardiac output, myoccardial contractility and coronary blood flow) are associated with CRH-R2 activation (54, 55). Various factors may lead to the reduced expression of urocortin receptors in the heart. Administration of IL-1 or TNF-α was found to reduce CRH-R2 expression in murine cardiomyocytes (56). It has been suggested that proinflammatory cytokines may be responsible for downregulation of CRH-R2 in the heart by increasing the expression of Ucn1 (10). Similarly, inadequate food intake and acute or chronic stress can downregulate expression of CRH-R2 in the atria and the aorta (57, 58). Similar to CRH-R1, activation of CRH-R2 modulates numerous cellular pathways, including activation of adenyl cyclase, PKC, phosphatidylinositol 3-OH kinase (PI3K), MAPK, protein kinase A (PKA), protein kinase B (PKB/Akt). Activation of these pathways is responsible for vasodilation, cardioprotection and inhibition of apoptosis (59-61).

HEMODYNAMIC EFFECTS OF UROCORTINS

Urocortins, acting on isolated murine cardiac ventricular muscle cause dilation of coronary arteries and have positive inotropic and lusitropic effects (55). The effects of urocortins on the conduction system appear to have distinct mechanisms in different species and may be facilitated by activation of various cellular pathways. The effect of Ucn1 on isolated rat myocytes is independent of the activation of PKA and is related mainly to activation of PKC, MAPK and exchange protein activated by cAMP (Epac) as well as to increased intracellular calcium levels (62). Ucn2 increases contractility of ventricular myocytes in mice by activation of CRH-R2 in a cAMP/PKA-Ca2+ calmodulin-dependent protein kinase (Ca2+/CaMKII)-dependent pathway (63). On the other hand, it may induce PKA- and CaMKII-mediated arrhythmia (63). Ucn2 administration has been shown to activate PKA signaling in rabbit myocytes (64). Furthermore, urocortins cause cardiomyocyte hypertrophy, increase in cellular surface area, protein synthesis levels and amount of collagen in cardiomyocytes in rats. The mechanisms of these effects are not clearly understood, but the effects of urocortins on cardiomyocytes may be regulated by Akt, PI3K and MAPK downstream signaling. Out of the three urocortins, the highest hypertrophic effect is attributed to Ucn3 (65).

Administration of urocortins in mice causes increase in heart rates and inotropic as well as lusitropic effects, all mediated by CRH-R2. Moreover, it was observed that administration of a beta-blocker (esmolol) did not interfere with the inotropic and lusitropic effects of urocortins, thus indicating that these actions do not involve activation of beta adrenergic receptors (66). Similarly, both central and peripheral administration of urocortins to conscious sheep caused an increase in heart rates and cardiac contractility (54, 67, 68).

Administration of Ucn1, Ucn2 and Ucn3 in sheep, both healthy and with induced heart failure, resulted in increased cardiac output, decreased peripheral resistance and decreased left ventricular (LV) pressure (67, 69, 70), while increased heart rates

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**Fig. 1.** Structure properties of CRH peptides. (A) Ribbon representation of urocortin II NMR structure (PDB:2RMG) (18). N-terminal and C-terminal helices are depicted in blue and pale green respectively. (B) Crystal structure of human CRH-R2 alpha extracellular domain in complex with C-terminal helix of Ucn 2 (pale green) (PDB:3N95) (149). (C) Sequence alignment of CRH peptides. Residues strictly conserved - red background, residues well conserved – red letters. Alignment was prepared by ClustalW (150) and visualized in ESPript 3.0 (151).
were observed only in healthy animals. Similar hemodynamic effects were observed in mice with dilated cardiomyopathy, where administration of Ucn2 resulted in improved LV function and decreased peripheral resistance (66). The effects of Ucn2 persisted over the entire period of administration of the peptide, suggesting that no desensitization of Ucn receptors has occurred (71). Administration of Ucn1 at the early stages of heart failure induction in sheep resulted in delayed disease progression, which was manifested by decreased cardiac output, increased peripheral resistance and increased LV pressure (72). In patients with heart failure treated with urocortins, increased cardiac output and improved LV function were observed; these were accompanied by a decrease in peripheral resistance and fall of LV pressures and arterial blood pressures (73). Administration of Ucn1, Ucn2 and Ucn3 in animals with heart failure causes natriuresis, which results in an increase in renal creatinine clearance and urine volume compared to a control group (67, 69, 70).

Urocortins in combination with loop diuretics (furosemide), mineralocorticoid receptor antagonists and beta-blockers were studied in animal models of heart failure (74-76). Combining Ucn2 with furosemide resulted in increased diuresis and natriuresis, without the concomitant reduction in potassium levels and increase in creatinine levels (74). Moreover, the above combination produced a greater reduction in LV pressures than either furosemide or Ucn2 alone (74). Furthermore, adding Ucn2 to furosemide did not cause a reduction in mean arterial pressure (MAP), as would be expected in the case of the loop diuretic alone. The reduction in peripheral resistance observed during the combined treatment with both agents was comparable to that produced by Ucn2 alone (74). On the other hand, combination of a beta-blocker (metoprolol) with Ucn2 in acute heart failure improved cardiac output, caused a rise in MAP without increasing heart rate, and reduced peripheral resistance and left atrial pressure (75).

In sheep models with pacing-induced congestive heart failure, administration of Ucn2 alone or in combination with an aldosterone receptor antagonist increased diuresis and natriuresis and reduced plasma potassium levels (76). Combination treatment with Ucn2 and aldosterone receptor antagonist caused an additional reduction in peripheral resistance and left atrial pressure, while increasing stroke volume without producing a drop in blood pressures compared with the aldosterone receptor antagonist alone (76).

**ANTIHYPERTENSIVE EFFECTS OF UROCORTINS**

Urocortins are significant and distinctive regulators of vascular smooth muscle that have both direct and indirect paracrine and autocrine effects on the vascular system (77).

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**Fig. 2. Potential mechanisms mediated by urocortins in cardiovascular disease.**
A number of mechanisms responsible for urocortin-mediated vasodilation have been proposed, which could be attributed to the direct effects of urocortins on the vascular wall, either dependent or independent of the endothelium. A mechanism involving the endothelium is believed to be mediated by CRH-R2 activation, which leads to increased cAMP production and Ca\(^{2+}\) related phosphorylation of extracellular signal-regulated kinase (ERK), p38 and Akt, resulting in increased NO production (78). The endothelium-dependent component of the relaxation is primarily mediated through endothelial NO that in turn likely activated K\(_C\) channels in vascular smooth muscle cells in a cyclic GMP-dependent manner (79, 80) and subsequent vasodilation. The above mechanism was confirmed both in animal models (rat coronary arteries) and in humans (internal mammary artery) (79). On the other hand, the mechanisms independent of the endothelium have yet to be clearly defined. In vitro studies on isolated rat small pulmonary arteries have shown that vasodilation mediated by urocortins partly depends on the activation of a sodium-calcium exchanger located in the sarcolemma, which is inhibited by nickel (nickel-sensitive Na\(^+-\)Ca\(^{2+}\) exchanger) (81). Urocortins acting on rat coronary arteries cause endothelium-independent relaxation via the activation of potassium channels and a cAMP-dependent signaling pathway (82). They were also observed to cause vasodilation in rat thoracic aortic walls through the activation of CRH-R2 as well as PKA and MAPK pathways (83).
Furthermore, studies on aortic smooth muscle have shown that urocortins, acting through CRH-R2, caused PKA activation and increased cAMP levels (84). A direct vasodilatory effect on isolated endothelium of human saphenous vein and internal mammary artery of urocortins has been noted, and this was explained by urocortin-mediated activation of potassium channels by calcium ions in the vascular smooth muscle (79, 85). Elevated MAP, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were observed in CRH-R2–/– knock-out mice when compared to that in wild-type animals. Moreover, intravenous administration of Ucn1 did not cause blood pressure reduction in CRH-R2–/– mice when compared with control group (86). A study by Dieterle et al. showed that treatment with Ucn2 in hypertensive rats (Dahl salt sensitive rats, DSS) resulted in blood pressure reduction without an increase in heart rate (87). Long-term treatment of these rats with urocortins also prevented mesenteric artery remodeling, manifested by a decrease in media-lumen ratio and an increase in lumen diameter (88). Intra-arterial infusion of Ucn2 and Ucn3 in healthy humans resulted in a dose-dependent vasodilation. The administration of Ucn2 produced maximal vasodilation after approximately 10 minutes, while in the case of Ucn3 the effect was immediate (89). The return to baseline values took longer in the case of Ucn2 than in the case of Ucn3 and tachyphylaxis was not observed. Furthermore, this study revealed that the effects of urocortins are partly dependent on NO signaling (89). In humans with congestive heart failure, high levels of Ucn2 also produced reductions in MAP and peripheral vascular resistance (73).

As urocortins influence the activity of the renin-angiotensin-aldosterone (RAA) system, their hypotensive and vasodilatory effects may in part be caused by reduced levels of vasoconstrictive factors described below and/or an increase in intravascular volume.

**EFFECTS OF UROCORTINS ON HORMONE PROFILE**

A number of studies have demonstrated the effect of urocortins on the hormone profiles, and particularly the RAA system, in various species. Intravenous administration of urocortins in healthy rats caused a dose-proportional decrease in serum (sACE) levels. At the same time, long-term urocortin treatment resulted in an increase in tissue (tACE) levels (90). In subsequent studies by the same group of investigators it was demonstrated that long-term urocortin administration in hypertensive rats resulted in reduced serum ACE activity and angiotensin II levels, with a concomitant increase in chymase activity, tissue ACE activity, angiotensin 1-7 and NO levels, all leading to a decrease in MAP (91). In both cases, the postulated mechanism has been linked with ERK1/2 activation (90, 91).

The effects of urocortins (Ucn1, Ucn2, Ucn3) were studied on sheep models of congestive heart failure (67, 69, 70). When the effects of administration of urocortins in healthy sheep was compared with the sheep with congestive heart failure, significant

| Table 1. Role of urocortins in cardiovascular system. |
|-----------------|-----------------|-----------------|
| **Subject** | **Effect** | **Mechanism** |
| **in vitro/ in vivo** | | |
| Rat’s isolated heart | Vasodilatation of coronary blood vessels | PKC, MAPK |
| | Positive inotropic | Epac, Increase of intracellular calcium concentration (55, 62) |
| | Positive lusitropic | |
| Mouses’s cardiomyocytes | Positive inotropic | cAMP/PKA, Ca²⁺/CaMKII – dependent (63) |
| Rabbit’s cardiomyocytes | Positive inotropic | PKA (64) |
| Neonatal rat’s cardiomyocytes | Hypertrophia | Akt/ PI3-K/MAPK (65) |
| **in vivo** | | |
| Healthy rats | Increased of tACE, Decreased of sACE | Erk1/2 (90) |
| Hypertensive rats | Increased of: chymase, tACE, angiotensin 1-7, NO | Erk ½ (87, 88, 91) |
| Decreased of: sACE, ANGII, Hypotension, Prevent from remodeling of artery’s walls | |
| Sheep (health and with congestive heart failure) | Positive inotropic and chronotropic, Decreased of systemic peripheral resistance and pressure in left atrium, Increased of cardiac output | Natureuris, Increased creatine clearance and volume of urine |
| | | Increased of ACTH and cortisol concentration (54, 67, 69, 70) |
| Healthy sheep | Increased of BNP and AVP | Increased of ANP (67, 69, 70) |
| Sheep with congestive heart failure | Decreased of: ARO, aldosterone, ET-1, cAMP, ANP, BNP, AVP, adrenaline (67, 69, 70) | |
reductions in serum renin activity, as well as serum aldosterone, adrenaline and cAMP levels, were observed in the congestive heart failure group, without a notable reduction of the parameters in healthy sheep. Reduction in atrial natriuretic peptide (ANP) levels was significantly higher in sheep with heart failure compared to that in healthy animals. On the other hand, an opposite trend was observed in the case of vasopressin and brain natriuretic peptide (BNP), with increased levels in healthy animals and a reduction in animals with heart failure. Norpinephrine levels in healthy animals remained unchanged. In summary, administration of urocortins in sheep with congestive heart failure led to reduction in both vasoconstrictor factors and/or factors responsible for increasing intravascular volume, that is: angiotensin, norepinephrine, epinephrine, vasopressin, aldosterone, renin and endothelin-1. Furthermore, a reduction in ANP and BNP levels was also observed.

Adding Ucn2 to furosemide not only counteracted the furosemide-induced increase in plasma renin activity (PRA), but also produced a significant reduction of this parameter, similar to that observed in the case of vasopressin and the natriuretic peptides (74). As seen in combined therapy with Ucn2 and an aldosterone receptor blocker in sheep models of induced congestive heart failure, there was a significant reduction in aldosterone levels, plasma renin activity and the levels of vasopressin and angiotensin II. It is proposed that the effects of urocortins may counteract those of aldosterone; this implies that urocortins have antioxidant and anti-inflammatory properties, and inhibit fibrosis (76). Taking into consideration the presence of CRH-R2 in the adrenal glands (92), it is believed that urocortins may also have a direct inhibitory effect on the secretion of aldosterone. Further studies in sheep with induced congestive heart failure revealed that Ucn2 in combination with an ACE-inhibitor (captopril) reduced aldosterone levels as well as plasma renin activity, adrenaline, vasopressin and endothelin 1 (ET-1) levels (93). The above inference indicates that urocortins play a protective role in congestive heart failure, while the role of these peptides in a healthy organism is rather marginal (54, 94). Urocortins are also known to influence the regulation of the hypothalamus-pituitary-adrenal axis, as the injection of all three peptides in healthy animals as well as animals with induced heart failure, there was a significant increase in blood pressure, heart rate and catecholamine levels, that is: noradrenaline, adrenaline and cAMP levels, were observed in the studies on human small cell lung carcinoma, in tumor cells, and stimulated apoptosis (113). Similar effects were observed on lung neoplasms conducted in humans and on animal models have contributed to a better understanding of the effects of urocortins on angiogenesis. Administration of Ucn2 in mice with Lewis lung Carcinoma inhibited angiogenesis and proliferation of tumor cells, and stimulated apoptosis (113). Similar effects were observed in the studies on human small cell lung carcinoma, in which Ucn2, acting through CRH-R2, inactivated p38 and Akt, thus causing a reduction of VEGF secretion (114).

**CARDIOPROTECTION AND UROCORTINS**

Urocortins protect myocytes against ischemia/reperfusion injury. This effect results from a reduction in the infarct size (61), as well as from an improvement of ventricular function by
obtained and PI3-kinase PKB/Akt (117-119). Similar results were demonstrated that PKC α/β II is completely deprived of the cardioprotective effects of cardiomyocytes, unlike those in the control group, were completely deprived of the cardioprotective effects of urocortins, which confirms that the above mechanism is crucial for cardioprotection of urocortins (123). It has been demonstrated that PKCε has an indirect inhibitory effect on the pro-apoptotic effects of BAD (11). It is believed that cardioprotective effects of urocortins are mediated by mechanisms similar to those observed in ischemic preconditioning of the heart, involving the opening of sarcoplasmic potassium channels. Urocortins amplify expression of the gene encoding the Kir 6.1 subunit of the ATP-sensitive K⁺ channel and its blockade results in reduction of the cardioprotective effects of these peptides in rat cardiomyocytes (124). Exposing adult or neonatal rat cardiomyocytes to urocortins, similarly to such exposure of an isolated rat heart, inhibited the opening of the mitochondrial permeability transition pore (MPTP) during the process of reperfusion (125). In vitro studies in rats showed reduced infarct size, decreased incidence of cardiac arrhythmias and reduced levels of reactive oxygen species with the simultaneous increase of the levels of anti-oxidant enzymes (glutathione reductase and SOD) during reperfusion, following prior administration of a urocortin (126). It has also been shown that Ucn1 influenced the expression of one of the genes of the inhibitor of apoptosis family of proteins (IAP)-X-linked inhibitor of apoptosis protein (XIAP), which inhibit apoptosis induced by reactive oxygen species, by stimulating the expression of antioxidant and by reducing caspase levels (127). Furthermore, urocortins also play a role in gene regulation. Stimulation of nuclear factor, erythroid 2-like 1 (NFE2L1) and glutaredoxin 2 (GLRX2), or suppression of Ras-related C3 botulinum toxin substrate 2 (RAC2) is responsible for reducing the levels of reactive oxygen species (127). Administration of Ucn1 also reduces the levels of a cytotoxic activator of transcription 3 (STAT3), which is also known to be cytoprotective in an acute phase of ischemia-induced cardiac muscle injury (131). Urocortins also have the ability to stimulate gene expression and increase levels of mRNA levels and protein cardiotropin-1 (CT-1), all of which are involved in the cardioprotective effects mediated by the activation of PI3K/Akt and p42/44MAPK pathways (132). These protective effects of urocortins are modulated by estrogens. In ovariectomised rats, the cardiomyocyte levels of mRNA for CRH-R2 are reduced (133). Administration of estrogens stimulates the expression of CRH-R2; this effect is mediated by activation of estrogen receptor α (ERα) and specific protein 1 (SP1; a transcription factor) (134). Of note, the cardioprotective effects of urocortins were observed following their administration both before ischemia and in its early phase (135).

In summary, activation of CRH-R2 in cardiac myocytes exposed to ischemia and reperfusion stimulates the activation of intracellular kinases, which promote cytoprotection and cell survival by influencing mitochondrial function, activation of translation and transcription factors and eventually influencing gene expression (11).

**EXPERIMENTAL STUDIES ON UROCORTINS IN HUMANS**

In the recent decade numerous studies on the functions of urocortins in humans were conducted (73, 89, 136, 137). Most of the effects of urocortins observed in animal models have been confirmed in humans. The vasodilatory effects of urocortins were observed on an intraoperative sample of the internal thoracic artery, obtained during coronary artery bypass surgery. The same (although less pronounced) effect was also observed in the artery after separation of the endothelium. Administration of potassium channel blockers, such as tetroethylamilimonium (TEA), charybdotoxin (CTX), and iberiotoxin (IBX) inhibited the vasodilation; this also suggests the involvement of these channels in mediating of the effects of urocortins (79, 138). AAAs are characterized by chronic inflammation and extensive disruption of extracellular matrix, resulting in a reduction in tensile strength. Moreover, secondary fibrinolysis also plays an important role in the pathogenesis of AAAs (139). Another relevant element in the development of AAAs is an altered response to α-adrenergic receptor stimulation (140). Ucn 2 also seems to have an effect on the formation of AAAs. In patients undergoing surgical treatment of AAA, intraoperative samples of vascular *muscularis propria* were obtained from the area of a maximal dilation of the aortic lumen as well as from the neck of the aneurysm. In patients with AAA increased serum levels of Ucn2 were observed along with an increased expression of Ucn2 in samples obtained from the AAA body. Ucn2 suppresses proliferation of the *muscularis propria* cells in the vascular *tunica media* and also causes an increase in IL-6 production, which may be directly responsible for the progression of the aneurysm (111).

Cardioprotective effects of urocortins have been studied in patients undergoing cardiac surgery (coronary artery bypass graft, CABG) involving extracorporeal circulation using warm blood cardioplegia. The right atrial tissue samples were obtained twice - immediately after cardioplegia and ten minutes after releasing the aortic clamp. The duration of ischemia was approximately 40–100 minutes. In the samples obtained after reperfusion elevated levels of Ucn1 in viable myocytes were
observed compared with the samples obtained during the initial stage of ischemia. It was concluded that the myocardial cells that underwent apoptosis as a result of ischemia lacked Ucn1 expression (141). The mechanisms of cardioprotection depend on an increased expression of PKCε and the Kir 6.1 subunit of the potassium channel (141, 142). These results are similar to those observed in animal models (123).

Moreover, evidence from studies evaluating serum urocortin levels in groups of healthy individuals and patients is also available. It has been observed that in patients with heart failure, urocortin levels were significantly higher compared with the control group and revealed a positive correlation with severity of heart failure (143). Ng et al. (136) demonstrated, that in a group of 119 patients with congestive heart failure serum Ucn1 levels were significantly higher compared with the control group of 212 sex- and age-matched persons (men 50.2 pmol/L versus 19.5 pmol/L; women 21.8 pmol/L versus 14.2 pmol/L). Topal et al. (144) evaluated serum Ucn2 levels in groups of patients with systolic and diastolic dysfunction and ischemic heart disease. Higher serum levels of Ucn2 were observed in patients with mild and moderate congestive heart failure (classified on the basis of ejection fraction). In the group of patients with ischemic heart disease without prior myocardial infarction and in those with diastolic dysfunction, serum Ucn2 levels were comparable to those in the control group. Yildirim et al. (145) also evaluated Ucn1 levels in patients with congestive heart failure, while studying the trends of various parameters in relation to Ucn1. They concluded that the rise in Ucn1 levels may be associated with progression of heart failure and ventricular dysfunction and concluded that Ucn1 may therefore be used as an effective biomarker of systolic heart failure.

Several studies reported clinical effects of exogenous Ucn1 and Ucn2 administration in healthy volunteers (137, 146). In a study by Davis et al. (137), eight healthy male volunteers were given 50 µg of Ucn1 in one-hour intravenous infusion. Increased levels of ACTH, cortisol, ANP and decreased levels of ghrelin were observed in the group receiving Ucn1 compared with the placebo control group. The above dose and method of administration did not produce significant hemodynamic or renal effects in healthy individuals; however, the observed decrease in ghrelin levels may explain the anorexia induced by Ucn1. In another study by the same investigators (146), two intravenous doses (25 µg and 100 µg) of Ucn2 were administered to healthy male volunteers and hemodynamic, renal and neurohormonal effects were assessed. In the Ucn2 group dose-dependent increases in cardiac output, heart rate and left ventricle ejection fraction (LVEF), as well as decreased peripheral vascular resistance were observed in comparison to the control group. The Ucn2 group also demonstrated a fall in DBP and MAP, without a statistically significant fall in SBP. Increases in plasma renin activity, as well as angiotensin II and noradrenaline levels were observed after administration of higher doses of Ucn2. Moreover, minor reductions of urine output and urinary sodium excretion were observed (146).

Clinical effects of Ucn2 and Ucn3 were also studied by Venkatasubramanian et al. (89) in a double-blind, randomized placebo-controlled study of 18 healthy male volunteers. The subjects received increasing intraarterial doses of Ucn2 and Ucn 3 in 7-day intervals, peptides alone and in combination with substance P, aspirin, an inhibitor of NO synthase and flunazolone. Urocortin infusion produced significant vasodilation, which persisted despite administration of inhibitors of vasodilation. A transient erythema of the upper extremity (the infused forearm) was observed, accompanied by mild tachycardia and decrease in DBP, which were observed immediately after the infusion of the highest dose of Ucn3. In 2005 Davis et al. (147) published the effects of a study on intravenous Ucn1 infusion in 8 individuals with congestive heart failure (LVEF <40%). Ucn in a dose of 50 µg was associated with higher ACTH and cortisol levels compared with the placebo group. No changes in the levels on ANP or ghrelin, or any significant hemodynamic or renal effects were observed.

A similar study on Ucn2 was conducted, including on 8 individuals with congestive heart failure (LVEF <40%, 6 subjects with non-ischemic etiology and 2 with ischemic cardiomyopathy) who received low and high doses (25 µg and 100 µg) of intravenous urocortin (73). The Ucn group showed increases in cardiac output and LVEF that proportionally correlated with Ucn doses. These were accompanied by a reduction in MAP, systemic peripheral resistance and cardiac work (73).

In an ongoing phase II study administration of Ucn3 in patients with congestive heart failure (LVEF <35%) is assessed. It was demonstrated that administration of increasing doses of Ucn3 caused dose-dependent increases in cardiac index and reduction in systemic vascular resistance, without any effects on pulmonary capillary wedge pressure, heart rate or SBP (148).

The above reports show that urocortins can be considered as therapeutic agents in cardiac diseases. Their cardioprotective, vasodilatory, anti-hemostatic, anti-thrombotic and neurohormonal effects are interesting as a possible intervention in the pathogenesis of the cardiovascular diseases. Urocortins and CRH-R2 may be used as potential therapeutic targets in patients with coronary heart disease, congestive heart failure and hypertensive.

The CRH family of peptides as well as CRH-R1 and CRH-R2 are widely distributed in the cardiovascular system and have hemodynamic, neurohormonal and cardioprotective effects that have been demonstrated both in animal models and in humans. The observed effects could be beneficial in the treatment of cardiovascular diseases and they suggest that further studies on urocortins may have potential therapeutic significance. The mechanisms of these effects have yet to be clearly understood and the knowledge of the functions of urocortins in humans is still insufficient. In spite of several studies confirming the positive effects of urocortins on the cardiovascular system, there is also some evidence of adverse effects, mainly proinflammatory, prothrombotic as well as inhibition of proliferation of the vascular muscularis propria. In conclusion, further experimental and clinical studies are warranted to fully explain the effects of urocortins.

Abbreviations: CRH: corticotropin releasing hormone; CRH-R1: corticotropin releasing hormone receptor 1; CRH-R2: corticotropin releasing hormone receptor 2; CRH-BP: corticotropin releasing hormone binding protein; GPCR: G-protein-coupled receptor; MMP-9: metalloproteinase-9; PKC: protein kinase C; Pselectin (P-selectin); P-selectin glycoprotein ligand 1; NO synthase; FLNC: filamin C; MMP: matrix metalloproteinase; C5aR: complement 5a receptor; C-reactive protein; sICAM: soluble intracellular adhesion molecule; hsCRP: high sensitive C-reactive protein; MEK: extracellular signal-regulated protein kinase 1 and 2; Hsp90: heat shock protein 90; MEK: extracellular signal-regulated kinases; Hsp90: heat shock protein 90; MAPK: mitogen-activated protein kinase; PKA: protein kinase A; PKB/Akt: protein kinase B; ERK: extracellular signal-regulated kinases; PKB: protein kinase B; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; MAP: mean arterial pressure; RAA: renin-angiotensin-aldosterone system; ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; sACE: serum angiotensin converting enzyme; CRH: corticotropin releasing hormone; CRH-R1: corticotropin releasing hormone receptor 1; CRH-R2: corticotropin releasing hormone receptor 2; C: Ca(2+); CaMKII: Ca(2+)-calmodulin dependent protein kinase; sACE: serum angiotensin converting enzyme; TACE: tissue angiotensin converting enzyme; PRA: plasma renin activity; ET-1: endothelin 1; SAM: sympatho-adrenalomedullary system; CSNA: cardiac sympathetic nerve activity; SOD: superoxide dismutase; HDHF: heparinase degraded heparin fragments; t-PA: tissue plasminogen activator; TF: tissue factor pathway inhibitor; TF: tissue factor; sICAM: soluble intracellular adhesion molecule; VCAM: vascular cell adhesion molecule; PECAM: platelet endothelial cell adhesion molecule; hsCRP: high sensitive C-reactive protein; MEK: ERK activator kinase; Hsp90: heat shock protein 90.
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