Review

Urocortin and cardiovascular protection

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ABSTRACT

Urocortin and other hypothalamus corticotropin-releasing factor (CRF) polypeptides play biologically diverse roles in the stress, cardiovascular and inflammatory responses by acting on central and peripheral CRF receptors. Urocortin shows a significantly high sequence homology to CRF, and the concurrent expression of type-2 CRF (CRF_2) receptors with urocortin in the heart suggests that urocortin may play a physiological role in the cardiac function. Urocortin is thought to be the endogenous agonist producing the cardiovascular actions previously attributed to CRF. This review highlights the current novel findings on the molecular and cellular mechanisms by which urocortin may exert its cardiovascular protective action.

UROCORTIN AND CRF RECEPTORS

Urocortin, a 40 amino acid peptide belongs to the hypothalamic CRF family, which also include CRF, urotensin and sauvagine. CRF polypeptides play biologically diverse roles in the stress responses by acting on central neurons expressing CRF receptors. CRF receptor agonists acting on the peripheral CRF receptors contribute to the regulation of cardiovascular and inflammatory responses. Abnormal CRF receptor-mediated cellular signaling might be closely associated with the pathophysiology of stress-related centrally controlled disorders such as anxiety, depression and impaired cardiovascular function.

Urocortin, first identified in rat[1] and later in man[2,3], is the second mammalian member of the CRF family to be found and displays 45 % amino acid sequence homology to CRF. Urocortin is more conserved than CRF across species. The CRF and urocortin precursor genes consist of two exons with the entire precursor protein encoded within the second exon[3]. The search for new members of CRF family has led to the cloning of stresscopin and stresscopin-related peptide from human cDNA[4] and urocortin II and urocortin III from mouse cDNA[5,6]. These new peptides containing 38 amino acids share high degree of sequence homology. The human myocardium is immunohistochemically positive for urocortin and immunoreactive intensity is greater in the failing heart[7]. A recent study on the human heart obtained at autopsy reported that urocortin-like immunoreactivity was detected in all four cham-

Abbreviations: BK channel, large-conductance Ca^{2+}-activated K^+ channel; CREB, cyclic AMP response element-binding protein; CRF, corticotropin-releasing factor; CRF-R, CRF receptor; ERK, extracellular signal-regulated kinase; K_{ATP} channel, ATP-sensitive K^+ channel; MAPK, mitogen-activated protein kinase; MEK, MAP kinase kinase; NO, nitric oxide; NOS, nitric oxide synthase; PI3K, phosphatidylinositol 3-OH kinase; PKA, cyclic AMP-dependent protein kinase; PKG, cyclic GMP-dependent protein kinase; STAT, signal transducer and activator of transcription; TEA^+, tetraethylammonium ion.

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bers with the highest intensity in the left ventricle[8]. In contrast, CRF-like immunoreactivity or CRF mRNA is basically undetectable in the human heart[8]. These results suggest that urocortin can be endogenously synthesized in the human heart and may exert its cardiac action in an autocrine and/or paracrine manner, even though the cellular location of urocortin is not known. Urocortin but not CRF has been proposed to act as an endogenous ligand mostly for CRF_2 receptor in the mammalian cardiac myocytes[8,9].

The physiological actions of CRF polypeptides are believed to be mediated through G protein-coupled seven-transmembrane receptors (CRF_1 and CRF_2) derived from two distinct genes[10,11]. Urocortin selectively binds to CRF_2 receptors with over 100-fold greater affinity than CRF[9]. CRF_2 receptor mRNA is widely expressed in peripheral tissues including cardiac myocytes[12,13]. Two subtypes of CRF_2 receptors are cloned from cardiac tissues, $CRF_{2(\alpha)}$ receptors in human and $CRF_{2(\beta)}$ receptors in the rat[14,15]. $CRF_{2(\alpha)}$ receptor mRNA is detected in the human heart and $CRF_{2(\beta)}$ receptor mRNA is predominantly expressed in the left atrium[8].

Activation of CRF_2 receptors elevates the cellular contents of cyclic AMP through the G_s protein-adenylate cyclase pathway[16]. Urocortin also stimulates the phosphorylation of cyclic AMP response element-binding protein (CREB) in cells expressing CRF_2 receptors, and the cyclic AMP-dependent protein kinase (PKA) inhibitor blocks formation of phosphorylated CREB[17]. Like β-adrenoceptor agonists, urocortin exerts its iontropic or chronotropic effects likely via a PKA-dependent mechanism.

VASODILATATION

Urocortin produces a potent and long-lasting hypotensive action in conscious rats[1], probably due to reduced peripheral vascular resistance. Its vasodilator effect was also reported in the human perfused placenta and saphenous veins[18,19], in the rat cerebral, tail and coronary arteries *in vitro*[20-22], and in the sheep coronary arteries *in vivo*[23]. The non-selective CRF receptor antagonists such as α-helical CRF (9-41) and astressin inhibit the vasorelaxant effects of urocortin, suggesting that CRF receptors mediate the vascular response of the peptide. With development of more selective antagonists for CRF receptor subtypes, the relevant contribution of $CRF_{2(a)}$ and $CRF_{2(b)}$ receptors to urocortin-mediated vasodilatation ought to be revealed. $CRF_{2(b)}$ receptors are expressed in blood vessels of both non-pregnant and pregnant rats, which may mediate the vasorelaxant effect of the CRF polypeptides[24]. The potent hypotensive effect produced by urocortin in wild-type mice is completely lost in CRF_2 receptor knockout mice[25]. Nevertheless, the physiological role of urocortin in vascular function has not been established.

The endothelium appears to play varied roles in urocortin-induced relaxation in some vascular beds. Removal of endothelium does not affect the relaxant response to urocortin in the isolated rat tail and basilar arteries[20,21]. In contrast, the reduced relaxant response to urocortin was observed in the endothelium-denuded rat coronary arteries[22]. Removal of endothelium also blunts CRF-induced relaxation of the pregnant rat uterine artery[26] and the non-pregnant rat aorta[27]. Endothelial cells express predominantly $CRF_{(2b)}$ receptors in the rat aorta with low expression in the smooth muscle layer[24], implicating a role of endothelium in CRF-mediated vasorelaxation. Treatment with inhibitors of nitric oxide synthase (NOS) or guanylate cyclase reduces relaxations to urocortin in the rat coronary artery[22] or to CRF in the rat aorta and uterine artery[26,27]. N^G-nitro-*L*-arginine, a NOS inhibitor attenuates the vasodilator effect of CRF in the isolated rat heart[28] and in human foetal-placental vessels[29]. These data suggest a NO-mediated cyclic GMP-dependent mechanism involved the vasorelaxation to CRF polypeptides. However, in the isolated rat heart NOS inhibition does not diminish the urocortin-induced reduction in coronary perfusion pressure at a constant flow rate[30]. In contrast,urocortin-induced NO-mediated relaxation can be achieved at concentrations below 30 nmol/L in the rat coronary artery[22]. However, whether coronary vasodilatation is also a consequence of metabolic relaxation due to an increased cardiac contractility remains to be investigated.

CRF_2 receptors, also expressed in vascular smooth muscle[24] may mediate urocortin-induced endothelium-independent vasodilatation. Upon stimulation of $CRF_{2(b)}$ receptors, cyclic AMP and subsequent PKA-dependent cellular process are proposed to mediate the cardiovascular effects of the CRF polypeptides[1,13]. Urocortin increases intracellular cyclic AMP production in a rat aortic cell line, A7r5[31]. The PKA inhibitors (Rp-cAMPS, KT 5720 or SQ22536) reduce urocortin-induced relaxation in isolated rat tail, basilar and coronary arteries[20,21,32]. In contrast, the cyclic GMP-dependent

mechanism is unlikely involved since KT 5823 (an inhibitor of cyclic GMP-dependent protein kinase) has no effect[32]. A markedly reduced vasorelaxant effect of urocortin or CRF in elevated extracellular potassium was observed in the rat uterine[26], coronary[22], cerebral[20], and pulmonary arteries (unpublished observation). This raises a possibility that urocortin may hyperpolarize vascular smooth muscle cells via activation of K^+ channels. Urocortin-induced relaxation is attenuated to a similar extent by iberiotoxin, a potent blocker of large-conductance Ca^{2+}-activated K^+ (BK) channels and by tetraethylammonium ion (TEA^+) at concentrations that inhibited single arterial BK channels[33]. In the human saphenous veins the relaxation to urocortin is also reduced by TEA^+ [19]. These new results support the notion that urocortin may activate BK channels, and subsequent membrane hyperpolarization inhibits Ca^{2+} influx via arterial voltage-gated Ca^{2+} channels.

Both ATP-sensitive (K_{ATP}) and BK channels are activated by PKA stimulation in porcine or rabbit coronary artery smooth muscle cells. Urocortin-induced coronary relaxation is unaffected by glibenclamide[32] at a concentration that blocks vascular K_{ATP} channels[34], indicating a negligible role of K_{ATP} channels. Similar results were reported in the rat cerebral[20] and uterine artery[26]. It appears that a larger portion of urocortin-induced relaxation is mediated by stimulation of receptor-coupled adenylate cyclase, which results in activation of BK channels. Similarly, both TEA^+ and iberiotoxin reduced the relaxant response to forskolin, which relaxes vessels through a cyclic AMP-dependent mechanism[32]. In single non-vascular smooth muscle cells, urocortin elicits PKA-dependent increases in Ca^{2+}-activated K^+ currents[35].

Urocortin plasma concentration is around 1 pmol/L in humans[36]. Urocortin relaxes blood vessels with varied effectiveness. The rank order of relaxing potency (indicated by the IC_{50} values) is human placental artery (about 0.03 nmol/L) > human saphenous vein (about 0.1 nmol/L) > rat basilar artery (about 0.5 nmol/L) > rat coronary artery (about 2.3 nmol/L) > rat tail artery (about 2.6 nmol/L)[18-22]. It is apparent that vascular sensitivity to urocortin is different among vessels or species with more potent vasodilator effect in human vessels. The vasodilator and inotropic effects of urocortin seem to occur at pharmacological concentrations since the plasma levels of urocortin used in animal studies are far greater than that circulating in blood. However, it is difficult to determine the actual concentration of urocortin at the receptor sites, which may be much higher than its circulating levels. Nevertheless, the exact physiological role of urocortin in control of vessel tone and blood pressure is yet to be established. No information has so far been available on the mechanisms by which urocortin is metabolized.

Intravenous injection of urocortin produces vasodilatation[1], an effect more potent than that of CRF[18], which is accompanied by flushing and itching[37], indicating that CRF polypeptides may be also involved in the inflammatory response to stressful stimulation. Urocortin is one of the most potent triggers of rat mast cell degranulation and skin vascular permeability[38]. This *in situ* effect is not mediated through the peripheral nervous system, but histamine seems to account mostly for the vasodilating effect of urocortin via vascular H_1-histaminergic receptors[38]. The notion that urocortin can act as an immune-inflammatory mediator is further supported by the findings that the expression of urocortin mRNA is detected in synovium of patients with rheumatoid arthritis and osteoarthritis[39].

CARDIAC PROTECTION

Urocortin exerts both positive chronotropic and inotropic actions in the heart and elevates coronary blood flow[23,30]. These effects are associated with elevated formation of cyclic AMP in the cardiac tissue[40]. Urocortin, when administered intravenously to the rats, produces a slowly developing decrease in mean arterial blood pressure, which parallels with a rise in heart rate and cardiac output. A more significant increase in cardiac contractility is seen with intravenous administration of urocortin to sheep as reflected by greater elevation in both maximal aortic blood flow and aortic dF/dt [41]. Urocortin is more potent than CRF in enhancing the cardiac performance[30,41] and α-helical CRF 9-41, a non-selective CRF receptor antagonist diminishes the cardiac effects of urocortin[30]. The difference in the cardiac action between CRF and urocortin may reflect the difference in the binding affinity of the two peptides for CRF receptors, as previously described[1]. Systemic administration of urocortin fails to enhance cardiac performance and to reduce blood pressure in CRF_2 receptor-knockout mice. This supports a central role of CRF_2 receptors in mediating urocortin-induced peripheral haemodynamic effects[42]. CRF_2 receptors may also contribute to cardiovascular homeostasis because the CRF_2 receptor-knockout mice have elevated basal blood

pressure[42]. Urocortin mRNA is detected in both cultured neonatal cardiac myocytes and the adult heart of rats and urocortin protects the intact rat heart against the damaging effects of ischemia/reperfusion injury[43,44]. A most recent study demonstrates that urocortin exerts profound and sustained cardiovascular, hormonal, and renal effects in experimental heart failure of sheep and these effects include reduced peripheral resistance, cardiac preload and afterload, and augmented cardiac output. This hemodynamic alteration is associated with inhibition of vasoconstricting/volume-retaining factors (vasopressin, angiotensin II, endothelin-1 and aldosterone) and with persistent increase in natriuresis, diuresis and glomerular filtration[45]. All these indicate a considerable importance of urocortin in the (patho)physiology of the cardiovascular system.

CRF receptors, widely expressed in mammalian brain and pituitary might contribute to generation of the human stress response and the pathophysiology of stress-related disorders such as increased anxiety, depression, and decreased appetite[16]. However, it is yet to be determined what role of the central CRF receptors may play in the cardiovascular action produced by systemic CRF polypeptides. Intravenous CRF-induced peripheral vasodilatation in rats consistently leads to a fall in mean arterial pressure and subsequent reflex tachycardia[46]. This centrally mediated chronotropic effect is also observed at relatively high doses in humans[47] and monkeys[48]. The onset of increased cardiac contractility precedes any change in blood pressure or central venous pressure in sheep, indicating that the positive inotropic action of urocortin is unlikely a result of a reflex response to cardiac preload or afterload [41]. It is likely that urocortin increases cardiac contractility via a direct mechanism which is not secondary to an enhanced coronary vasodilatation[30,49].

The positive inotropic effect of CRF polypeptides may be related to elevated cardiac cyclic AMP production. CRF elicits a significant rise in the intracellular concentration of cyclic AMP in isolated neonatal rat cardiomyocytes, which was inhibited by α-helical CRF (9-41)[40]. Urocortin stimulates increase in cardiac cyclic AMP content with an EC_{50} of 0.1 nmol/L[7] and the rat heart may contain 1 nmol/L of urocortin[50], a concentration that produces a maximal rise in cellular cyclic AMP content in cardiomyocytes *in vitro*[7]. It is proposed that urocortin binds predominantly to CRF_2 receptors, leading to an increased production of cyclic AMP which exerts a positive inotropic effect. Immunoreactivity of urocortin is also detected in the human hearts[7]. Given the high affinity of urocortin for CRF_2 receptor, urocortin at concentrations in picomolar and nanomolar ranges should have an important role as a local regulator of cardiac function. However, little is known about the exact cellular source of urocortin in the physiological conditions and additional second messengers that may have been also involved.

The expression of urocortin mRNA in a rat cardiac cell line or in primary cultures of cardiomyocytes is increased 12-18 h after thermal injury. Urocortin protected cardiac myocytes from cell death induced by hypoxia. It is suggested that urocortin is an endogenous cardiomyocyte peptide which modulates the cellular response to stress[51]. Urocortin exerts a protective effect in primary cardiac myocyte culture exposed to lethal simulated hypoxia/ischemia and this effect is rapid, occurring 30 min after urocortin administration[44]. Urocortin is also cardioprotective when added at the point of reoxygenation[44]. In isolated perfused rat hearts *ex vivo*, urocortin reduces infarct size of the intact rat heart when administered before or after a simulated ischemic injury[44]. This protection may be in part mediated by activation of extracellular signal-regulated kinase (ERK) 1/2-p42/44 signaling pathway, as previously demonstrated for CRF since it can be abolished by the MAP kinase kinase (MEK)1 inhibitor, PD98059. MEK1/2 is the upstream activator of the p42/44 MAP kinase (MAPKs) and its activity is inhibited by PD98059[52]. Activation of p38 MAPKs induces apoptosis in neonatal rat cardiac myocytes against ischemia and a specific inhibitor of p38 MAPK, SB 203580 suppresses activation of caspase-3, a key enzyme involved in an apoptotic process[53]. However, SB 203580 does not inhibit the cardioprotective effect of urocortin, suggesting that the p38 MAPKs signaling pathway play little role.

Hypoxia/ischemia is probably the main physiological stress to the heart and increased expression of heat shock proteins, hsp27 and hsp70, is related to the cardiac protection against hypoxic stress. Hsp70 expression triggered by thermal or ischemic preconditioning results in reduction in infarct size in animal hearts[54,55]. In transgenic mice models over-expression of hsp70 improves functional recovery and decreases infarct size *in vitro* after myocardial ischemia and reperfusion[55-57]. Urocortin-stimulated hsp90 expression is inhibited by PD98059 and cycloheximide. Both inhibitors are found to reduce urocortin-induced cardioprotection[58]. These studies demonstrate a direct positive correlation between

the amount of heat shock protein and the degree of myocardial protection, thus indicating that activation of MEK1/2 initiates several signaling pathways to mediate the cardioprotective effect of urocortin.

Ischemia induces cardiac cell death, which is accompanied by phosphorylation and increased expression and transcriptional activity of signal transducer and activator of transcription-1 (STAT-1)[59]. Induction of STAT-1 demonstrated by immunofluorescence in cardiomyocytes co-localizes with TUNEL-positive apoptotic cells[59], indicating that STAT-1 may participate in promoting ischemia/reperfusion-induced cardiac cell apoptosis[60]. However, urocortin has no stimulatory effect on STAT pathway since STAT-1 and STAT-3 tyrosine phosphorylation is undetectable in cardiomyocytes in the presence of urocortin at the same concentration range that up-regulates MAPK-dependent pathway [44]. It is however unknown whether urocortin could inhibit the ischemia-induced stimulation of STAT-1 pathway.

Additional to MEK1/2 and p42/p44 MAPK, activation of phosphatidylinositol 3-OH kinase (PI3K) plays a crucial role in the regulation of cardiac cell survival and apoptosis. For example, a PI3K-dependent mechanism mediates the survival-promoting and anti-apoptotic effect of cardiotrophin-1 on cultured ventricular myocytes[61]. Insulin prevents neonatal rat cardio-myocytes from oxidative stress-mediated apoptosis via activation of both PI3K and the putative downstream effector, the serine-threonine kinase Akt. Akt activation preserves cardiac function and prevents injury after transient ischemic insult *in vivo*[62]. Wortmannin, a specific PI3K inhibitor or over-expression of dominant negative mutant of PI3K abolished the cardioprotective effect of insulin[63]. Urocortin-induced cardiac protection against hypoxia/reoxygenation is also mediated through activation of protein kinase B/Akt since blockade of the PI3K pathway by chemical inhibitors, wortmannin and LY294002 attenuates the cardioprotective effect of urocortin in both neonatal and adult cardiomyocytes[64]. Akt induces increased myocardial contractility and cell size *in vivo* in transgenic mice without directly altering ß-adrenoceptor signaling capacity[65]. Both Akt and protein kinase B, the important downstream targets of PI3K, increase heart size, which is associated with a comparable increase in single myocyte cell size in constitutively active Akt transgenic mice as compared with the non-transgenic mice[66]. These new findings support a role of PI3K/Akt, in addition to the p42/44 MAPK pathway in mediating urocortin-induced cardioprotection, *eg,* preventing ischemia/hypoxia-induced cardiomyocyte apoptosis *ex vivo* and *in vivo*, and preserving cardiac function against ischemia/reperfusion injury.

In vivo, loss of ventricular myocytes by apoptosis leads to heart failure and down-regulation of anti-apoptotic (survival) signals or over-expression of pro-apoptotic signals is closely involved in disease processes. Any endogenous substances such as urocortin that could enhance survival or prevent cell death in cardiomyocytes possess potential therapeutic usefulness in the treatment of heart failure. The endogenous level of urocortin is increased in cardiomyocytes after ischemia/reperfusion injury[51], which may involve the preconditioning effect. Urocortin promotes metabolic recovery by partial reservation of intracellular ATP and creatine phosphate levels at the end of ischemia or reperfusion, suggesting that urocortin may preserve mitochondrial function by maintaining the respiratory transport chain[67]. Amounting evidence implicates that activation of mitochondrial K_{ATP} channels protects the cardiac cell death against ischemia/hypoxia[68,69]. It is unknown whether endogenous urocortin, like K^+ channel openers, could stimulate the activity of mitochondrial K_{ATP} channels. A most recent study demonstrates that urocortin specifically increases gene expression of the Kir 6.1 cardiac K^+ channel subunit and urocortin-induced cardioprotection can be blocked by mitochondrial K_{ATP} channel blockers[70]. A role of enhanced mitochondrial K_{ATP} channel activity is further supported by the increased ischemia-mediated apoptosis after inhibition of the Kir 6.1 channel subunit in cardiomyocytes[70].

Urocortin and cardiotrophin-1 utilize similar cellular mechanisms underlying cardiac protection. They include MEK1/2, p42/44MAPK, PI3K, and Akt signaling pathways[44,64,71,72]. The molecular and pharmacological evidence indicates that urocortin may act as an endogenous cardioprotective agent in response to cardiac injury, and therefore may possess potential therapeutic activity in the treatment of myocardial infarction and heart failure.

Urocortin mRNA expression is higher in the hypertrophic hearts as compared to normal hearts, whereas CRF-R2β mRNA expression is reduced in ventricular hypertrophy[7], suggesting that urocortin may have a negative regulatory effect on mRNA expression of CRF-R2β in the hypertrophic hearts. It appears that urocortin can induce myocardial hypertrophy as evidenced by the ability of the peptide to increase protein

and DNA syntheses at concentrations that stimulate cyclic AMP production[7]. Urocortin also potentiates endothelin-1-induced increase in protein synthesis in neonatal rat cardiomyocytes[73]. Although it is becoming clear that multiple signaling pathways participate in the cardioprotective response to ischemia/hypoxia insult, there is little information on the role of these signals in the hypertrophic effect of urocortin. Possible crosstalk between these pathways and PKA remains to be elucidated.

CONCLUDING REMARKS

Amounting evidence suggest that urocortin plays a significant role in the control of cardiovascular function and may become one of the primary factors involved in the cardiovascular response to stressful stimulation. Both CRF_2 receptor and the proposed naturally occurring agonist, urocortin are expressed in the cardiovascular system. The potent vasodilator effect of urocortin is probably mediated through activation of PKA-dependent vascular K^+ channels. Immunoreactivity of urocortin detected in the endothelial cells of the rat arteries[22] suggests that urocortin may be locally synthesized in blood vessels. If this occurs in the endothelial cells, urocortin may act as a new candidate as an endothelium-derived relaxing factor. Urocortin has now emerged as a potentially important hormone in the regulation of cardiac function, and urocortin acts directly on the cardiomyocytes via binding to CRF_2 receptors and subsequent activation of multiple intracellular signal transduction pathways that result in the positive inotropic and cardioprotective actions. The coronary vasodilatation together with the potential benefits in the cardiac system may highlight a potential of developing urocortin and CRF-related peptides into therapeutic agents against the damaging effect of ischemia/reperfusion injury to the heart if these effects were to be confirmed in primates and humans (Fig 1).

In addition to the positive inotropism, the hypertrophic effect of urocortin may represent a compensatory mechanism by which cardiac function could be increased in response to the failing heart. However, this adaptive mechanism may eventually impair ventricular performance when sustained with increasing oxygen demand. Therefore, the hypertrophic response, if it ought to occur in the human hearts, could reduce the potential usefulness of urocortin in the treatment of ischemic heart disease. Another potential undesirable effect of urocortin is cardiac fibrosis since the peptide

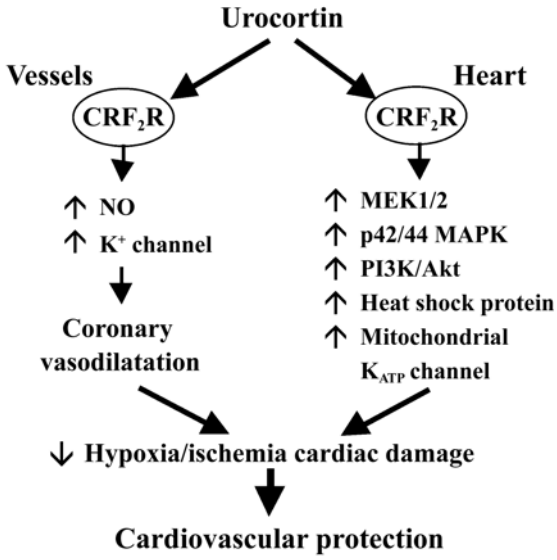

Fig 1. Urocortin exerts a cardiovascular protective action by acting on CRF_2 receptors in the cardiovascular system. Urocortin dilates the systemic arteries through stimulation of endothelial NO release and activation of smooth muscle K^+ channels. Coronary vasodilatation improves coronary blood flow during hypoxia/ischemia insult. In the heart, urocortin activates several intracellular signaling pathways to prevent cardiac cell injury or death resulting from hypoxia/ischemia. These pathways include MEK1/2, p42/44 MAPK, PI3K/Akt, heat shock proteins (hsp70 and hsp90) and mitochondrial K_{ATP} channels.

released by both cardiac non-myocytes and myocytes acts as an autocrine/paracrine growth factor to proliferate both cell types[74]. With increasing understanding of the intracellular transduction pathways involved in the cardiovascular protective effects of urocortin, further studies may lead to the development of new analogues of urocortin, which do not initiate a hypertrophic response.

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