A Comparison between the Cardiovascular Actions of Urocortin 1 and Urocortin 2 (Stresscopin-Related Peptide) in Conscious Rats

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ABSTRACT

The aims of the study were, in conscious Sprague-Dawley rats, to compare the effects of stresscopin-related peptide (SRP) and urocortin (UCN) 1 on blood pressure, heart rate, and regional hemodynamics; to determine whether or not there were residual tachycardic effects of SRP or UCN1 after cardiac autonomic blockade; and to investigate a possible involvement of corticotropin releasing factor type 1 (CRF1) receptor-mediated histamine release in the vasodilator actions of UCN1. SRP and UCN1 (both at 3 nmol/kg i.v.) caused hypotension, tachycardia, and mesenteric and hindquarters vasodilatation, but the magnitude and/or duration of the effects of UCN1 were generally greater than those of SRP. Pretreatment with atropine plus propranolol abolished the tachycardic effects of SRP and UCN1, and, under those conditions, the hypotensive effect of SRP, but not that of UCN1, was enhanced, probably because the hindquarters vasodilator effect of the latter was also reduced. Pretreatment with mepyramine plus cimetidine had no effect on the hemodynamic actions of either SRP or UCN1. It is concluded that, in conscious rats, the tachycardic effects of SRP and UCN1 are due to autonomic nervous activation mainly through baroreflex mechanisms. There is no evidence for an involvement of CRF1 receptor-mediated histamine release in the vasodilator actions of UCN1, but a propranolol-sensitive hindquarters vasodilator action of UCN, but not of SRP, was identified.

Four members of the corticotropin-releasing factor (CRF) family of peptides have been identified, namely CRF, urocortin (UCN) 1, UCN2 (analogous to stresscopin-related peptide (SRP)), and UCN3 (analogous to stresscopin). In addition, two receptor types have been cloned, namely, CRF1 and CRF2. The CRF1 receptor is expressed largely, but not exclusively, in the central nervous system, whereas CRF2 receptor expression is mainly localized to peripheral tissues (for review, see Dautzenberg and Hauger, 2002). The binding affinities of the CRF peptides for the different receptors vary, with CRF showing more affinity for CRF1 than CRF2 receptors, UCN1 showing equal affinity at CRF1 and CRF2 receptors, and UCN2 and UCN3 showing almost exclusive binding to CRF2 receptors (for review, see Dautzenberg and Hauger, 2002).

In the original description of the identification of UCN1 (Vaughan et al., 1995), hypotensive and tachycardic effects were described in conscious rats. The hypotension has since been attributed to vasodilatation, and the tachycardia has been suggested to be a reflex response to the hypotension because it was absent in thiobutabarbital-anesthetized rats (Abdelrahman and Pang, 2003). However, this suggestion is not consistent with the finding that, in sheep, the tachycardic effects of UCN1 are unaffected by ganglion blockade (for review, see Parkes et al., 2001).

Hypotensive (Chen et al., 2003; Mackay et al., 2003) and vasodilator (Gardiner et al., 2005) effects of UCN2/SRP have also been described in conscious rats and shown to be mediated by CRF2 receptors. In the latter study, it was noted that there was dissociation between the extent of the hypotension and the degree of tachycardia. Specifically, CRF had modest hypotensive effects relative to an equimolar dose of UCN2 but caused the same degree of tachycardia, suggesting that the tachycardia may not be entirely reflexive in origin. Others have reported cardiac effects of UCN1 (Terui et al., 2001) and UCN2 (Bale et al., 2004) that were insensitive to β-adrenoceptor antagonism. Thus, the first aim of the present study was to compare the integrated cardiovascular effects of UCN1 and SRP in the absence and presence of cardiac autonomic blockade, with atropine and propranolol, to determine...
whether or not there were any tachycardic effects independent of the autonomic nervous system.

The similarity between the cardiovascular actions of UCN1 and UCN2, together with the peripheral localization of CRF₂ receptors and the effectiveness of selective CRF₂ receptor antagonism, has led to the conclusion that the cardiovascular actions of all peripherally administered UCN peptides are likely to be CRF₂ receptor-mediated (for review, see Hashimoto et al., 2004). However, there is evidence to suggest that activation of CRF₁ receptors causes degranulation of mast cells (for review, see Theoharides et al., 2004) and that the resulting release of histamine contributes to the vasodilator effects of CRF in human skin (Crompton et al., 2003). Thus, it is feasible that CRF₁ receptor-mediated histamine release may contribute to the cardiovascular actions of UCN1, but not UCN2, because the latter has no affinity for CRF₁ receptors (see above). This was the second hypothesis tested in the current study in which we assessed the effects of histamine (H₁ and H₂) receptor antagonism on the cardiovascular actions of UCN1 and SRP.

Materials and Methods

Animals and Surgery. Before any surgical intervention, male Sprague-Dawley rats (350–400 g; Charles River, Margate, Kent, UK) were housed in the Biomedical Services Unit, University of Nottingham for at least 10 days after delivery, with free access to standard rat chow (Teklad Global 18% protein rodent diet; Bicester, Oxon, UK) and water. Room temperatures were maintained at 21 ± 2°C, and there was a 12-h light/dark cycle (6:00 AM to 6:00 PM).

Surgery was performed in two stages under general anesthesia (fentanyl and medetomidine, 300 µg/kg of each i.p.). Anesthetic reversal and the provision of analgesia were achieved using atipamazole and buprenorphine (0.5 and 0.05 mg/kg, respectively, s.c.). Firstly, miniaturized pulsed Doppler flow probes were sutured around the left renal and superior mesenteric arteries and around the distal abdominal aorta (to monitor hindquarters flow). Secondly, catheters were implanted in the distal abdominal aorta (via the ventral caval artery) for monitoring arterial blood pressure and heart rate and in the right jugular vein for the administration of substances. The surgical stages were separated by at least 10 days, and between stages, the fitness of the animals was certified by the named veterinary surgeon. The procedures were approved by the University of Nottingham Ethical Review Committee and were performed under Home Office Project and Personal License authority.

After catheterization, the animals were fitted with custom-designed harnesses with a counterbalanced spring attached to protect the catheters and allow the animals freedom of movement in their home cage with access to food and water ad libitum. The arterial catheters were connected to fluid-filled swivels for overnight i.a. infusion of heparinized (15 U/ml, 0.4 ml/h) saline to maintain catheter patency.

Data Acquisition and Analysis. Experiments began 24 h after catheterization. On each experimental day, continuous recordings of cardiovascular variables (heart rate, arterial blood pressure, renal, mesenteric and hindquarters Doppler shifts (flow)) were made using a customized, computer-based system (Hemodynamics Data Acquisition System, University of Limburg, Maastricht, The Netherlands) connected to the transducer amplifier (Gould model 13-4615-50; Gould Instrument Systems Inc., Cleveland, OH) and the Doppler flowmeter (Crystal Biotech VF-1 mainframe (pulse repetition frequency, 125 kHz) fitted with high-velocity (HVFD-20) modules; Crystal Biotech, Holliston, MA). Data were sampled by the Hemodynamic Data Acquisition System every 2 ms, averaged each cardiac cycle, and stored to disc every 5 s throughout the experimental period.

Data were analyzed off-line (Datview; University of Maastricht). Measurements were made under resting conditions and at intervals up to 240 min after peptide administration. Data are expressed as mean ± S.E.M. Within-group analyses were carried out by a non-parametric equivalent of analysis of variance allowing for multiple comparisons (Friedman’s test) (Theodorsson-Norheim, 1987). Between-group analyses were performed on the maximal changes and the times at which these occurred (calculated for each animal) and on the integrated responses (areas under or over curves) using Wilcoxon’s test (paired) or Mann-Whitney U test (unpaired) as appropriate. P ≤ 0.05 was taken as significant.

Experiment 1: Regional Hemodynamic Effects of UCN1 and SRP in the Absence and Presence of Atropine and Propranolol. Two groups of rats were used (n = 8 in each), one group receiving UCN1 and SRP in the presence of saline, and the other group receiving UCN1 and SRP in the presence of atropine and propranolol. On day 1, either UCN1 or SRP was administered at a dose of 3 nmol/kg i.v., 90 min after the onset of administration of either saline (0.1 ml bolus, 0.4 ml/h infusion) or a combination of propranolol (1 mg/kg bolus, 0.5 mg/kg/h infusion) plus atropine (1 mg/kg bolus, 1 mg/kg/h infusion). As a time control, on day 2, saline (0.1 ml) was administered 90 min after the start of saline (as above) or atropine plus propranolol (as above). On day 3, the animals that had received UCN1 on day 1 were given SRP and vice versa in the presence of either saline or atropine plus propranolol. In an additional group of animals (n = 7), the effects of atropine alone on responses to UCN1 and SRP were examined in a protocol that involved administration of UCN1 or SRP in the presence of saline (as above) on days 1 (n = 3 UCN, n = 4 SRP) and 2 (n = 4 UCN, n = 3 SRP) and in the presence of atropine (as above) on days 3 and 4.

Experiment 2: Regional Hemodynamic Effects of UCN1 and SRP in the Absence and Presence of Mepyramine and Cimetidine. Rats were given 3 nmol/kg UCN1 (n = 6) or SRP (n = 5) after administration of saline on day 1, and the same peptide was readministered on day 3, 15 min after the end of administration (1 ml infused i.v. over 30 min) of a combination of i.v. mepyramine (3 mg/kg) and cimetidine (30 mg/kg) (Vleeming et al., 2000). No substances were administered on day 2.

Experiment 3: Effects of Antisauvagine-30 on the Hemodynamic Responses to UCN1. In the light of the findings in the initial experiments (see Results), an additional experiment was performed (n = 6) in which the effects of UCN1 (3 nmol/kg) were tested 30 min after the onset of a primed infusion of the CRF₂ receptor antagonist, antisauvagine-30 (50 µg/kg bolus, 50 µg/kg/h infusion) (Gardiner et al., 2005).

Peptides and Drugs. Urocortin 1 (human) was from Bachem (St. Helens, UK) and SRP (human) was from the Peptide Institute Inc. (Scientific Marketing Associates, Barnet, UK). Stock solutions of peptides were made up in sterile water for injection and diluted in sterile saline. Injection volumes were 0.1 ml, and infusion rates were 0.4 ml/h except in the case of mepyramine/cimetidine, which was given as a mixture at a rate of 2 ml/h for 30 min. Atropine methyl nitrate, propranolol hydrochloride, mepyramine maleate, and cimetidine were from Sigma Chemical (Poole, Dorset, UK); pentanal citrate was from Janssen-Cilag (High-Wycombe, UK); medetomidine hydrochloride (Domitor) and atipamazol hydrochloride (Antisedan) were from Pfizer (Sandwich, Kent, UK); and buprenorphine (Vetergesic) was from Alstoe Animal Health (York, UK).

Results

Experiment 1: Regional Hemodynamic Effects of UCN1 and SRP in the Absence and Presence of Atropine and Propranolol. In rats receiving saline infusion, resting cardiovascular variables immediately before administration of UCN1 and SRP were not different (Table 1). Bolus injection of SRP (3 nmol/kg) caused tachycardia, hypotension, and marked vasodilatation in the mesenteric and...
hindquarters vascular beds, with little or no change in the renal vascular bed (Fig. 1a), as described previously (Gardiner et al., 2005). The effects of UCN1 (3 nmol/kg) were qualitatively similar to those of SRP, but there were some differences in the time to peak, maximal change, and duration of action (Fig. 1b). Thus, for heart rate and blood pressure, the maximal effects of SRP (+166 ± 9 beats/min, −50 ± 2 mm Hg) occurred at 6.2 ± 1.2 and 9.6 ± 0.6 min, respectively, whereas for UCN1, the maximal changes (+170 ± 6 beats/min, −32 ± 3 mm Hg) were similar but occurred significantly (P < 0.001, Wilcoxon’s test) later (at 14 ± 1.1 and 29 ± 2.3 min, respectively) and lasted longer (compare Fig. 1, a with b). The maximal increase in hindquarters vascular conductance in response to SRP also occurred significantly (P < 0.001) sooner (at 17 ± 2.7 min) than the corresponding response to UCN1 (at 102 ± 15.3 min); however, in addition, the magnitude of change was greater for UCN1 (+160 ± 9%) than that for SRP (+95 ± 9%; P < 0.001), and the latter was more long-lasting (compare Fig. 1, a with b). In contrast, the maximal increase in mesenteric vascular conductance following SRP (+116 ± 9% at 4.1 ± 0.6 min) occurred at the same time and was of similar magnitude to the response to UCN1 (+89 ± 7% at 4.9 ± 0.9 min), although the duration of action of UCN1 on mesenteric vascular conductance was greater than SRP (compare Fig. 1, a with b). As a result of these differences, the integrated (0–240 min) increase in heart rate (+17,454 ± 1871 beats), fall in blood pressure (−6106 ± 398 mm Hg), and increase in mesenteric vascular conductance (+10,258 ± 1421% min) and hindquarters vascular conductance (+27,240 ± 1576% min) in response to UCN1 were significantly (Wilcoxon’s test) greater than the corresponding changes following SRP (+7134 ± 618 beats; −3196 ± 276 mm Hg min; +6058 ± 1615% min; +10,953 ± 1093% min). In the renal vascular bed, UCN1 caused an initial small vasodilatation followed by vasoconstriction (Fig. 1b).

In rats receiving atropine plus propranolol, cardiovascular variables immediately before administration of UCN1 or SRP were not different and did not differ from baseline variables in the saline-infused rats (Table 1). In the presence of UCN1 or SRP, heart rate was increased by 7% at 4.9 min and 11% at 4.1 min, respectively, whereas for SRP, the maximal increase in hindquarters vascular conductance was greater than SRP (compare Fig. 1, a with b). As a result of these differences, the integrated (0–240 min) increase in heart rate (+17,454 ± 1871 beats), fall in blood pressure (−6106 ± 398 mm Hg), and increase in mesenteric vascular conductance (+10,258 ± 1421% min) and hindquarters vascular conductance (+27,240 ± 1576% min) in response to UCN1 were significantly (Wilcoxon’s test) greater than the corresponding changes following SRP (+7134 ± 618 beats; −3196 ± 276 mm Hg min; +6058 ± 1615% min; +10,953 ± 1093% min). In the renal vascular bed, UCN1 caused an initial small vasodilatation followed by vasoconstriction (Fig. 1b).
of atropine plus propranolol, the tachycardic response to SRP was virtually abolished, and the mesenteric and hindquarters vasodilatations were unaffected, but the integrated (0–240 min) fall in blood pressure (−4167 ± 453 mm Hg min) was significantly greater than in the presence of saline (Fig. 1a). In contrast, although the tachycardic response to UCN1 was virtually abolished by atropine and propranolol, the integrated fall in blood pressure was unaffected, possibly because the integrated increase in hindquarters vascular conductance (+15,991 ± 2521% min) was significantly reduced (Fig. 1b).

In rats receiving atropine before administration of SRP or UCN1, resting heart rate was significantly elevated (369 ± 8 and 363 ± 11 beats/min, respectively) compared with the corresponding values in the presence of saline (339 ± 7 and 325 ± 8 beats/min), but all other cardiovascular variables were not different (data not shown). In the presence of atropine, the tachycardic responses to SRP (+46 ± 9 beats/min at 30 min) and UCN1 (+97 ± 10 beats/min at 30 min) were reduced by approximately 50% (P < 0.05) compared with the corresponding changes in the presence of saline (+71 ± 10 and +135 ± 23 beats/min, respectively), but the hypotensive and regional vascular changes were not different (data not shown).

Experiment 2: Regional Hemodynamic Effects of UCN1 and SRP in the Absence and Presence of Mepyramine and Cimetidine. Resting cardiovascular variables in the two groups of animals before administration of UCN1 and SRP are shown in Table 1. The cardiovascular responses to SRP and UCN1 in these groups of animals were as described above, and the combination of cimetidine and mepyramine had no effect on the hemodynamic responses to either peptide (Fig. 2).

Experiment 3: Effects of Antisauvagine-30 on the Hemodynamic Responses to UCN1. In the presence of antisauvagine-30, the hemodynamic effects of UCN1 were markedly reduced or abolished. Thus, 30 min after administration of UCN1, there was no significant change in blood pressure (+2 ± 3 mm Hg), renal vascular conductance (−5 ± 7%), or mesenteric vascular conductance (−1 ± 2%), and the changes in heart rate (+13 ± 6 beats/min) and hindquarters vascular conductance (+15 ± 6%) were substantially less than in animals not given antisauvagine-30 (see Figs. 1 and 2).

Discussion

The design of these experiments enabled, firstly, a direct comparison of the regional hemodynamic effects of equimolar doses of UCN1 and SRP in the same animals, secondly, an evaluation of the extent to which the tachycardic effects of these two peptides are dependent on the autonomic nervous system, and thirdly, assessment of the possible involvement of CRF1 receptor-mediated histamine release in the vasodilator effects of UCN1. The following discussion will deal with each of these aspects of the study in turn.

Comparison of the Hemodynamic Effects of UCN1 and SRP. In conscious sheep, integrated cardiovascular effects of UCN1 (Rademaker et al., 2002), UCN2 (Rademaker et al., 2005), and UCN3 (Rademaker et al., 2006) have been described in separate studies. Qualitatively, the effects of the three urocortin peptides are similar in this large animal model, and although those studies did not provide a direct comparison between them, Rademaker et al. (2005) noted that the hemodynamic effects of UCN2 took less time to reach a maximum and were of shorter duration of action than UCN1. They suggested that the difference could be explained by differences in volume of distribution and clearance of the peptides (Rademaker et al., 2005), although, given their similarities in size (UCN1, 40 amino acids; UCN2, 38 amino acids), it is not clear why this should be.

Consistent with the observations of Rademaker and colleagues, the present study shows, for the first time in the same animals, that the regional hemodynamic effects of UCN1 and SRP in conscious rats were qualitatively similar,
but the effects of UCN1 on heart rate, blood pressure, and hindquarters vascular conductance took longer to reach a maximum and were more long-lasting than those of an equimolar dose of UCN2. Interestingly, the same did not apply to the mesenteric vasodilator response, which, for both peptides, reached a maximum within the first 5 min of administration, although the duration of effect of UCN1 was greater. Elsewhere, we have commented on the difference in time course of onset and duration of effect of UCN2 in the mesenteric and hindquarters vascular beds, with the response in the latter being slower in onset and of longer duration than that in the mesenteric vascular bed (Gardiner et al., 2005). In that study, we suggested that the mesenteric vasodilator effect might represent a direct action at vascular CRF₂ receptors, whereas the hindquarters vasodilatation could be secondary to a metabolic action (Gardiner et al., 2005). The present findings are consistent with UCN1 and SRP having similar affinity for CRF₂ receptors in the mesenteric vascular bed, but with the two peptides having differential effects on the putative indirect mechanisms responsible for the hindquarters vasodilatation (see below). The longer lasting hypotensive effect of UCN1 corresponded with the more persistent hindquarters vasodilatation.

Effects of Atropine and Propranolol on Responses to UCN1 and SRP. The tachycardic effects of both peptides were abolished in the presence of atropine and propranolol, consistent with the increase in heart rate being due to sympathoexcitation and vagal withdrawal, and experiments involving administration of atropine alone indicated a significant and similar contribution from both components of the autonomic nervous system. This contrasts with findings in sheep where the tachycardic effects of UCN1 were reported to be unaffected by ganglion blockade (Parkes et al., 2001), but, in that species, UCN1-induced tachycardia was accompanied by a rise rather than a fall in blood pressure, with no peripheral vasodilatation (Rademaker et al., 2002). Given the closeness of the time course of blood pressure and heart rate changes in the present study, it seems likely that the tachycardia was a reflex response to the fall in blood pressure, although we cannot exclude a contribution from centrally mediated sympathoexcitation to the response to UCN1, as shown for CRF (Overton and Fisher, 1991).

In the presence of atropine and propranolol, the hypotensive effects of SRP were enhanced, whereas the mesenteric and hindquarters vasodilator effects were unchanged, indicating that reflex cardiac responses to SRP normally acted to oppose the hypotension. In contrast, the hypotensive response to UCN1 was not enhanced when the tachycardia was abolished in the presence of atropine and propranolol, most probably because the hindquarters vasodilator response to UCN1 was reduced under those conditions. Theoretically, the inhibition of the hindquarters vasodilator response could have been due to propranolol antagonizing adrenomedullary, β₂-adrenoceptor-mediated, vasodilatation, or to atropine inhibiting cholinergic vasodilatation. However, because an additional experiment showed that administration of atropine alone had no effect on the hindquarters vasodilator effect of UCN1, it is most likely that the effects we observed with atropine plus propranolol were due to an effect of propranolol. Therefore, the results indicate that UCN1 may cause sympatho-adrenal activation that contributes to its hindquarters vasodilator and hypotensive effects but that this effect is not seen with SRP. The results of the experiment performed using antisauvagine-30 indicate that UCN1-induced cardiovascular effects are primarily, if not exclusively, mediated via CRF₂ receptors, consistent with our previous work using CRF (Gardiner et al., 2005). Therefore, the additional, propranolol-sensitive vasodilator effect of UCN1 observed here is likely to be CRF₂ receptor-mediated and may be explained by a more effective activation of sympatho-adrenal activity by that ligand, possibly due to different pharmacokinetics (see above and Rademaker et al., 2005).

Effects of Mepyramine and Cimetidine on Responses to UCN1 and SRP. As explained in the Introduction, findings in the literature are consistent with activation of CRF₁ receptors causing mast cell degranulation and histamine release, and given the affinity of UCN1 for CRF₁ receptors and the lack of such affinity of SRP, it is feasible that the cardiovascular effects of UCN1 involved histamine release, whereas those of SRP did not. However, in the presence of mepyramine and cimetidine, to block H₁ and H₂ receptors, respectively, there was remarkably little change in the responses to UCN1 or SRP, indicating no involvement of endogenous histamine via H₁ or H₂ receptors in the vasodilator responses. Histamine can also affect cardiac function importantly through H₃ receptors (see Levi and Smith, 2000), and because we did not use an H₃ receptor antagonist, we cannot exclude an effect of histamine via this receptor subtype. Furthermore, vasoactive mediators other than histamine may be released from mast cells, and we cannot exclude a contribution from those to the effects observed, although histamine has been shown to be the principal mediator of CRH-induced skin vasodilatation in man (Crompton et al., 2003).

In conclusion, the present results are consistent with SRP and UCN1 causing similar CRF₂ receptor-mediated mesenteric vasodilator responses and indirect, autonomically mediated, tachycardic effects. Although both peptides cause hindquarters vasodilatation that is resistant to propranolol and atropine, an additional propranolol-sensitive vasodilator effect of UCN1 has been identified.

References
Theodorsson-Norheim E (1987) Friedman and Quade tests: BASIC computer pro-
gram to perform nonparametric two-way analysis of variance and multiple compa-
Vleeming W, Hendriksen CFM, van de Kuil A, van den Hout JWE, and de Wildt DJ (2000) Mepyramine but not cimetidine or clobenpropit blocks pertussis toxin-

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