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# Regional hemodynamic actions of selective $CRF_2$ receptor ligands in conscious rats

Sheila M Gardiner, Julie E March, Philip A Kemp Anthony P Davenport[1],

Katherine E Wiley[1] & Terence Bennett

Centre for Integrated Systems Biology & Medicine

School of Biomedical Sciences

University of Nottingham

Nottingham NG7 2UH

(SMG, JEM, PAK, TB)

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Corresponding author: Professor SM Gardiner

School of Biomedical Sciences

University of Nottingham NG7 2UH. UK

Tel: 01159709476 Fax: 01159709259

e-mail: sheila.gardiner@nottingham.ac.uk

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AII, angiotensin II; AVP, arginine vasopressin; CRF, corticotropin releasing factor; HDAS, hemodynamic data acquisition system; hUCN2, human urocortin 2; L-NAME, $N^G$ nitro-L-arginine methylester; mUCN2, mouse urocortin 2; NO, nitric oxide; SD, Sprague-Dawley.

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**ABSTRACT**

In conscious, male, Sprague-Dawley rats, we compared regional hemodynamic actions of the selective corticotropin-releasing factor type 2 ($CRF_2$) receptor ligands, human and mouse urocortin 2 (hUCN2, mUCN2), 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 3 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. Pre-treatment with the non-selective CRF receptor antagonist, astressin, or the selective $CRF_2$ receptor antagonist, antisauvagine 30, abolished all the cardiovascular actions of all 3 peptides. Indomethacin had no effect on responses to hUCN2, and there was no evidence for any involvement of 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 $CRF_2$ receptors and do not involve NO or prostanoids.

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There are four members of the mammalian corticotropin-releasing factor (CRF) family. The 41 amino acid peptide, CRF, was isolated and characterised in 1981 (Vale et al., 1981) and is now known to play a pivotal role in neuroendocrine, autonomic, immune and behavioural responses to stress (see review by Grammatopolous and Chrousos, 2002). Subsequently, the 40 amino acid peptide, urocortin (now known as urocortin 1, UCN1), which is the mammalian homologue 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), while 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 quite 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 (see Hauger et al., 2003 for review of structures and recommended nomenclature).

Two types of G protein-coupled CRF receptor have been identified ($CRF_1$ and $CRF_2$), with at least three splice variants of $CRF_2$ (see Grammatopoulos and Chrousos, 2002; Hauger et al., 2003). The receptors show 69% amino acid sequence homology, but differ in tissue distribution and ligand binding. Thus, CRF shows higher affinity for $CRF_1$ than $CRF_2$ receptors, but UCN1 has equal affinity for $CRF_1$ and $CRF_2$ receptors, whereas UCN2 and 3 bind selectively to $CRF_2$ receptors (Hauger et al., 2003).

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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 vasoconstriction at higher doses (Gardiner et al., 1988). However, at that time, it was not known which CRF receptor type was responsible for the effects observed. Since then, evidence has accumulated in favour of the $CRF_2$ receptor being responsible for the hypotensive actions of CRF in rats (see Chen et al., 2003; Mackay et al., 2003), although the regional vascular consequences of selective $CRF_2$ receptor activation in vivo are not known. Therefore, the aims of the present experiments were, in conscious, chronically-instrumented, male Sprague-Dawley (SD) rats:- 1) to characterise the regional hemodynamic profiles of a range of doses of the $CRF_2$ receptor ligands, human and mouse UCN2 (hUCN2, mUCN2), and to compare them with those of CRF given under identical conditions; 2) to determine the effects of the non-selective CRF receptor antagonist, astressin (Gulyas et al., 1995), and the selective $CRF_2$ receptor antagonist, antisauvagine 30 (Rühmann et al., 1998) on responses to the $CRF_2$ receptor-selective agonists and to CRF; 3) with hUCN2 as the exemplar, to assess the possible involvement of nitric oxide (NO) and prostanoids in the vasodilator responses to $CRF_2$ receptor-selective ligands, using the non-selective NO synthase inhibitor, $N^G$ nitro-L-arginine methyl ester (L-NAME), and the cyclo-oxygenase inhibitor, indomethacin, and 4), to determine the extent to which activation of endogenous vasoconstrictor systems counteracted the vasodilator effects of hUCN by measuring the effects of hUCN2 alone or in the presence of endothelin and angiotensin receptor antagonism (with SB 209670 and losartan).

## METHODS

### *Animals and surgical preparation*

Experiments were performed in adult, male, SD rats (380 – 450g) obtained from Charles River (Margate, Kent. U.K). Animals were housed in the Biomedical Services Unit for at least 10 days after delivery before any surgical interventions took place. Room temperatures were maintained at 21 ± 2°C, there was a 12h light/dark cycle (06.00h to 18.00h), 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, 300μg $kg^{-1}$ of each i.p.). Anesthetic reversal and the provision of analgesia was 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 re-anesthetised (as 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 Licence authority.

### *Cardiovascular recordings*

Cardiovascular recordings began on the day following catheterisation, 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 (HDAS), 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 (HVPD-20) modules).

***Experimental protocols***

*Experiment 1. Regional hemodynamic effects of increasing doses of hUCN2, mUCN2 or CRF*

Rats (n=12) were randomised to receive bolus i.v. injections (0.1ml) of either hUCN2 or mUCN2 (3, 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 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 re-administered 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*

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Rats (n=8) were given 3000 pmol $kg^{-1}$ hUCN2 90min 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 co-infusion 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 (10mM $Na_2$ $CO_3$) on Day 1, and 90 min after the onset of administration of indomethacin (5mg $kg^{-1}$ $h^{-1}$ 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 90min 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 HDAS every 2ms, averaged each cardiac cycle and stored to disc every 5s. Offline, data were analysed (Datview, University of 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 prior to administration of the peptide, across 20s epochs around 1, 2, 3, 4, and 5 min after drug administration, and thereafter across 1-2 min epochs around 10, 20, 25 and 30min for the low doses (3 and 30 p mol $kg^{-1}$), a further 40, 50 60 min for the 300 pmol $kg^{-1}$ dose, and additionally at 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 non-parametric equivalent of ANOVA (Friedman's test), (Theodorsson-Norheim, 1987). Between-group analyses were performed on the integrated responses measured over the first 30min following peptide administration, using Wilcoxon's test, or Mann-Whitney U test, as appropriate. $P \leq 0.05$ 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 Associates, Barnet, UK). Angiotensin II, arginine vasopressin, astressin and antisauvagine 30 were from Bachem (St Helens, UK), L-NAME ($N^G$ nitro-L-arginine methyl ester) was from Sigma (Poole, Dorset, UK) and indomethacin was from Merck Biosciences Ltd (Nottingham, UK). SB 209670 ([(+)-(1S, 2R, 3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-13,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SKB, U.S.A.). Losartan potassium was a gift from Dr. R.D. Smith (DuPont, U.S.A.). All drugs were dissolved in sterile saline with the exception of indomethacin which was dissolved in 10mM sodium carbonate. Stock solutions of peptides were made up in sterile water for injection, and diluted in sterile saline. Injection volumes were 0.1ml and infusion rates were 0.4ml $h^{-1}$.

Fentanyl citrate was from Janssen-Cilag (High-Wycombe, UK); medetomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were from Pfizer (Sandwich, Kent, UK); nalbuphine hydrochloride (Nubain) was from Bristol-Myers-Squibb (Hounslow, UK).

**RESULTS**

*Experiment 1. Regional hemodynamic effects of increasing doses* of *hUCN2, mUCN2 or CRF*

Resting cardiovascular variables prior to 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 hUCN2, mUCN2 or CRF at 3 and 30 pmol $kg^{-1}$ (data not shown).

At 300 pmol $kg^{-1}$, all 3 peptides caused tachycardia, increases in mesenteric Doppler shift and vascular conductance, and increases in hindquarters vascular conductance (Figure 1). The integrated (0-30 min) increases in mesenteric vascular conductance in response to hUCN2 (+418 ± 62 % min) and mUCN2 (+397 ± 58 % min) were greater ($P \leq 0.05$) than those to CRF (+277 ± 82 % min), and there was an accompanying fall blood pressure with hUCN2 and mUCN2 which did not occur with CRF (Figure 1). The integrated tachycardic effect of 300 pmol $kg^{-1}$ hUCN2 (+797 ± 172 beats) was greater ($P \leq 0.05$) than that of mUCN2 (+334 ± 127 beats) and CRF (+455 ± 142 beats).

At a dose of 3000 pmol $kg^{-1}$, all 3 peptides caused hypotension, tachycardia, and hyperemic vasodilatations in the mesenteric and hindquarters vasculature, accompanied by falls in renal Doppler shift and biphasic changes in renal vascular conductance, with short-lived vasodilatation giving way to vasoconstriction (Figure 2). The integrated (0-30min) hypotensive effect of hUCN2 (-770 ± 30 mmHg min) was greater ($P \leq 0.05$) than that of mUCN2 (-387 ± 63 mmHg min) which was greater ($P \leq 0.05$) than that of CRF (-274 ± 77 mmHg 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 ($P \leq 0.05$) between CRF and hUCN2. The integrated (0-30 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 ($P \leq 0.05$) smaller (+780 ± 125 % min). The integrated (0-30 min) increase in hindquarters vascular conductance in response to hUCN2 (+1760 ± 326 % min) was greater ($P \leq 0.05$) than that of mUCN2 (+1018 ± 142 % min) and CRF (+926 ± 191 % min). In the renal vascular bed, the integrated (0-30min) 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 ($P \leq 0.05$) with CRF (+13±4 % min), whereas the fall in vascular conductance, which occurred later, was greater ($P \leq 0.05$) 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 Figure 2 with Figures 3a-c). There were no cardiovascular changes associated with administration of either astressin or antisauvagine 30, hence cardiovascular variables immediately prior to 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 (Figures 3a-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 prior to administration of hUCN2 in the 2 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 co-infusion of AII with AVP although the tachycardia was unaffected (compare Figures 3a & 4). Thus, there were no differences between the cardiovascular effects of hUCN2 in the presence of either L-NAME or AII plus AVP (Figure 4).

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 (Figure 5).

*Experiment 4. Effects of hUCN in the absence and presence of SB 209670 and losartan*

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

159 and + 1178 ± 239 [kHz $mmHg^{-1}$] $10^3$ min, hindquarters +587 ± 96 and +533 ± 63 [kHz $mmHg^{-1}$] $10^3$ 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 $CRF_1$ 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 (e.g., 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 following 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 anesthetised (Chen et al., 2003) rats. Thus, in the study of Mackay and colleagues, a fall in blood pressure of approximately 20mmHg was seen following 2.4nmol$kg^{-1}$ mUCN2, whereas Chen et al. (2003) reported a similar fall in blood pressure following a lower dose (0.6nmol$kg^{-1}$) of hUCN2, with a greater fall (47mmHg) following a 10-fold higher dose. In our study, hUCN2 caused a greater fall in blood pressure (~30 mmHg) than mUCN2 (~20mmHg) at the highest dose (3nmol $kg^{-1}$).

It is notable that i.v. injection of all 3 peptides caused rapid-onset, but transient, mesenteric vasodilatation, whereas the vasodilatation in the hindquarters was slower to develop and markedly more persistent. Recently, $CRF_2$ receptors associated with skeletal myotubes have been linked to generation of cAMP, and $CRF_2$ 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, hyperaemic hindquarters vasodilatation caused by the UCN2 peptides is secondary to an initial metabolic effect. Hinkle et al (2003a, b) suggested that $CRF_2$ receptor ligands may be useful in promoting skeletal muscle growth in wasting conditions, but our results indicate that the anabolic effects may be inseparable from the hemodynamic effects – a situation analogous to that seen with the $\beta_2$-adrenoceptor agonist, clenbuterol (Sleeper et al., 2002).

Our results showed marked mesenteric vasodilatation, but modest, and inconsistent, renal vasodilator actions of the CRF ligands, although interestingly, the latter became more apparent under conditions where there was increased basal tone (see Figure 4). In vitro, it has been demonstrated that renal artery segments pre-contracted with endothelin relax in response to UCN1 (Sanz et al., 2003), although, in vivo, the hypotensive effect of UCN1 is not accompanied by renal vasodilatation (Abdelrahman & Pang, 2003). One possible explanation for the lack of consistent, overt renal vasodilatation with the $CRF_2$ receptor ligands was that compensatory vasoconstrictor mechanisms were activated by the hypotension which overcame any modest, direct renal vasodilator action. However, co-administration 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 $CRF_2$ receptors in the renal vascular bed than in the mesentery. We know of no studies in which renal and mesenteric vasodilator

responses to $CRF_2$ 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 & Corder, 1999) and coronary (Grunt et al., 1993; Terui et al., 2001; Huang et al., 2002) vasodilatation, 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 & 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, which appeared to involve long-lasting activation of endothelial NO synthase (Barker & 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 co-infusion. Interestingly, we saw no secondary, long-lasting, mesenteric vasodilator response of the sort described by Barker & 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 hUCN2 and mUCN2 than CRF are consistent with the known affinity of these ligands for $CRF_2$ 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 papers showed that the hypotensive and tachycardic effects of mUCN2 (Mackay et al., 2003) and hUCN2 (Chen et al., 2003) were abolished by $CRF_2$ receptor antagonism. Those studies corroborated an earlier report which showed that i.v. administration of the CRF2 receptor antagonist, K41498, abolished the hypotensive 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 $CRF_2$ receptor ligands, and of CRF, are as effectively abolished by the $CRF_2$ receptor-selective antagonist, antisauvagine 30, as by the non-selective CRF receptor antagonist, astressin. There were no residual effects of CRF in the presence of antisauvagine 30 which could be attributed to $CRF_1$-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 $CRF_2$ receptor-mediated tone (Mackay et

al., 2003). Interestingly, even under conditions where others have reported up-regulation of $CRF_2$ receptors in skeletal muscle, i.e., endotoxaemia (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 prostanoids. 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 $CRF_2$ receptor-mediated vasodilator tone in vivo, in conscious, unrestrained, normotensive rats.

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**FOOTNOTES**

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Some of these results have been presented to the British Pharmacological Society.

http://www.pa2online.org/Vol1Issue4abst019P.html

b) Reprint requests to Professor SM Gardiner, School of Biomedical Sciences, Floor E, Medical School, University of Nottingham NG7 2UH. UK

c) [1] Clinical Pharmacology Unit, University of Cambridge, Centre for Clinical Investigation, Addenbrooke's Hospital, Cambridge CB2 2QQ

**Figure legends**

**Figure 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.

**Figure 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.

**Figure 3**. Hemodynamic effects of i.v. administration (3000 pmol $kg^{-1}$) of hUCN2 (a, left hand panels), mUCN2 (b, middle panels) and CRF (c, right hand panels), in the absence (closed circles) or in the presence of primed i.v. infusion (50 μg $kg^{-1}$ bolus, 50μg $kg^{-1}$ $h^{-1}$ infusion) of either astressin (open circles) or antisauvagine 30 (open squares), in conscious Sprague-Dawley rats. Values are mean and vertical bars show S.E.M.

**Figure 4**. 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 L-NAME (3 mg $kg^{-1}$ $h^{-1}$, n=8, open circles), or AII plus AVP (200 and 20 ng $kg^{-1}$ $h^{-1}$, respectively, n=8, closed circles) to match the hemodynamic effects of L-NAME. Values are mean and vertical bars show S.E.M.

**Figure 5.** 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 indomethacin (5 mg $kg^{-1}$

$h^{-1}$, n=8, open circles) or vehicle (10mM $Na_2CO_3$ at 0.4 ml $h^{-1}$, n=8, closed circles). Values are mean and vertical bars show S.E.M.

**Figure 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 (0.4ml $h^{-1}$, 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.

Table 1. Resting cardiovascular variables (Experiment 1)

| | Saline | hUCN2 | mUCN2 | CRF |
|---|---|---|---|---|
| | n=12 | n=12 | n=12 | n=8 |
| Heart rate (beats $min^{-1}$) | 352±9 | 365±13 | 340±11 | 339±7 |
| Mean BP (mmHg) | 111±3 | 109±2 | 111±2 | 108±2 |
| Renal Doppler Shift (kHz) | 9.1±0.5 | 8.8±0.6 | 8.9±0.6 | 8.8±0.5 |
| Renal VC ([kHz $mmHg^{-1}$]$10^3$) | 81±3 | 80±4 | 81±4 | 82±4 |
| Mesenteric Doppler Shift (kHz) | 10.8±0.7 | 10.8±0.7 | 11.1±0.8 | 9.2±0.7 |
| Mesenteric VC ([kHz $mmHg^{-1}$]$10^3$) | 98±7 | 99±6 | 102±9 | 86±7 |
| Hindquarters Doppler Shift (kHz) | 3.7±0.4 | 4.6±0.4 | 3.8±0.1 | 3.6±0.3 |
| Hindquarters VC ([kHz $mmHg^{-1}$]$10^3$) | 34±4 | 43±5 | 35±1 | 34±3 |

Values (mean ± S.E.M.) are those obtained prior to administration of the first dose of the peptide or saline.

VC=vascular conductance.

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Table 2. Resting cardiovascular variables (Experiment 2)

| | hUCN2 | mUCN2 | CRF |
|---|---|---|---|
| | n=10 | n=8 | n=8 |
| Heart rate (beats $min^{-1}$) | 334±8 | 322±9 | 336±11 |
| Mean BP (mmHg) | 104±2 | 102±2 | 103±4 |
| Renal Doppler Shift (kHz) | 6.6±0.5 | 6.8±0.5 | 7.4±0.3 |
| Renal VC ([kHz $mmHg^{-1}$]$10^3$) | 63±4 | 67±4 | 72±3 |
| Mesenteric Doppler Shift (kHz) | 7.4±0.5 | 7.5±0.5 | 6.4±0.8 |
| Mesenteric VC ([kHz $mmHg^{-1}$]$10^3$) | 72±6 | 73±5 | 62±7 |
| Hindquarters Doppler Shift (kHz) | 3.9±0.4 | 3.9±0.5 | 4.1±0.6 |
| Hindquarters VC ([kHz $mmHg^{-1}$]$10^3$) | 38±3 | 39±5 | 40±5 |

Values (mean ± S.E.M.) are those obtained on Day 1 prior to administration of the peptide. VC=vascular conductance.

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Table 3. Baseline cardiovascular variables (Experiment 3)

| | -L-NAME | +L-NAME | -AII+AVP | +AII+AVP |
|---|---|---|---|---|
| | n=8 | n=8 | n=8 | n=8 |
| Heart rate (beats $min^{-1}$) | 324±11 | 281±16* | 346±8 | 322±13* |
| Mean BP (mmHg) | 100±2 | 137±8* | 103±3 | 134±3* |
| Renal Doppler Shift (kHz) | 8.0±0.5 | 6.3±0.9* | 9.0±1.0 | 8.2±1.0* |
| Renal VC ([kHz $mmHg^{-1}$]$10^3$) | 79±4 | 47±7* | 88±11 | 61±8* |
| Mesenteric Doppler Shift (kHz) | 7.5±0.6 | 4.7±0.5* | 8.2±0.4 | 5.5±0.4* |
| Mesenteric VC ([kHz $mmHg^{-1}$]$10^3$) | 76±7 | 36±5* | 80±5 | 42±4* |
| Hindquarters Doppler Shift (kHz) | 3.3±0.2 | 2.5±0.3* | 3.6±0.3 | 3.5±0.3 |
| Hindquarters VC ([kHz $mmHg^{-1}$]$10^3$) | 33±1 | 19±2* | 35±4 | 27±3* |

Values (mean ± S.E.M.) are those obtained before and 90min after the onset of infusion of L-NAME or angiotensin plus vasopressin (AII+AVP). VC=vascular conductance. * denotes a significant change within the group (Wilcoxon's test)

Figure 1

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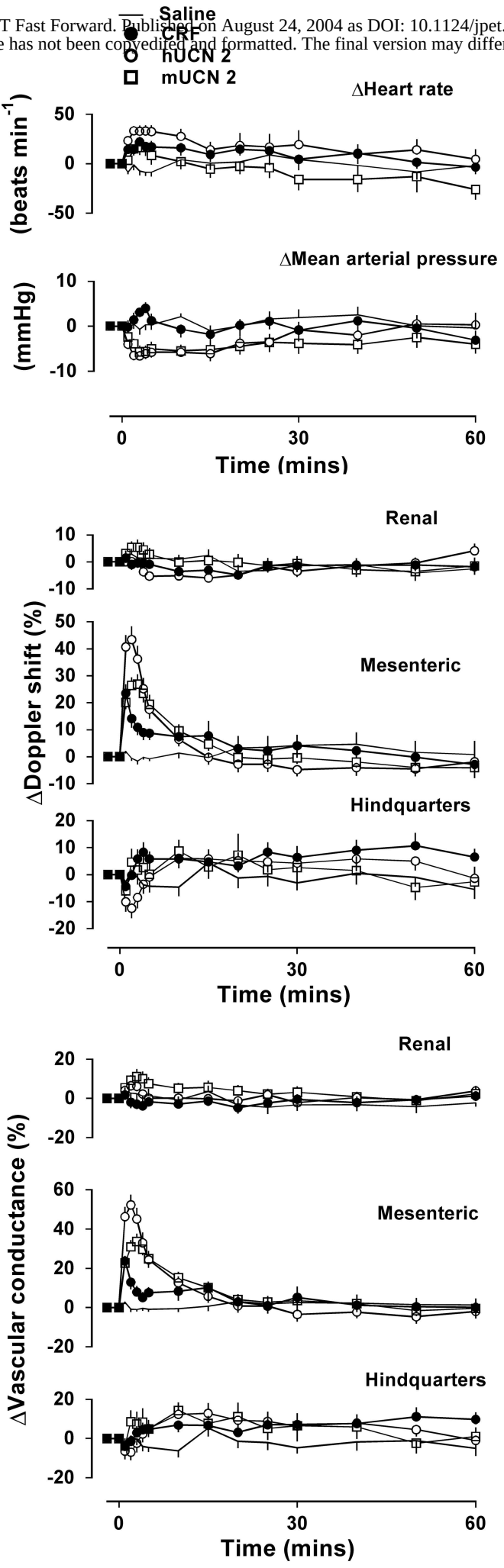

Figure 2

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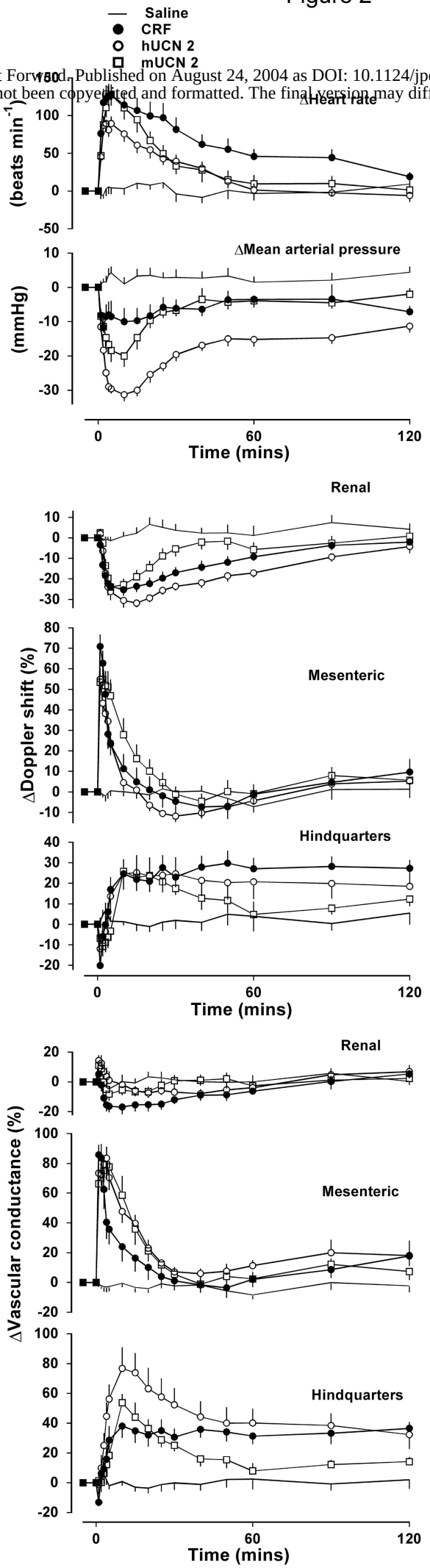

Figure 3

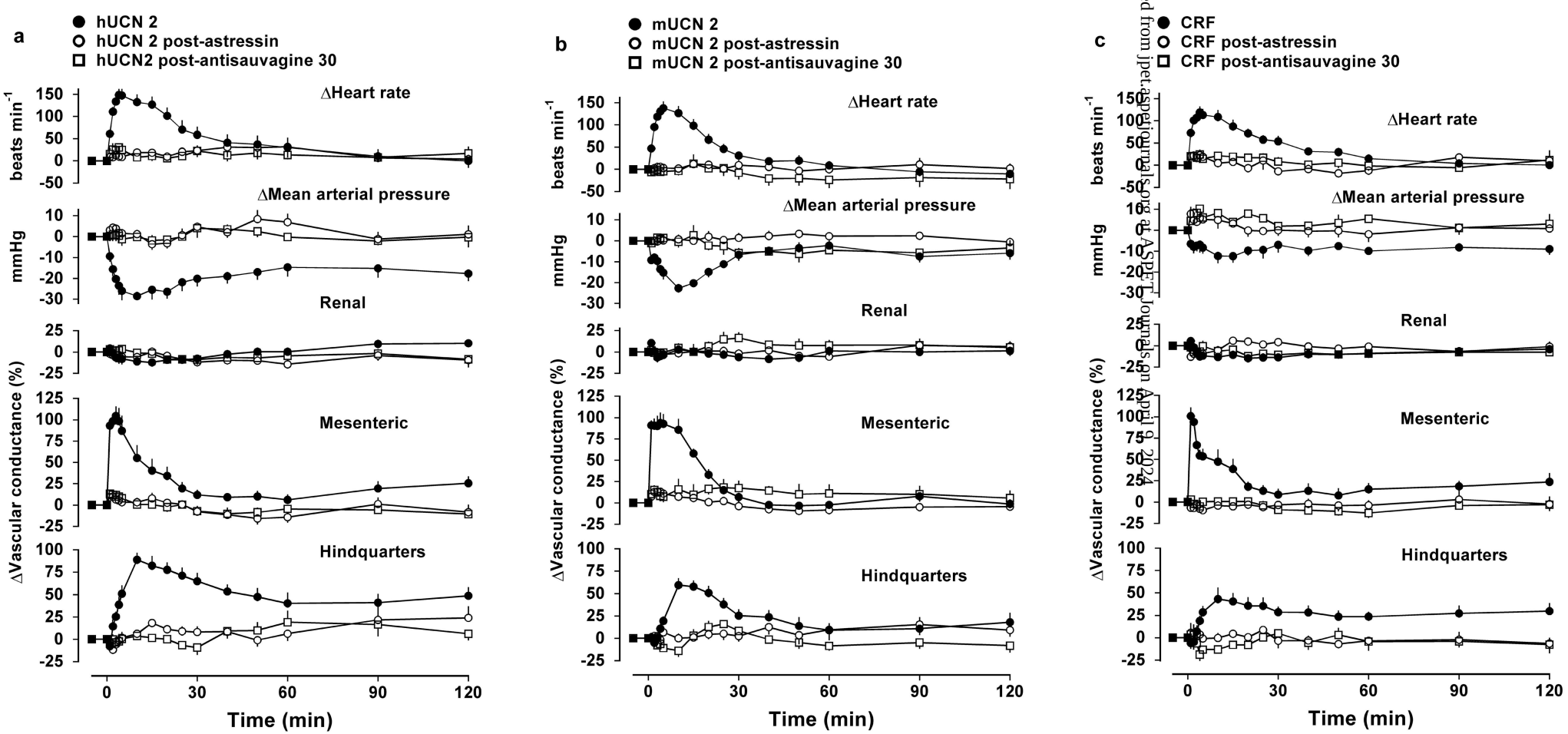

Figure 4

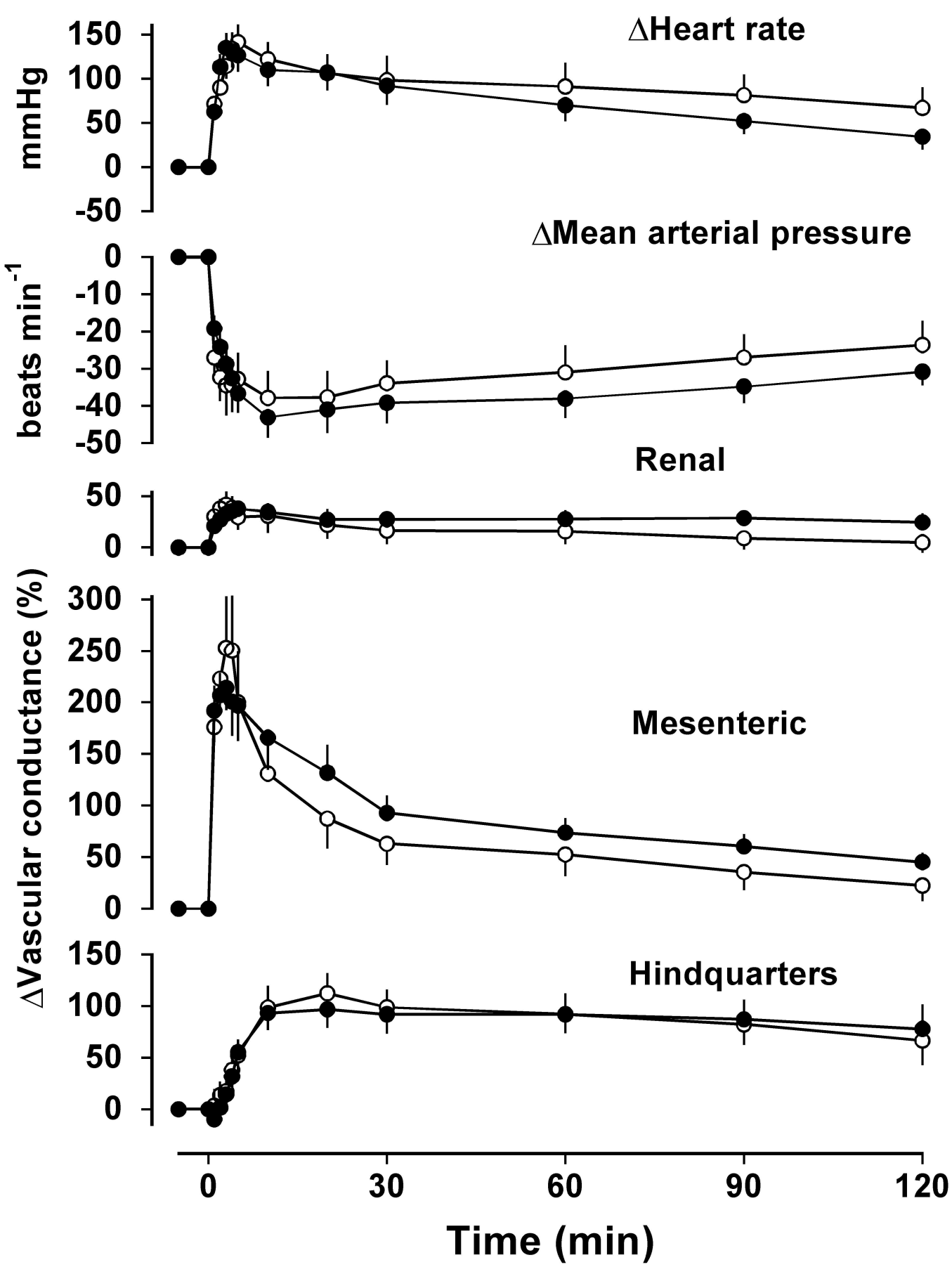

Figure 5

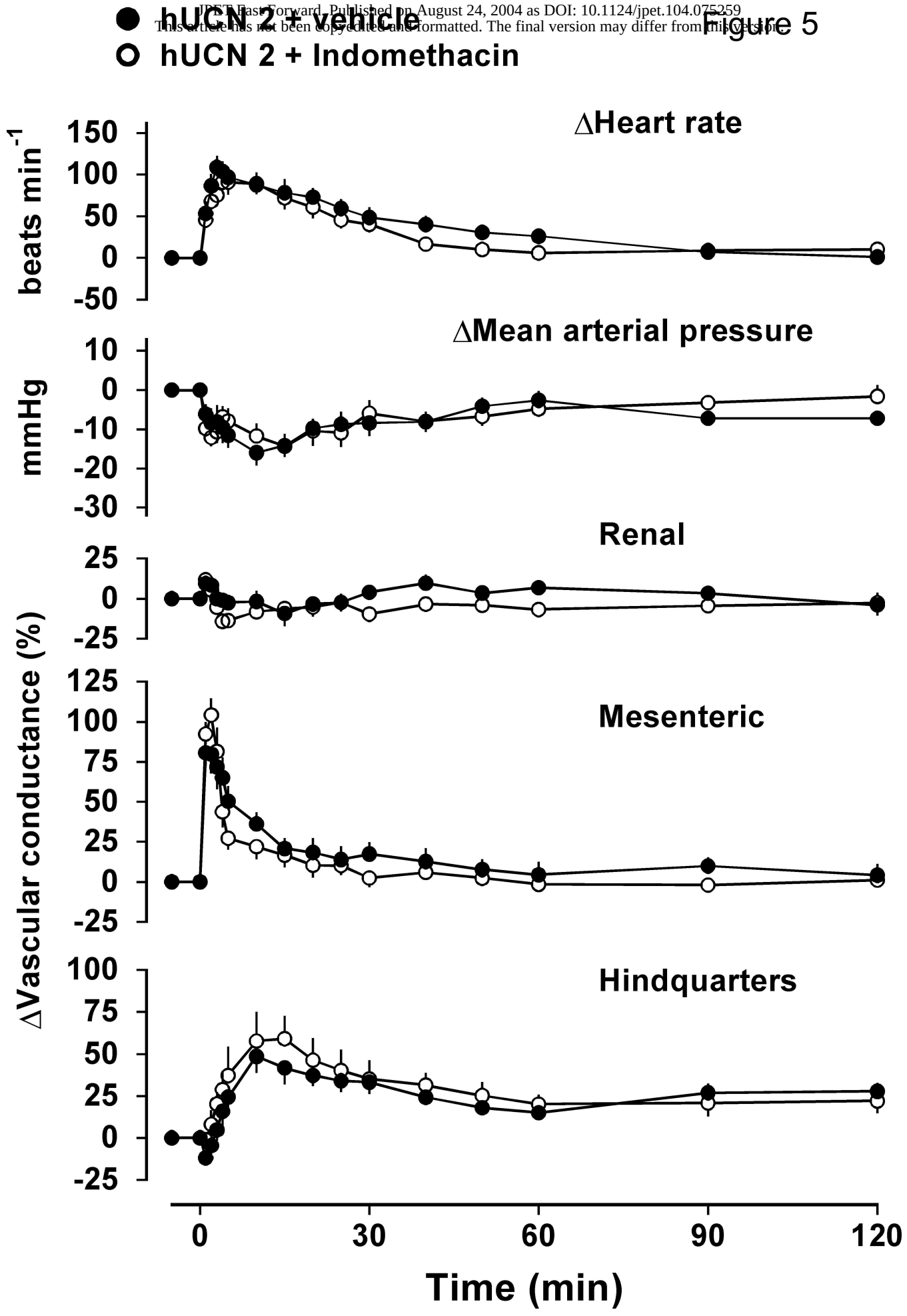

Figure 6