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Influence of urocortin and corticotropin releasing factor on venous tone in conscious rats

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Abstract

The corticotropin releasing factor (CRF) family includes CRF and urocortin. The effects of urocortin and CRF (0.3, 1, 3 and 10 nmol/kg, i.v.) relative to those of vehicle (0.9% NaCl) on mean arterial pressure and mean circulatory filling pressure (index of venous tone) were examined in conscious, unrestrained rats that were either intact (unblocked) or ganglion-blocked by treatment with mecamylamine (10 mg/kg, i.v.) followed by noradrenaline (4 μg/kg/min, i.v.) to increase vasomotor tone. Both urocortin and CRF dose-dependently decreased mean arterial pressure in intact rats and ganglion-blocked rats. The depressor action of urocortin was greater than that of CRF at all doses. In intact rats, neither compound reduced mean circulatory filling pressure. In ganglion-blocked rats, urocortin and the highest dose of CRF decreased mean circulatory filling pressure. In conclusion, both urocortin and CRF are vasodepressor agents with venodilator action.

Keywords: Urocortin; Corticotropin releasing factor; Mean arterial pressure; Mean circulatory filling pressure; Venous; Capacitance vessel

1. Introduction

The corticotropin releasing factor (CRF) family includes CRF, a 41 amino acid peptide, and urocortin, a 40 amino acid peptide (Vaughan et al., 1985). The actions of CRF, urocortin and related peptides are mediated through the activation of CRF_1 and CRF_2 receptors. The location and function of these receptors are still uncertain. CRF_1 receptor mRNA is widely expressed in the brain and pituitary (Potter et al., 1994). CRF_2 receptors have three splice isoforms: $CRF_{2\alpha}$ mRNA is detected in the brain (Lovenberg et al., 1995) and heart (Kimura et al., 2002), $CRF_{2\beta}$ mRNA is present in the heart, skeletal muscle, lung, intestine, brain (Lovenberg et al., 1995; Kageyama et al., 2000) and aorta (Kageyama et al., 2000), whereas $CRF_{2\gamma}$ mRNA is detected in the brain (Kostich et al., 1998). CRF induces a sustained (5–8 min) vasodepressor response which is accompanied by sustained tachycardia in conscious rats (Gardiner et al., 1988), or transient (<1 min) bradycardia in anaesthetized rats (Lei et al., 1993). In vitro studies show that CRF increases coronary arterial flow (Grunt et al., 1993), causes dilatation of isolated perfused rat mesenteric arteries (Barker and Corder, 1999), and relaxes preconstricted mesenteric and cerebral arteries (Lei et al., 1993). Urocortin also causes a long-lasting depressor response which is accompanied by tachycardia in conscious and anaesthetized rats (Parkes et al., 2001; Vaughan et al., 1985) and anaesthetized mice (Cohen et al., 2000). The depressor action of urocortin is due to generalized vasodilatation of organs and tissues that include the heart, gastrointestinal organs, skeletal muscle and skin (Abdelrahman and Pang, 2003). Urocortin also induces vasodilatation of isolated rat coronary (Huang et al., 2002), basilar (Schilling et al., 1998) and tail (Lubomirov et al., 2001) arteries, isolated perfused human fetal placental artery (Leitch et al., 1998) and isolated rat heart (Terui et al., 2001).

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The venous system plays a crucial role in the regulation of cardiac output. The aim of this study was to investigate if urocortin and CRF decreased mean circulatory filling pressure, the driving force of venous return. Mean circulatory filling pressure is the pressure which would occur throughout the circulation if all pressures were abruptly brought to an equilibrium (Guyton, 1955). In the absence of a change in blood volume, a decrease in mean circulatory filling pressure denotes a reduction in body venous tone (Pang, 2001). The effects of CRF and urocortin on venous tone were examined in conscious, intact (unblocked) rats and in ganglion-blocked rats infused with noradrenaline to increase vascular tone. Noradrenaline was used in this study because the vasodilator action of a drug is more clearly seen in animals with a high venous tone (Abdelrahman and Pang, 1990, 1992).

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (350–450 g) were anaesthetized with halothane (4% in air for induction and 1.5% for maintenance). Polyethylene (PE50) catheters were introduced into the left iliac artery to record mean arterial pressure by a pressure transducer (PD23DB, Gould, Statham, CA, USA) and the right iliac vein for the administration of drug or vehicle. Heart rate was derived electronically from the upstroke of the arterial pulse pressure by a Grass 7P4G tachograph. The left iliac vein was also cannulated to allow the insertion of a catheter into the inferior vena cava for the measurement of central venous pressure using another pressure transducer (P23DB, Gould Statham, CA, USA). A saline-filled, balloon-tipped catheter was advanced into the right atrium through the right external jugular vein. The proper positioning of the balloon was tested by transiently inflating it, which when correctly placed resulted in a simultaneous decrease in mean arterial pressure to 20–25 mm Hg and an increase in central venous pressure within 5 s of circulatory arrest. All cannulae were filled with heparinized normal saline (25 I.U./ml) and tunneled to the back of the neck, exteriorized and secured. The rats were allowed 6 h to recover from the effects of surgery and anaesthesia before further use.

2.2. Measurements of mean circulatory filling pressure

The method for measuring mean circulatory filling pressure has been described in detail elsewhere (Pang, 2001). Briefly, steady-state readings of mean arterial pressure and central venous pressure were noted at 4–5 s after temporarily stopping the circulation by inflation of the atrial balloon. To correct for the incomplete equilibration of arterial and venous pressures during circulatory arrest, mean circulatory filling pressure was calculated by the following equation: Mean circulatory filling pressure=Venous plateau pressure+1/60 (Final arterial pressure−Venous plateau pressure), where 1/60 represents the ratio of arterial to venous compliance.

2.3. Experimental protocol

The rats were divided into six groups (n=6–9 each). Mean arterial pressure, heart rate and central venous pressure were continuously displayed on a Grass Polygraph (Model RPS 7C8). The conscious rats were given 30 min to stabilize before baseline haemodynamic measurements were taken. Three groups of rats were given i.v. bolus injections of urocortin (0.3, 1, 3, 10 nmol/kg,), CRF (0.3, 1, 3 and 10 nmol/kg) or an equivalent volume of the vehicle (0.9% saline) at intervals of 10 min. Another three groups of rats were pretreated with mecamylamine (10 mg/kg, i.v. bolus) followed 8 min later by noradrenaline (4 μg/kg/min, i.v. infused), and measurements were again taken at 10 min after the start of noradrenaline infusion. This was followed by i.v. bolus injections of either urocortin (0.3, 1 and 3 nmol/kg), CRF (0.3, 1 and 3 nmol/kg) or vehicle at 10 min intervals. Mean circulatory filling pressure readings were taken at 9 min after the start of administration of urocortin, CRF or the vehicle, at the plateau phase of response to the depressor agents.

2.4. Statistical analysis

All data are presented as mean±S.E.M. The data were analyzed by one-way (among groups of rats) or two-way (within the same group) repeated measures analysis of variance followed by multiple comparisons of group data using the Tukey test (SigmaStat statistical software), with $P<0.05$ selected as the criterion for statistical significance.

2.5. Drugs

Urocortin and CRF (Sigma, St. Louis, MO, USA) were dissolved in distilled water, and kept in aliquots at −20 °C until the day of the experiment when they were diluted with normal saline (0.9% NaCl). Mecamylamine and noradrenaline (Sigma, St. Louis, MO, USA) were also dissolved in normal saline.

3. Results

3.1. Effects of CRF and urocortin on mean arterial pressure, heart rate and mean circulatory filling pressure in conscious rats

There were no significant differences in baseline readings of mean arterial pressure, heart rate and mean circulatory filling pressure among the three groups of intact rats (I, II and III in Table 1). Urocortin caused a dose-dependent

decrease in mean arterial pressure, an increase in heart rate and it had no effect on mean circulatory filling pressure (Fig. 1). CRF also caused a dose-dependent decrease in mean arterial pressure but had no effect on the heart rate and mean circulatory filling pressure (Fig. 1). Curve analysis show that the depressor effect of urocortin was significantly greater than that of CRF. The vehicle did not have any significant effect on any measured variables during the whole protocol indicating that inflating the atrial balloon and temporarily stopping the blood circulation did not affect haemodynamic parameters (Fig. 1). In other words, the haemodynamic parameters were stable during the whole experimental protocol.

3.2. Effects of mecamylamine and noradrenaline on mean arterial pressure, heart rate and mean circulatory filling pressure

Mecamylamine significantly decreased mean arterial pressure, heart rate and mean circulatory filling pressure. The subsequent infusion of noradrenaline increased mean arterial pressure, mean circulatory filling pressure and heart rate in all groups (IV, V and VI in Table 1).

3.3. Effects of urocortin and CRF on mean arterial pressure, heart rate and mean circulatory filling pressure in ganglion-blocked rats

Saline did not significantly alter mean arterial pressure, heart rate or mean circulatory filling pressure in the time-control group treated with mecamylamine and noradrenaline (Fig. 2). Both urocortin and CRF dose-dependently reduced mean arterial pressure but did not affect heart rate relative to the changes in the time-control rats (Fig. 2). Curve analysis showed that the depressor responses to both urocortin and CRF were significantly greater in the mecamylamine-treated rats than in the intact rats (significance not shown in the Figures). In addition, similar to the results of mean arterial pressure in the intact rats, the depressor response to CRF was significantly less than that to urocortin in the mecamylamine-treated rats (Fig. 2). The highest two doses of urocortin, and only the high dose of CRF (3 nmol/kg), significantly decreased mean circulatory filling pressure relative to responses in the time-control group treated with vehicle (Fig. 2).

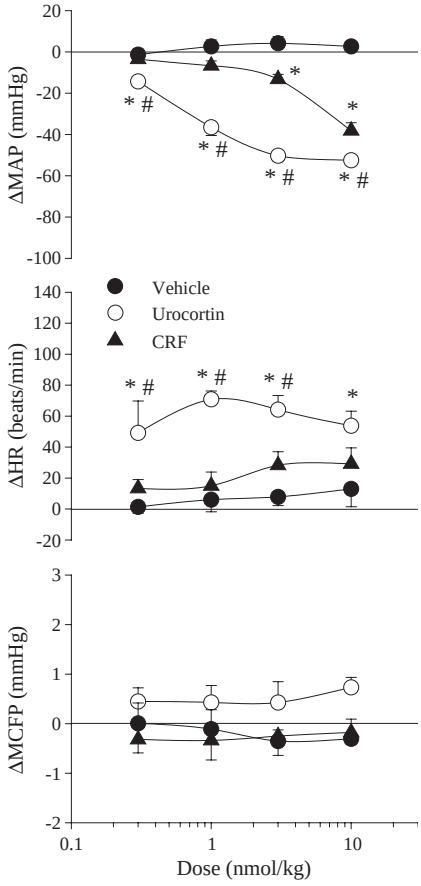

Fig. 1. Effects (mean±S.E.M, n=6 per group) of urocortin, corticotropin releasing factor (CRF) and an equal volume of the vehicle (0.9% NaCl) on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious, intact rats. *Significantly (P<0.05) different from the saline group. #Significantly different from the CRF group.

Table 1
Baseline values (means±S.E.M.) of mean arterial pressure, heart rate and mean circulatory filling pressure in conscious rats (n=6–9 per group)

	Mean arterial pressure (mm Hg)	Heart rate (beats/min)	Mean circulatory filling pressure (mm Hg)
Group I—saline	104±2	406±12	6.2±0.3
Group II—urocortin	106±2	407±8	5.6±0.4
Group III—CRF	100±4	403±13	6.6 ± 0.2
Group IV—Mec-NA-saline			
Baseline	110±3	421±13	6.2±0.3
After mecamylamine	79±3[a]	369±17[a]	4.4±0.3[a]
After noradrenaline	150±3[a,b]	487±14[a,b]	8.7±0.5[a,b]
Group V—Mec-NA-urocortin			
Baseline	114±2	409±10	5.5±0.2
After mecamylamine	79±3[a]	379±9[a]	3.5±0.4[a]
After noradrenaline	153±4[a,b]	489±9[a,b]	8.7±0.5[a,b]
Group VI—Mec-NA-CRF			
Baseline	110±4	419±7	6.2±0.3
After mecamylamine	70±3[a]	378±11[a]	4.2±0.4[a]
After noradrenaline	151±4[a,b]	482±6[a,b]	9.1±0.4[a,b]

The rats received mecamylamine (10 mg/kg, i.v. bolus) and noradrenaline (4 μg/kg/min, i.v. infusion).
[a] Significantly different (P<0.05) from the baseline value.
[b] Significantly different (P<0.05) from mecamylamine treatment.

4. Discussion

Urocortin decreased mean arterial pressure in a dose-dependent manner and increased heart rate in conscious, intact rats. In conscious rats pretreated with mecamylamine

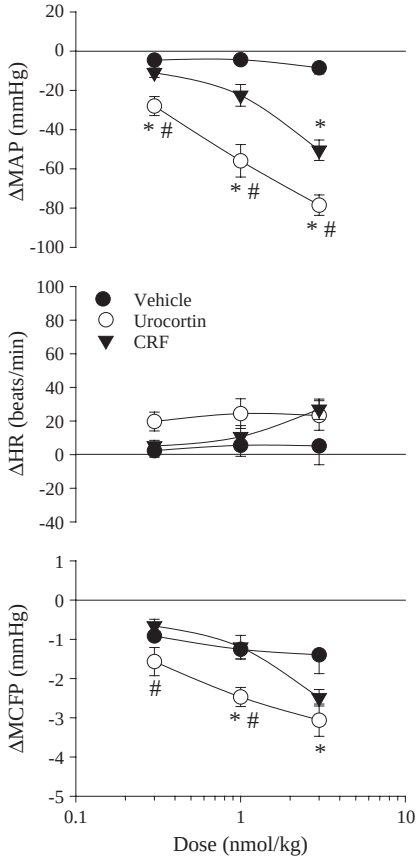

Fig. 2. Effects (mean±S.E.M.) of urocortin (n=7), corticotropin releasing factor (CRF, n=9) and an equal volume of vehicle (0.9% NaCl, n=8) on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious rats pretreated with mecamylamine (10 mg/kg, i.v.) and noradrenaline (4 μg/kg/min, i.v.). *Significantly (P<0.05) different from the saline group. #Significantly different from the CRF group.

to block the ganglia, followed by noradrenaline to increase vascular tone, urocortin also caused a dose-dependent decrease in mean arterial pressure but had no significant effect on heart rate. The ability of mecamylamine to abolish the tachycardic effect of urocortin suggests that the tachycardia was baroreflex-mediated. However, we cannot rule out the possibility that, in the ganglion blocked rats, the heart rate was already relatively high (~490 beats/min) and could not be surmounted by the (weak) tachycardic effect of urocortin. The vasodepressor effect of urocortin has been reported in anaesthetized rats (Parkes et al., 2001), conscious rats (Vaughan et al., 1985) and anaesthetized mice (Cohen et al., 2000). Urocortin did not alter mean circulatory filling pressure in conscious, intact rats, but significantly decreased mean circulatory filling pressure in rats pretreated with mecamylamine and noradrenaline. These results suggest that urocortin does have a venodilator action, but this is masked when autonomic reflexes are functional when blood pressure is reduced acutely. This is in accordance with our previous findings which show that vasodilators such as verapamil (Ca^{2+} channel blocker, Waite et al., 1988), nitroglycerine or sodium nitroprusside (nitrovasodilator, D'Oyley et al., 1989) either induced no change in mean circulatory filling pressure or increased mean circulatory filling pressure in conscious, intact rats, and that the venodilator action of these compounds is clearly seen following partial ganglionic blockade. Our present results show that urocortin has a dilator action on arterial resistance as well as capacitance vessels. These results are consistent with the observation that urocortin causes relaxation of the preconstricted human saphenous vein (Sanz et al., 2002).

In the present study, the vasodilator action of urocortin was compared to that of equimolar doses of CRF. Similar to urocortin, CRF also produced a dose-dependent decrease in mean arterial pressure in intact rats as well as in ganglion-blocked rats, but its depressor action was significantly less than that of urocortin in both conditions. The depressor response of CRF has been reported (Gardiner et al., 1988; Lei et al., 1993). These findings are in accordance with those of Vaughan et al. (1985), which showed that the depressor response of CRF is less in magnitude and shorter in duration than that of urocortin in conscious rats. CRF produced no significant increase in heart rate in the intact rats and did not affect heart rate in the ganglion-blocked rats. These results suggest that CRF also has no direct chronotropic action. CRF did not affect mean circulatory filling pressure in intact rats; however, the high dose of CRF (3 nmol/kg) reduced mean circulatory filling pressure in ganglion-blocked rats. These results suggest that CRF has the ability to dilate arterial and capacitance vessels. The venodilator potency of CRF appears to be less than that of urocortin.

There are indications that the depressor actions of urocortin and CRF may both be mediated via the activation of CRF_2 receptors. First, $CRF_{2\beta}$ mRNA is present in vascular tissues such as arterioles (Lovenberg et al., 1995) and the aorta (Kageyama et al., 2000), and urocortin does not cause a depressor response in mice with deficient CRF_2 receptors (Bale et al., 2000; Coste et al., 2000). Interestingly, mice with deficient CRF_2 receptors have elevated blood pressure (Coste et al., 2000), thereby suggesting that CRF_2 receptors are physiologically involved in blood pressure regulation. Secondly, urocortin is 10-fold more potent than CRF in increasing cAMP levels in cells expressing $CRF_{2\beta}$ receptors, whereas CRF is twice as potent as urocortin in increasing cAMP in cells expressing CRF_1 receptors (Vaughan et al., 1985). Therefore, if CRF_1 receptors were the mechanism underlying vasodilatation by both peptides, CRF should have been more potent than urocortin in decreasing mean arterial pressure and mean circulatory filling pressure; however, this was clearly not true in the present study.

In conclusion, urocortin and CRF decrease both mean arterial pressure and mean circulatory filling pressure in conscious rats showing that they have in vivo arterial and venous dilator actions. The depressor and venodilator effects of urocortin are greater than those of CRF.

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