Regional Hemodynamic Actions of Selective Corticotropin-Releasing Factor Type 2 Receptor Ligands in Conscious Rats

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Received July 30, 2004; accepted August 20, 2004

ABSTRACT

In conscious male Sprague-Dawley rats, we compared regional hemodynamic actions of the selective corticotropin-releasing factor type 2 (CRF₂) receptor ligands human and mouse urocortin 2 (hUCN2 and mUCN2, respectively) with those of CRF. Bolus i.v. doses of 3 and 30 pmol kg⁻¹ hUCN2, mUCN2, or CRF had no significant hemodynamic actions, but at doses of 300 and 3000 pmol kg⁻¹, all three peptides caused dose-dependent tachycardia and hypotension, with rapid-onset, short-duration, mesenteric vasodilatation and slower-onset, more prolonged hindquarters vasodilatation but little or no change in renal vascular conductance. Pretreatment with the nonselective CRF receptor antagonist astressin or the selective CRF₂ receptor antagonist antisauvagine 30 abolished all the cardiovascular actions of all three peptides. Indomethacin had no effect on responses to hUCN2, and there was no evidence for any involvement of nitric oxide (NO) in the vasodilator actions of hUCN2. There was no evidence that recruitment of angiotensin- and endothelin-mediated vasoconstrictor mechanisms counteracted the vascular actions of hUCN2. The results indicate that the hemodynamic effects of i.v. hUCN2, mUCN2, and CRF depend on activation of CRF₂ receptors and do not involve NO or prostanoids.

There are four members of the mammalian corticotropin-releasing factor (CRF) family. The 41-amino acid peptide CRF was isolated and characterized in 1981 (Vale et al., 1981) and is now known to play a pivotal role in neuroendocrine, autonomic, immune, and behavioral responses to stress (for review, see Grammatopoulos and Chrousos, 2002). Subsequently, the 40-amino acid peptide urocortin (now known as urocortin 1, UCN1), which is the mammalian homolog of urotensin I and sauvagine in fish and amphibians, was isolated, initially from specific regions of the brain (Vaughan et al., 1995). Subsequently, it has been shown to be expressed in a number of peripheral locations, including the heart (Kageyama et al., 1999). More recently, two 38-amino acid isoforms of urocortin were cloned from mouse and human cDNA libraries by two independent groups, one of which named the peptides urocortin 2 (UCN2; Reyes et al., 2001) and urocortin 3 (UCN3; Lewis et al., 2001), whereas the other used the names stresscopin (UCN3) and stresscopin-related peptide (UCN2) (Hsu and Hsueh, 2001). It is notable that although the amino acid number is similar for the four mammalian CRF peptides, the sequence homology is relatively low; indeed, only four amino acids are completely conserved, suggesting that secondary structure, rather than sequence homology, probably determines the biological activity (for review, see Hauger et al., 2003 concerning structures and recommended nomenclature).

Two types of G protein-coupled CRF receptor have been identified (CRF₁ and CRF₂), with at least three splice variants of CRF₂ (Grammatopoulos and Chrousos, 2002; Hauger et al., 2003). The receptors show 69% amino acid sequence homology, but they differ in tissue distribution and ligand binding. Thus, CRF shows higher affinity for CRF₁ than CRF₂ receptors, but UCN1 has equal affinity for CRF₁ and CRF₂ receptors, whereas UCN2 and 3 bind selectively to CRF₂ receptors (Hauger et al., 2003).

Several years ago, we examined the cardiovascular responses to peripheral administration of CRF in conscious rats and showed dose-dependent hypotension, tachycardia, and marked, early onset mesenteric vasodilatation, with later onset hindquarters vasodilatation and renal vasocon-
striction at higher doses (Gardiner et al., 1988). At that time, however, it was not known which CRF receptor type was responsible for the effects observed. Since then, evidence has accumulated in favor of the CRF2 receptor being responsible for the hypotensive actions of CRF in rats (Chen et al., 2003; Mackay et al., 2003), although the regional vascular consequences of selective CRF2 receptor activation in vivo are not known.

Therefore, the aims of the present experiments were, in conscious, chronically instrumented male Sprague-Dawley rats, 1) to characterize the regional hemodynamic profiles of a range of doses of the CRF2 receptor ligands, human and mouse UCN2 (hUCN2, mUCN2), and compare them with those of CRF given under identical conditions; 2) to determine the effects of the nonselective CRF receptor antagonist astressin (Gulyas et al., 1995) and the selective CRF2 receptor antagonist antisauvagine 30 (Rühmann et al., 1998) on responses to the CRF2 receptor-selective agonists and to CRF; 3) with hUCN2 as the exemplar, to assess the possible involvement of nitric oxide (NO) and prostanooids in the vasodilator responses to CRF2 receptor-selective ligands, using the nonselective NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME), and the cyclooxygenase inhibitor indomethacin; and 4) to determine the extent to which activation of endogenous vasoconstrictor systems counteracted the vasodilator effects of hUCN2 by measuring the effects of hUCN2 alone or in the presence of endothelin and angiotensin receptor antagonism (with SB 209670 and losartan).

Materials and Methods

Animals and Surgical Preparation

Experiments were performed in adult male Sprague-Dawley rats (360–450 g) obtained from Charles River (Margate, Kent, UK). Animals were housed in the Biomedical Services Unit for at least 10 days after delivery before any surgical interventions took place. Room temperature was maintained at 21 ± 2°C, and there was a 12-h light/dark cycle (6:00 AM to 6:00 PM), and animals had free access to standard rat chow (Beekay Feeds, Hull, England) and water throughout the study.

Surgery was performed in two stages under general anesthesia (fentanyl and medetomidine, 300 μg kg⁻¹ of each i.p.). Anesthetic reversal and the provision of analgesia were achieved using atipamezole and nalbuphine, respectively (1 mg kg⁻¹ of each s.c.). At the first surgical stage, 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). At least 10 days later, after the fitness of the animals had been certified by the named Veterinary Surgeon, animals were reanesthetized (as described above), and catheters were implanted in the distal abdominal aorta (via the ventral caudal artery) for monitoring arterial blood pressure and heart rate, and in the right jugular vein for the administration of substances. The procedures were approved by the University of Nottingham Ethical Review Committee and were performed under Home Office Project License authority.

Cardiovascular Recordings

Cardiovascular recordings began on the day after catheterization, when the animals were fully conscious and freely moving, with access to food and water ad libitum. 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) and the Doppler flowmeter [Crystal Biotech VF-1 mainframe (pulse repetition frequency 125 kHz) fitted with high-velocity (HVFD-20) modules].

Experimental Protocols

Experiment 1. Regional Hemodynamic Effects of Increasing Doses of hUCN2, mUCN2, or CRF. Rats (n = 12) were randomized to receive bolus i.v. injections (0.1 ml) of either hUCN2 or mUCN2 at 30, 300, and 3000 pmol kg⁻¹ on day 1 and the other peptide on day 3, with control saline injections being given to all animals on day 2. The doses of the peptides were given in ascending order, with 30 min between the first and second dose, 30 min between the second and third dose, and 60 min between the third and fourth dose.

A separate group of rats (n = 8) was given CRF at the same doses and the same time intervals as mentioned above.

Experiment 2. Effects of CRF Receptor Antagonists on Responses to hUCN2, mUCN2, and CRF. Rats were given 3000 pmol kg⁻¹ hUCN2 (n = 10), mUCN2 (n = 8), or CRF (n = 8) on day 1, and the same peptide was readministered on day 3, 30 min after the onset of a primed infusion (50 μg kg⁻¹ bolus, 50 μg kg⁻¹ h⁻¹ infusion) of either astressin or antisauvagine 30 (hUCN2, n = 5; mUCN2, n = 4; CRF, n = 4 in each group). No substances were administered on day 2.

Experiment 3. Effects of L-NAME or Indomethacin on Responses to hUCN2. Rats (n = 8) were given 3000 pmol kg⁻¹ hUCN2 90 min after the onset of infusion of L-NAME (3 mg kg⁻¹ h⁻¹). To control for the baseline hemodynamic actions of L-NAME, a separate group of rats (n = 8) was given 3000 pmol kg⁻¹ hUCN2, 90 min after the onset of coinfusion of angiotensin II (AII; 200 ng kg⁻¹ h⁻¹) and arginine vasopressin (AVP; 20 ng kg⁻¹ h⁻¹).

A third group of rats (n = 8) was given 3000 pmol kg⁻¹ hUCN2 in the presence of indomethacin vehicle (10 mM Na2CO3) on day 1 and 90 min after the onset of administration of indomethacin (5 mg kg⁻¹ h⁻¹ infusion) on day 3. No treatments were given on day 2.

Experiment 4. Effects of hUCN in the Absence and Presence of SB 209670 and Losartan. Rats (n = 8) were given 3000 pmol kg⁻¹ hUCN2 in the presence of saline on day 1 and 90 min after the onset of treatment with the endothelin antagonist SB 209670 (600 μg kg⁻¹ bolus, 600 μg kg⁻¹ h⁻¹ infusion), and the angiotensin receptor antagonist losartan (10 mg kg⁻¹), on day 3. No treatments were given on day 2.

Data Analysis

Data were sampled by hemodynamics data acquisition system every 2 ms, averaged each cardiac cycle, and stored to disc every 5 s. Offline, data were analyzed (Datview; University of Maastricht, Maastricht, The Netherlands) using electronically derived averages across times selected on the basis of the profile of response to the peptides. Hence, measurements were made under resting conditions across a 5-min epoch before administration of the peptide; across 20-s epochs around 1, 2, 3, 4, and 5 min after drug administration; and thereafter, across 1- to 2-min epochs around 10, 20, 25, and 30 min for the low doses (3 and 30 pmol kg⁻¹), a further 40, 50, and 60 min for the 300 pmol kg⁻¹ dose, and an additional 90 and 120 min for the highest dose. These data were exported into a custom-designed statistical analysis package. Data are expressed as mean ± S.E.M.

Within-group analyses were carried out by a nonparametric equivalent of analysis of variance (Friedman’s test), (Theodorsson-Norheim, 1987). Between-group analyses were performed on the integrated responses measured over the first 30 min after peptide administration using Wilcoxon’s test or Mann-Whitney U test as appropriate. P ≤ 0.05 was taken as significant.

Peptides and Drugs

Urocortin 2 (mouse), urocortin 2 (human), and CRF (rat, human) were from the Peptide Institute Inc. (Scientific Marketing Associ-
Cardiovascular Effects of CRF₂ Ligands in Conscious Rats

55

Results

Experiment 1. Regional Hemodynamic Effects of Increasing Doses of hUCN2, mUCN2, or CRF. Resting cardiovascular variables before administration of saline, hUCN2, mUCN2, and CRF were not significantly different (Table 1). There were no consistent cardiovascular effects associated with administration of saline or UCN2, mUCN2, or CRF at 3 and 30 pmol kg⁻¹ (data not shown).

At 300 pmol kg⁻¹, all three peptides caused tachycardia, increases in mesenteric Doppler shift and vascular conductance, and increases in hindquarters vascular conductance (Fig. 1). The integrated (0- to 30-min) increases in mesenteric vascular conductance in response to hUCN2 (+418 ± 62% min) and mUCN2 (+397 ± 58% min) were greater (P < 0.05) than those to CRF (+277 ± 82% min), and there was an accompanying fall in blood pressure with hUCN2 and mUCN2 that did not occur with CRF (Fig. 1). The integrated tachycardic effect of 300 pmol kg⁻¹ hUCN2 (+797 ± 172 beats) was greater (P < 0.05) than that of mUCN2 (+334 ± 127 beats) and CRF (+455 ± 142 beats).

At a dose of 3000 pmol kg⁻¹, all three peptides caused hypotension, tachycardia, and hyperemic vasodilatations in the mesenteric and hindquarters vascular bed, accompanied by falls in renal Doppler shift and biphasic changes in renal vascular conductance, with short-lived vasodilatation giving way to vasoconstriction (Fig. 2). The integrated (0- to 30-min) hypotensive effect of hUCN2 (−770 ± 30 mm Hg min) was greater (P < 0.05) than that of mUCN2 (−387 ± 63 mm Hg min), which was greater (P < 0.05) than that of CRF (−274 ± 77 mm Hg min). Interestingly, for the accompanying tachycardia, the rank order of potency tended to be reversed with the effects of CRF (+3120 ± 476 beats) being greater than those of mUCN2 (+2452 ± 316 beats), which were greater than those of hUCN2 (+1836 ± 234 beats), although the difference was only significant (P < 0.05) between CRF and hUCN2. The integrated (0- to 30-min) increases in mesenteric vascular conductance in response to 3000 pmol kg⁻¹ hUCN2 and mUCN2 were not different (+1172 ± 127, +1188 ± 231% min, respectively), but the effect of CRF was of shorter duration and hence the integrated response was significantly (P < 0.05) smaller (+780 ± 125% min). The integrated (0- to 30-min) increase in hindquarters vascular conductance in response to hUCN2 (+1760 ± 326% min) was greater (P < 0.05) than that of mUCN2 (+1018 ± 142% min) and CRF (+926 ± 191% min). In the renal vascular bed, the integrated (0- to 30-min) response was biphasic. During this 30-min period, the increase in vascular conductance was similar for hUCN2 (+125 ± 71% min) and mUCN (+114 ± 42% min) but less (P < 0.05) with CRF (+13 ± 4% min), whereas the fall in vascular conductance that occurred later was greater (P < 0.05) for CRF (−426 ± 104% min) than for hUCN2 (−201 ± 46% min) and mUCN2 (−216 ± 54% min).

Experiment 2. Effects of CRF Receptor Antagonists on Responses to hUCN2, mUCN2, and CRF. Resting cardiovascular variables in the rats used in this experiment are shown in Table 2. The cardiovascular effects of 3000 pmol kg⁻¹ hUCN2, mUCN2, and CRF on day 1 were generally similar to those seen in experiment 1, although the changes in the renal vascular bed were less marked (compare Fig. 2 with Fig. 3, a–c). There were no cardiovascular changes associated with administration of either astressin or antisauvagine 30; hence, cardiovascular variables immediately before administration of hUCN, mUCN, and CRF in the presence of either astressin or antisauvagine 30 were not different (data not shown). In the presence of either astressin or antisauvagine 30, all the cardiovascular effects of hUCN2, mUCN2, and CRF were abolished (Fig. 3, a–c).

Experiment 3. Effects of l-NAME or Indomethacin on Responses to hUCN2. Infusion of l-NAME or of AII plus AVP both caused similar degrees of hypertension, bradycardia, and vasoconstriction such that immediately before administration of hUCN2 in the two conditions, cardiovascular variables were not significantly different (Table 3). The hypotensive and mesenteric and hindquarters vasodilator effects of hUCN2 were enhanced equally by l-NAME and by coinfusion of AII with AVP, although the tachycardia was unaffected (compare Figs. 3a and 4). Thus, there were no differences between the cardiovascular effects of hUCN2 in the presence of either l-NAME or AII plus AVP (Fig. 4).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>hUCN2</th>
<th>mUCN2</th>
<th>CRF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td><strong>Heart rate</strong></td>
<td>352 ± 9</td>
<td>365 ± 13</td>
<td>340 ± 11</td>
<td>339 ± 7</td>
</tr>
<tr>
<td><strong>Mean BP (mm Hg)</strong></td>
<td>111 ± 3</td>
<td>109 ± 2</td>
<td>111 ± 2</td>
<td>108 ± 2</td>
</tr>
<tr>
<td><strong>Renal Doppler shift (kHz)</strong></td>
<td>9.1 ± 0.5</td>
<td>8.8 ± 0.6</td>
<td>8.9 ± 0.6</td>
<td>8.8 ± 0.5</td>
</tr>
<tr>
<td><strong>Renal VC (kHz mm Hg⁻¹10⁴)</strong></td>
<td>81 ± 3</td>
<td>80 ± 4</td>
<td>81 ± 4</td>
<td>82 ± 4</td>
</tr>
<tr>
<td><strong>Mesenteric Doppler shift (kHz)</strong></td>
<td>10.8 ± 0.7</td>
<td>10.8 ± 0.7</td>
<td>111 ± 0.8</td>
<td>9.2 ± 0.7</td>
</tr>
<tr>
<td><strong>Mesenteric VC (kHz mm Hg⁻¹10⁴)</strong></td>
<td>98 ± 7</td>
<td>99 ± 6</td>
<td>102 ± 9</td>
<td>86 ± 7</td>
</tr>
<tr>
<td><strong>Hindquarters Doppler shift (kHz)</strong></td>
<td>3.7 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td><strong>Hindquarters VC (kHz mm Hg⁻¹10³)</strong></td>
<td>34 ± 4</td>
<td>43 ± 5</td>
<td>35 ± 1</td>
<td>34 ± 3</td>
</tr>
</tbody>
</table>

VC, vascular conductance.

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Fig. 1. Hemodynamic effects of vehicle (saline; n = 12), CRF (closed circles; n = 8), hUCN2 (open circles; n = 12), and mUCN2 (open squares; n = 12) in conscious Sprague-Dawley rats. Peptides were given as an i.v. bolus dose of 300 pmol kg⁻¹. Values are mean and vertical bars show S.E.M. Statistical comparisons of integrated responses are given in the text.

Fig. 2. Hemodynamic effects of vehicle (saline; n = 12), CRF (closed circles; n = 8), hUCN2 (open circles; n = 12), and mUCN2 (open squares; n = 12) in conscious Sprague-Dawley rats. Peptides were given as an i.v. bolus dose of 3000 pmol kg⁻¹. Values are mean and vertical bars show S.E.M. Statistical comparisons of integrated responses are given in the text.
Indomethacin had no effect on baseline hemodynamic variables, and the cardiovascular effects of hUCN2 were not different in the presence of indomethacin or its vehicle (Fig. 5).

**Experiment 4. Effects of hUCN in the Absence and Presence of SB 209670 and Losartan.** Ninety minutes after the onset of combined administration of SB 209670 with losartan, just before administration of hUCN2, heart rate was higher (362 ± 13 beats min⁻¹), blood pressure was lower (85 ± 5 mm Hg), and regional vascular conductances were higher [renal, 113 ± 8; mesenteric, 138 ± 12; and hindquarters, 48 ± 6 (kHz mm Hg⁻¹)¹⁰³] than at the corresponding time in the presence of saline infusion [334 ± 11 beats min⁻¹, 103 ± 3 mm Hg; 77 ± 7, 74 ± 6, 34 ± 5 kHz mm Hg⁻¹)¹⁰³; P ≤ 0.05]. In the presence of SB 209670 plus losartan, the
The integrated (0- to 30-min) hypotensive and tachycardic effects of hUCN2 were not different to those seen in the presence of saline (Fig. 6). The integrated (0- to 30-min) percentage of increases in mesenteric (+842 ± 156% min) and hindquarters (+1200 ± 214% min) vascular conductances were smaller (P ≤ 0.05) in the presence of SB 209670 plus losartan than in the presence of saline (+1114 ± 169 and +1722 ± 145% min, respectively) (Fig. 6), but this was due to the difference in baseline values since, when expressed in absolute terms, the integrated (0- to 30-min) responses in the presence of saline or SB 209670 plus losartan were not different [mesenteric, +832 ± 159 and +1178 ± 239 (kHz mm Hg$^{-1}$) 10$^3$ min; hindquarters, +587 ± 96 and +533 ± 63 (kHz mm Hg$^{-1}$) 10$^3$ min, respectively].

**Discussion**

The present results indicate that the regional hemodynamic effects of i.v. injection of the CRF$_2$ receptor ligands hUCN2 and mUCN2 strongly resemble those of the CRF, and all are mediated by activation of CRF$_2$ receptors. Hence, the reported CRF$_2$ receptor-mediated changes in gastrointestinal function (Martinez et al., 1999, 2002; Million et al., 2002) and/or pituitary-adrenal activation (Rivier et al., 2003) are not likely to be involved in the cardiovascular effects seen here. In agreement with this suggestion, observations in sheep indicate that CRF-mediated pituitary-adrenal activation has no concomitant hemodynamic effects (Parkes et al., 1997). Furthermore, since central administration of CRF causes pressor and mesenteric vasoconstrictor effects (Grosskreutz and Brody, 1988; Overton and Fisher, 1991), and central administration of hUCN3 causes a rise rather than a fall in blood pressure (Chu et al., 2004), whereas we saw frank hypotension and mesenteric vasodilatation with hUCN2, it is unlikely that centrally mediated effects played a major part in the responses reported here. However, we cannot dismiss the possibility that there was a central component to some of the effects we observed since tachycardia and hindquarters vasodilatation can occur after central, as well as peripheral, administration of CRF (Overton and Fisher, 1991).

The degree of hypotensive effects of hUCN2 and mUCN2 reported here, and the differences in potency between mUCN2 and hUCN2, are consistent with recent studies in conscious (Mackay et al., 2003) and in anesthetized (Chen et al., 2003) rats. Thus, in the study of Mackay and colleagues, a fall in blood pressure of approximately 20 mm Hg was seen after 2.4 nmol kg$^{-1}$ mUCN2, whereas Chen et al. (2003) reported a similar fall in blood pressure after a lower dose.
CRF ligands, although interestingly, the latter became more apparent under conditions where there was increased basal tone (Fig. 4). In vitro, it has been demonstrated that renal artery segments precontracted with endothelin relax in response to UCN1 (Sanz et al., 2003) although, in vivo, the hypotensive effect of UCN1 is not accompanied by renal vasodilation (Abdelrahman and Pang, 2003). One possible explanation for the lack of consistent, overt renal vasodilation with the CRF2 receptor ligands was that compensatory vasoconstrictor mechanisms were activated by the hypotension that overcame any modest, direct renal vasodilator action. However, coadministration of SB 209670 and losartan to inhibit the vasoconstrictor actions of endothelin and angiotensin II, respectively, did not uncover a renal vasodilator effect of hUCN2. It is possible, therefore, that there is less effective coupling of CRF2 receptors in the renal vascular bed than in the mesentery. We know of no studies in which renal and mesenteric vasodilator responses to CRF2 receptor ligands have been compared in vascular preparations isolated from the same animals.

There is clear agreement in the literature that CRF ligands cause mesenteric (Rohde et al., 1996; Barker and Corder, 1999) and coronary (Grunt et al., 1993; Terui et al., 2001; Huang et al., 2002) vasodilation, but there is no consensus with regard to the mediators involved in those responses or the degree of their dependence on the endothelium. For example, Grunt et al. (1993) provided evidence in the isolated rat heart to suggest that the coronary vasodilator action of CRF involved the endothelial release of NO and prostacyclin. In contrast, Terui et al. (2001), using the same preparation but with UCN1 rather than CRF as the agonist showed an involvement of prostanoids but not NO. Furthermore, Huang et al. (2002) reported that NO, but not prostanoids, contributed to the relaxant effects of UCN1 in isolated segments of rat coronary artery.

The picture in the mesenteric circulation is also complex, as illustrated by the findings of Barker and Corder (1999) in rat isolated perfused mesenteric arterial bed. They showed an initial, transient, mesenteric vasodilator response to CRF, or sauvagine, that was unaffected by removal of the endothelium, or by l-NAME, but was slightly enhanced by indomethacin. Thereafter, a persistent mesenteric vasodilatation developed that seemed to involve long-lasting activation of endothelial NO synthase (Barker and Corder, 1999).

Our in vivo results were notable for the lack of involvement of either NO or prostanoids in the vasodilator effects of hUCN2. Thus, the rapid-onset, transient, but marked mesenteric vasodilator response to hUCN2 was unaffected by indomethacin or l-NAME, when baseline effects of l-NAME were allowed for by comparison with hUCN2 given during AII and AVP coinfusion. Interestingly, we saw no secondary, long-lasting, mesenteric vasodilator response of the sort described by Barker and Corder (1999) in vitro. We considered the possibility that activation of endogenous vasoconstrictor mechanisms by the initial hypotension might have limited the mesenteric (and renal, see above) vasodilator effect of hUCN2, but combined inhibition of the vasoconstrictor actions of endothelin and angiotensin II did not enhance but rather diminished hUCN2-induced mesenteric vasodilatation. It is likely that the diminution was because of the vasodilatation caused by SB 209670 and losartan.

Our results showing more marked hypotensive effects of

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Fig. 6. Hemodynamic effects of i.v. administration (3000 pmol kg⁻¹) of hUCN2 in conscious Sprague-Dawley rats, in the presence of i.v. infusion of saline (0.4 ml h⁻¹; n = 8; closed circles) or SB 209670 plus losartan (600 mg kg⁻¹ bolus, 600 mg kg⁻¹ h⁻¹ plus 10 mg kg⁻¹, respectively; n = 8; open circles). Values are mean and vertical bars show S.E.M. (0.6 nmol kg⁻¹) of hUCN2, with a greater fall (47 mm Hg) after a 10-fold higher dose. In our study, hUCN2 caused a greater fall in blood pressure (~30 mm Hg) than mUCN2 (~20 mm Hg) at the highest dose (3 nmol kg⁻¹).

It is notable that i.v. injection of all three peptides caused rapid-onset, but transient, mesenteric vasodilatation, whereas the vasodilatation in the hindquarters was slower to develop and markedly more persistent. Recently, CRF2 receptors associated with skeletal myotubes have been linked to generation of cAMP, and CRF2 receptor ligands have been shown to have trophic effects on skeletal muscle in vivo (Hinkle et al., 2003a,b). This raises the possibility that the gradual onset, prolonged, hyperemic hindquarters vasodilation caused by the UCN2 peptides is secondary to an initial, slow, delayed, prolonged mesenteric vasodilatation (Hinkle et al., 2003a,b). This raises the possibility that the activation of endogenous vasoconstrictor mechanisms by the initial hypotension might have limited the mesenteric vasodilator actions of endothelin and angiotensin II in vivo. Our results showed marked mesenteric vasodilatation but modest, and inconsistent, renal vasodilator actions of the
hUCN2 and mUCN2 than CRF are consistent with the known affinity of these ligands for CRF2 receptors (Hauger et al., 2003). The fact that their apparent potency for eliciting a tachycardic effect did not mirror that for the hypotension probably indicates that the tachycardia was only partly a reflex response to the fall in blood pressure. Others (Parkes et al., 2001) have reported direct cardiac effects of UCN1, including tachycardia.

Two recent articles showed that the hypertensive and tachycardic effects of mUCN2 (Mackay et al., 2003) and hUCN2 (Chen et al., 2003) were abolished by CRF2 receptor antagonism. Those studies corroborated an earlier report that showed that i.v. administration of the CRF2 receptor antagonist K41498 abolished the hypertensive response to i.v. rat UCN1 (Lawrence et al., 2002). Here, we have extended those earlier observations by showing that all the regional hemodynamic effects of the CRF2 receptor ligands, and of CRF, are as effectively abolished by the CRF2 receptor-selective antagonist antisauvagine 30, as by the nonselective CRF receptor antagonist astressin. There were no residual effects of CRF in the presence of antisauvagine 30 that could be attributed to CRF1 receptor-mediated actions. Furthermore, we have now shown that i.v. administration of the antagonists is without effect on any measured hemodynamic variable, supporting the suggestion that the lack of effect on blood pressure indicates a lack of CRF2 receptor-mediated tone (Mackay et al., 2003). Interestingly, even under conditions where others have reported up-regulation of CRF2 receptors in skeletal muscle, i.e., endotoxemia (Heldwein et al., 1997), we have found no hemodynamic effect of i.v. astressin (unpublished observations).

In conclusion, the depressor, tachycardic, and mesenteric and hindquarters vasodilator actions of mUCN2, hUCN2, and CRF depend on activation of CRF2 receptors and do not involve NO or prostanooids. The relative lack of a renal vasodilator response to the peptides is not due to opposing activation of the renin-angiotensin system and endothelin release, and there is no evidence that activation of these counter-regulatory systems limits the mesenteric or hindquarters vasodilator effects. Finally, there is no evidence for endogenous CRF2 receptor-mediated vasodilator tone in vivo, in conscious, unrestrained, normotensive rats.

References
Hauger RL, Grigoriadis DE, Dallman MF, Plotsky PM, Vale WW, and Dautzenberg