Coronary vasodilation and positive inotropism by urocortin in the isolated rat heart

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Abstract

Corticotropin-releasing factor (CRF) has a coronary vasodilator effect and a positive inotropic effect on the isolated rat heart. Recently, expression of CRF receptor type 2 (CRF-R2) has been demonstrated in the heart. In addition, urocortin (Ucn), a new member of the CRF family, has been reported to have much greater affinity for CRF-R2 than CRF. It is suggested that the cardiac effects of Ucn may be more potent than those of CRF.

We compared the effect of Ucn with that of CRF on isolated rat heart. The effects of Ucn were then analyzed to determine whether these effects were mediated by CRF receptors and/or any other mediators under the

following conditions: perfusion buffer containing (1) α -helical CRF 9–41, (2) indomethacin, (3) N^G-nitro-L-arginine methylester and (4) propranolol. Ucn exhibited a greater effect with a longer duration of action than CRF. Indomethacin significantly attenuated the vasodilator effects of Ucn ($P < 0.05$). CRF receptor antagonist diminished both coronary vasodilation and the positive inotropic effects of Ucn ($P < 0.05$).

These results suggest that the cardiac effects of Ucn may be mediated by a CRF receptor, and prostaglandins may be involved in the vasodilator effect.

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Introduction

Corticotropin-releasing factor (CRF), a 41 amino acid peptide, is a primary factor contributing to stress response in mammals (Vale *et al.* 1981, 1983). This peptide regulates the hypothalamic–pituitary–adrenal axis and exerts diverse effects on the central nervous, reproductive, immune and cardiovascular systems (Vale *et al.* 1983, Smith *et al.* 1989).

The effects of CRF on the cardiovascular system have been extensively studied. It has been shown to exhibit different cardiovascular effects *in vivo* when administered intravenously compared with those when administered into the cerebral ventricles. Heart rate, cardiac output and mean arterial pressure increased with intracerebro-ventricular administration of CRF (Fisher *et al.* 1983, Overton *et al.* 1990, Overton & Fisher 1991). In contrast, intravenous administration of CRF decreased mean arterial pressure (Fisher *et al.* 1982, Overton & Fisher 1991). In 1993, Grunt *et al.* showed that in isolated rat hearts perfused with Krebs–Henseleit buffer (KHB) the peptide caused a positive inotropic effect and an increase in coronary flow (Grunt *et al.* 1993). These reports suggest the

presence of specific CRF receptors in the cardiovascular system.

Recently, two subtypes of CRF receptor have been characterized and termed CRF receptor type 1 (CRF-R1) and CRF receptor type 2 (CRF-R2) (Chang *et al.* 1993, Potter *et al.* 1994, Wong *et al.* 1994, Kishimoto *et al.* 1995, Lovenberg *et al.* 1995b, Perrin *et al.* 1995, Stenzel *et al.* 1995, Kostich *et al.* 1998). It has been reported that CRF-R1 is expressed in brain and pituitary (Chang *et al.* 1993, Potter *et al.* 1994, Wong *et al.* 1994). However, CRF-R2 was expressed not only in the central nervous system (Lovenberg *et al.* 1995b, Kostich *et al.* 1998) but also in peripheral tissues, including the heart (Kishimoto *et al.* 1995, Lovenberg *et al.* 1995a, Perrin *et al.* 1995, Stenzel *et al.* 1995).

In 1995, Vaughan *et al.* cloned a new member of the mammalian CRF family named urocortin (Ucn), which is a 40 amino acid peptide cloned from the rat midbrain (Vaughan *et al.* 1995). Ucn is reported to bind and activate CRF receptors to a greater extent than CRF. In particular, Ucn has much greater affinity for CRF-R2. It is therefore suggested that Ucn may be a natural ligand for CRF-R2, and its cardiovascular effects might be more

Table 1 Results are shown as mean concentrations ($\pm$ S.E.M.) in the hearts between 15 s of injection and maximum decrease ($\pm$ S.E.M.) in CPP with CRF and Ucn at 1 μ g ($n=5$), 3 μ g ($n=5$) and 10 μ g ($n=5$)

Dose (μ g)	CRF		Ucn	
	Concentration (nM)	Decrease in CPP (%)	Concentration (nM)	Decrease in CPP (%)
1	88.0 $\pm$ 5.0	4.0 $\pm$ 1.4	86.3 $\pm$ 7.1	4.2 $\pm$ 0.9
3	214.2 $\pm$ 8.2	12.7 $\pm$ 2.2	205.9 $\pm$ 10.0	27.0 $\pm$ 5.0*
10	689.4 $\pm$ 18.4	29.8 $\pm$ 1.9	660.7 $\pm$ 12.6	38.8 $\pm$ 3.2*

* $P<0.05$ compared with CRF-treated hearts.

potent than those of CRF. Parkes *et al.* (1997) showed that Ucn produces long-lasting actions that increase coronary flow and enhance cardiac contractility in conscious sheep, but the effect of Ucn in the isolated heart has not yet been confirmed.

The purpose of this study was to determine whether Ucn possesses positive inotropic and coronary vasodilator effects on isolated rat hearts, as does CRF, and to determine whether these effects of Ucn are mediated by CRF receptors and/or any other mediators. The present study was therefore conducted to investigate the cardiovascular effects of Ucn and CRF on isolated rat heart preparations perfused with a constant coronary flow rate.

Materials and Methods

Isolated rat heart preparation

Male Sprague–Dawley rats (350–450 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). While maintaining artificial ventilation, the chest was opened at the median line of the sternum and the pericardium was opened widely. After administration of heparin (5000 U/kg, i.v.), both pulmonary hila and the superior vena cava were ligated simultaneously. A perfusion tube was immediately inserted into the ascending aorta to perfuse the coronary arteries with KHB (concentrations in mmol/l: NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, glucose 11.7, EDTA 0.03, ascorbic acid 0.06) equilibrated with 95% O₂ and 5% CO₂ (pH 7.4) at a constant temperature (37 °C). Interruption of coronary perfusion lasted no longer than 20 s. The inferior vena cava was then ligated and the heart was removed from the chest and suspended from the coronary perfusion tube. A small vent was inserted into the left ventricular apex to drain Thebesian outflow. A flexible tube was inserted into the right ventricle for coronary venous drainage. Coronary flow was measured by timed collections of coronary drainage into a 10 ml glass pipette. A collapsed, thin, high-density polyethylene balloon was

placed in the left ventricle through the mitral orifice. The balloon was connected to a pressure transducer and a 1 ml graduated syringe. Pacing electrodes connected to an electronic stimulator were attached to the surface of the left ventricle. Coronary perfusion pressure was measured with a pressure transducer connected to a side arm of the coronary perfusion tube. Coronary flow rate was adjusted to obtain a perfusion pressure similar to the *in situ* blood pressure, and kept constant throughout the experiment. The heart was placed in a chamber with a heating jacket, and the temperature of the heart was maintained at 35–37 °C.

Experimental protocol

After coronary perfusion pressure (CPP), coronary flow (CF), and left ventricular pressure (LVP) were stabilized, either rat Ucn or human/rat CRF dissolved in 100 μ l saline was injected into the perfusion tube just above the heart as a bolus for about 15 s. Recordings were made under the baseline condition and then repeated at 1-min intervals for 10 min after the injection of the peptide. The changes in the hemodynamic measurements are expressed by the percent changes from baseline. We examined the dose-related effects of 1, 3 and 10 μ g Ucn or CRF in 15 hearts. Because the molecular weight of Ucn (4707) is very similar to that of CRF (4757), the injections of each amount of Ucn and CRF produced almost the same concentrations of the two peptides in the hearts within about 15 s of the injections. The concentrations were calculated by the coronary flow (Table 1). The selected dose of the peptides was based on the report of Grunt *et al.* (1993). The vasodilator effects of Ucn (3 μ g) were studied in separate hearts perfused with unchanged KHB, KHB containing α -helical CRF 9–41 (1 or 10 nM) which blocks CRF-R, KHB containing indomethacin (30 μ M) which inhibits cyclo-oxygenase or KHB containing N^G-nitro-L-arginine methylester (L-NAME) (30 μ M) which inhibits nitric oxide synthase.

The inotropic effect of Ucn (10 μ g) was also determined in separate hearts perfused with unchanged KHB, KHB

Table 2 Means ($\pm$ S.E.M.) basal CF and CPP in hearts perfused with unchanged KHB ($n=34$), KHB containing L-NAME ($30\text{ }\mu\text{M}$, $n=5$), indomethacin ($30\text{ }\mu\text{M}$, $n=9$), propranolol ($3\text{ }\mu\text{M}$, $n=4$), α -helical CRF (1 nM , $n=5$), α -helical CRF (10 nM , $n=5$) or α -helical CRF (30 nM , $n=4$)

	CF (ml/min)	CPP (mmHg)
Unchanged KHB	12.1 $\pm$ 0.6	106.0 $\pm$ 1.8
L-NAME	12.4 $\pm$ 2.9	121.2 $\pm$ 1.8*
Indomethacin	10.2 $\pm$ 1.3	97.9 $\pm$ 2.5
Propranolol	11.7 $\pm$ 1.1	93.8 $\pm$ 2.6
α -helical CRF (1 nM)	13.1 $\pm$ 1.5	95.0 $\pm$ 2.5
α -helical CRF (10 nM)	12.3 $\pm$ 1.1	94.2 $\pm$ 4.1
α -helical CRF (30 nM)	11.1 $\pm$ 2.2	96.0 $\pm$ 2.1

* $P<0.05$ compared with hearts perfused with unchanged KHB.

containing α -helical CRF 9–41 (30 nM), KHB containing propranolol ($3\text{ }\mu\text{M}$), or KHB containing indomethacin ($30\text{ }\mu\text{M}$).

Statistical analysis

The data were analyzed by a repeated measures design ANOVA, or by a one-way ANOVA. When the F test indicated a significant difference among the conditions, the Student–Newman–Keuls test was used to test the significance of difference between specific conditions. All data are presented as means $\pm$ S.E.M. and $P<0.05$ was considered to indicate statistical significance.

Results

Basal CPP and CF

Table 2 summarizes the baseline of CPP and CF in hearts perfused under different conditions. L-NAME showed an increase of CPP compared with unchanged KHB.

Effects on coronary vascular resistance

Both Ucn and CRF decreased CPP at a constant flow rate, indicating that both induced coronary vasodilation. The decrease in CPP with CRF attained its maximum response at 1 min after injection, and CPP gradually returned to its initial level. Vasodilator response with Ucn culminated at 3–5 min after the injection and lasted for approximately 10 min. Table 1 summarizes the maximum values of percent decrease in CPP with CRF and Ucn at each dose. Both peptides decreased CPP in a dose-dependent manner. Ucn showed a significantly greater decrease in CPP than CRF at $3\text{ }\mu\text{g}$ and $10\text{ }\mu\text{g}$ ($P<0.05$) by one-way ANOVA. A repeated measures ANOVA showed a significant difference of the vasodilator effects between CRF and Ucn at each dose ($P<0.05$) (Fig. 1).

Indomethacin significantly attenuated the vasodilator effects of Ucn ($P<0.05$), but L-NAME did not (Fig. 2).

Effects on the increase in cardiac contractility

Both Ucn and CRF increased LVP and rate of left ventricular pressure increase (LVdP/dt). We chose $10\text{ }\mu\text{g}$ CRF or Ucn for the main experiments, because the positive inotropic effects with 1 or $3\text{ }\mu\text{g}$ of both peptides were not potent enough to measure. LVP reached the maximum value at 1 min after injection with CRF, so did LVdP/dt. However, the positive inotropic effect had a shorter duration than the coronary vasodilator effect and it lasted no longer than 5 min. With Ucn, the peak LVP increased to its maximum value at 3 min after injection and gradually returned to the baseline level thereafter. The maximum increases in LVP were $11.7 \pm 1.7\%$ with $10\text{ }\mu\text{g}$ CRF and $11.6 \pm 2.3\%$ with $10\text{ }\mu\text{g}$ Ucn. Peak changes in LVdP/dt were $14.5 \pm 3.0\%$ with $10\text{ }\mu\text{g}$ CRF and $23.3 \pm 5.9\%$ with $10\text{ }\mu\text{g}$ Ucn. There was a significant difference in the increase in cardiac contractility between CRF and Ucn at a dose of $10\text{ }\mu\text{g}$ ($P<0.05$) (Fig. 3).

Propranolol and indomethacin did not influence the positive inotropic effects of Ucn (Fig. 4).

Effect of α -helical CRF 9–41

Pretreatment with α -helical CRF 9–41 diminished the $3\text{ }\mu\text{g}$ Ucn-induced decrease in CPP (Fig. 5), as well as the $10\text{ }\mu\text{g}$ Ucn-induced increase in LVP and LVdP/dt ($P<0.05$) (Fig. 4).

Discussion

In this study, we have investigated the direct cardiovascular effects of Ucn and CRF in isolated rat hearts. The two peptides exerted a coronary vasodilator effect and positive inotropism. CPP showed the maximum decrease at 1 min after administration of CRF and then returned to the control level. Such a change in CPP was similar to CRF-induced increase in coronary flow (Grunt *et al.* 1993). However, Ucn-induced vasodilation attained its maximum response at 3–5 min after injection and plateaued thereafter. The maximum vasodilator responses from 3 and $10\text{ }\mu\text{g}$ Ucn were greater than those of CRF.

We also found that the increases in LVP and LVdP/dt with Ucn were more potent and lasted longer than those with CRF. We measured maximum LVdP/dt instead of E_{max} (an index of contractility) in order to estimate cardiac contractility. Since our experiments were carried out in isovolumetrically contracting heart preparations and the preload and afterload of the left ventricle were kept constant throughout the experiment, the maximum rate of increase in LVP (maximum LVdP/dt) should be an appropriate estimate for cardiac contractility. In practice, it

Figure 1 Effects of (A) CRF and (B) Ucn on CPP at 1 ($n=5$), 3 ($n=5$) and 10 ($n=5$) μg . Each point represents the mean $\pm$ S.E.M. * $P<0.05$ compared with CRF.

was difficult to measure E_{max} every minute since the index requires steady-state LVP recordings at several left ventricle volumes. We therefore focused on the time-course study of the maximum LVDP/dt after the injection of the peptides.

CRF receptor antagonist diminished the effects of Ucn. This result suggests that the cardiac effects of Ucn are mediated by a CRF receptor. Because α -helical CRF

9–41 is a CRF receptor antagonist which blocks both CRF-R1 and -R2 we cannot conclude that the cardiovascular effects of Ucn and CRF are exclusively mediated by CRF-R2. However, this study has shown that the vasodilator effect and positive inotropic effect of Ucn were more potent and lasted longer than those of CRF. The difference in the effects between Ucn and CRF may be due to the difference in the affinity of the two peptides

Figure 2 Effects of Ucn (3 µg) on CPP in hearts perfused with unchanged KHB ($n=5$), KHB containing 30 µM L-NAME ($n=5$) or KHB containing 30 µM indomethacin ($n=5$). Each point represents the mean $\pm$ S.E.M. * $P<0.05$ compared with hearts perfused with unchanged KHB.

for CRF-R2, as reported (Vaughan *et al.* 1995). Some investigators have recently generated CRF-R2-deficient mice. It was reported that intravenous administration of Ucn failed to enhance the cardiac contractility of those CRF-R2-deficient mice, in contrast to wild-type mice (Coste *et al.* 2000). Their results showed that the cardiac effects of Ucn depend on CRF-R2, which supports our findings.

Using *in situ* hybridization, it has been demonstrated that CRF-R2 was expressed on arterioles of the rat heart (Lovenberg *et al.* 1995a). This suggests that these receptors were present in endothelial cells and/or smooth muscle cells of the coronary artery. Given that CRF-R2 exists in the smooth muscle cells of coronary arteries, Ucn and

Figure 3 Effects of CRF ($n=4$) or Ucn ($n=4$) on (A) LVP and (B) LVdp/dt at 10 µg. Each point represents the mean $\pm$ S.E.M. * $P<0.05$ compared with CRF.

Figure 4 Effects of Ucn (10 µg) on LVdp/dt in hearts perfused with unchanged KHB ($n=4$), KHB containing 3 µM propranolol ($n=4$), KHB containing 30 µM indomethacin ($n=5$) or KHB containing 30 nM α -helical CRF 9-41 ($n=4$). Each point represents the mean $\pm$ S.E.M. * $P<0.05$ compared with hearts perfused with unchanged KHB.

CRF could directly relax the smooth muscle cells via CRF-R2 as other vasodilators do via their own receptors.

In this study, indomethacin, a cyclo-oxygenase inhibitor, diminished the Ucn-induced change in CPP. It has also been reported that CRF-induced vasodilation was diminished by indomethacin (Grunt *et al.* 1993). Since cyclo-oxygenase is a key enzyme for biosynthesis of prostaglandins, our finding implies that prostaglandins, such as prostacyclin, are involved in Ucn-induced coronary vasodilation. It has been reported that prostacyclin was the major prostaglandin released from the isolated rat heart (de Deckere *et al.* 1977). One explanation for the prostaglandin-induced coronary vasodilator effect of Ucn could be that it is due to prostacyclin being released from

Figure 5 Effects of Ucn (3 µg) on CPP in hearts perfused with unchanged KHB ($n=5$), KHB containing 1 nM ($n=5$) or 10 nM ($n=5$) α -helical CRF 9-41. Each point represents the mean $\pm$ S.E.M. * $P<0.05$ compared with hearts perfused with unchanged KHB.

the endothelial cells of coronary arteries. However, it was reported that in rat resistance artery preparations CRF-induced relaxation did not depend on endothelial cells (Lei *et al.* 1993). If the result holds in coronary arteries also, the prostaglandins could be released from cells other than endothelial cells, such as smooth muscle cells of the coronary artery.

Nitric oxide is a potent vasodilator released from endothelial cells. L-NAME did not significantly diminish the vasodilator effect of Ucn in this study. It is therefore unlikely that Ucn-induced vasodilation was mediated by nitric oxide. Grunt *et al.* (1993), however, showed that CRF-induced vasodilation was diminished by N^G-nitro-L-arginine which inhibits nitric oxide synthase. It is possible that CRF is different from Ucn in releasing nitric oxid and that the effect of CRF on the endothelial cells of coronary artery is distinct from that of resistance artery.

Intravenous administration of Ucn in conscious sheep has been found to cause a gradual increase in coronary flow and cardiac contractility which lasted for a few days (Parkes *et al.* 1997). The slow onset of the cardiac effect of Ucn implies an indirect action of the peptide on cardiomyocytes. In the present study, however, it took only a few minutes to exhibit the cardiac effects of Ucn in the isolated heart. Furthermore, indomethacin diminished the coronary dilator effects of Ucn but did not influence the positive inotropic effects of the peptide. Thus, it is suggested that Ucn increases cardiac contractility via a direct mechanism which is independent of coronary dilation caused by Ucn.

The positive inotropic effect is caused by stimulation of β -adrenoceptor in cardiomyocytes. Because propranolol, a β -adrenoceptor antagonist, did not significantly attenuate the inotropic effect of Ucn, its inotropic effect may not be mediated by β -adrenoceptor. Catecholamines bind to β -adrenoceptor in cardiomyocytes and lead to an increase in intracellular levels of cAMP. The positive inotropic effect of catecholamine via β -adrenoceptor depends on cAMP (Sobel & Mayer 1973). It has been reported that CRF-R2 mRNA is expressed in murine atrial cardiomyocyte tumor cells and that CRF causes an increase in intracellular levels of cAMP in freshly isolated cardiomyocytes of neonatal rats (Heldwein *et al.* 1996). This fact suggests that CRF- or Ucn-induced positive inotropic effects may be related to cAMP elevated by the stimulation of CRF-R. In 1998, it was reported that Ucn mRNA is expressed in a rat cardiac myocyte cell line by reverse transcription polymerase chain reaction (Okosi *et al.* 1998). In that study, Ucn was suggested to be a natural ligand of CRF-R in the heart and to have paracrine effects. It was postulated that the physiological role of Ucn might be protection of cardiomyocytes from stress imposed on the cardiovascular system, such as ischemia (Okosi *et al.* 1998, Brar *et al.* 1999, 2000). If that is so, the coronary vasodilation and the positive inotropism of Ucn would be a relevant response to protect cardio-

myocytes from the stress. In addition, it was shown that systemic administration of endotoxin down-regulated CRF-R2 mRNA levels in the heart (Heldwein *et al.* 1997, Kageyama *et al.* 2000). These results also suggest a possible contribution of CRF-R2 in the heart to the cardiovascular response to stress.

In summary, this study is the first to present the cardiac effects of Ucn in isolated heart preparations. Both Ucn and CRF produced coronary vasodilation and a positive inotropic effect. In addition, the effect of Ucn was greater with a longer duration of action than that of CRF. Because a CRF-R antagonist diminished the effects of Ucn, the effects are mediated by CRF-R in the heart. Since the vasodilator effect of Ucn was diminished by indomethacin, Ucn-induced vasodilation may partly depend on prostaglandins.

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