British Journal of Pharmacology (2006) 147, 612–621
www.nature.com/bjp

Regional heterogeneity in the haemodynamic responses to urotensin II infusion in relation to UT receptor localisation

*,[1]Sheila M. Gardiner, [1]Julie E. March, [1]Philip A. Kemp, [2]Janet J. Maguire, [2]Rhoda E. Kuc, [2]Anthony P. Davenport & [1]Terence Bennett

[1]Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, University of Nottingham, Nottingham NG7 2UH and [2]Clinical Pharmacology Unit, University of Cambridge, Cambridge

1 The aim of the study was to measure regional haemodynamic responses to 6 h infusions of human urotensin II (hUII), to identify possible mediators of the effects observed, and to relate the findings to the distribution of urotensin II receptors (UT receptors).

2 Male, Sprague–Dawley rats had pulsed Doppler flow probes and intravascular catheters implanted for measurement of regional haemodynamics in the conscious, freely moving state. Infusions of saline ($0.4\,ml\,h^{-1}$) or hUII (30, 300 and $3000\,pmol\,kg^{-1}\,h^{-1}$) were given i.v. for 6 h, and the effects of pretreatment with indomethacin ($5\,mg\,kg^{-1}\,h^{-1}$), N^G-nitro-L-arginine methyl ester (L-NAME, $3\,mg\,kg^{-1}\,h^{-1}$) or propranolol ($1\,mg\,kg^{-1}$; $0.5\,mg\,kg^{-1}\,h^{-1}$) on responses to hUII ($300\,pmol\,kg^{-1}\,h^{-1}$ for 6 h) were assessed. Cellular localisation of UT receptor-like immunoreactivity was determined in relevant tissues.

3 hUII caused dose-dependent tachycardia and hindquarters vasodilatation, accompanied by a slowly developing rise in blood pressure. Haemodynamic effects of hUII were attenuated by propranolol or L-NAME and abolished by indomethacin. UT receptor-like immunoreactivity was detected in skeletal and vascular smooth muscle.

4 The findings indicate that in conscious rats, infusions of hUII cause vasodilatation, which, of the vascular beds monitored, is selective for the hindquarters and dependent on cyclooxygenase products and nitric oxide. The pressor effect of hUII under these conditions is likely to be due to an increase in cardiac output, possibly due to a positive inotropic effect. UT receptor-like immunoreactivity present in skeletal muscle is consistent with the haemodynamic pattern.
British Journal of Pharmacology (2006) **147,** 612–621. doi:10.1038/sj.bjp.0706503;
published online 28 November 2005

Keywords: Urotensin II; rats; haemodynamics; UT receptors

Abbreviations: BP, mean arterial blood pressure; HR, heart rate; hUII, human urotensin II; L-NAME, N^G-nitro-L-arginine methyl ester; PBS, phosphate-buffered saline; UT receptor, urotensin II receptor

Introduction

Human urotensin II (hUII) has been identified as an endogenous ligand for the G protein-coupled receptor 14, and shown to have marked cardiovascular actions *in vitro* and *in vivo*, although the literature on the peptide to date is distinguished by the marked variability in the effects observed (for reviews see Maguire & Davenport, 2002; Douglas, 2003).

The widespread distribution of UII mRNA and urotensin II receptor (UT receptor) mRNA has been taken to indicate a possible endocrine role for the peptide in cardiovascular regulation (Thanassoulis *et al*., 2004), although others (Clozel *et al*., 2004) have suggested that it may act in a more autocrine/paracrine manner. Raised plasma levels of hUII have now been measured in a variety of pathological conditions (see Douglas, 2003), but whether or not chronic elevation of circulating levels of the peptide has a cardiovascular impact is unknown. With one very recent exception (Kompa *et al*., 2004), all published studies involving systemic administration of hUII in experimental animals have used bolus injections of hUII (Ames *et al*., 1999; Gardiner *et al*., 2001; Abdelrahman & Pang, 2002; Hassan *et al*., 2003; Watson *et al*., 2003; Zhu *et al*., 2004), and the experiments in man involved only short-term (20 min) infusions (Affolter *et al*., 2002).

Therefore, the aim of the present study was to define the regional haemodynamic effects of longer-term (6 h) intravenous infusions of a range of doses of hUII in conscious rats. Since those studies showed marked, dose-dependent hindquarters vasodilator responses to the peptide (see Results), we also assessed the effects of pretreatment with indomethacin or N^G-nitro-L-arginine methyl ester (L-NAME) on responses to hUII infusion, because we (Gardiner *et al*., 2004) have recently shown that the hindquarters vasodilator response to bolus injection of hUII is blocked by indomethacin and L-NAME. Since others have shown sympathoadrenal activation following central administration of hUII (Watson *et al*., 2003), we also assessed the possible involvement of β-adrenoceptor activation in the effects of hUII infusion. Furthermore, to determine if the regional heterogeneity in the haemodynamic response to hUII was a reflection of the distribution of UT receptors, we determined their localisation in cardiovascular tissues and skeletal muscle, using immunocytochemistry.

*Author for correspondence;
E-mail: sheila.gardiner@nottingham.ac.uk

Methods

Animals and surgical preparation

Male, Sprague–Dawley rats (400–450 g) were obtained from Charles River U.K., and were housed in the Biomedical Services Unit at Nottingham for at least 10 days after delivery before any surgical interventions took place. The procedures were approved by the University of Nottingham Ethical Review Committee, were performed under Home Office Project Licence authority, and conformed to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Surgery was performed in two stages under general anaesthesia (fentanyl and medetomidine, 300 $\mu g\,kg^{-1}$ of each i.p.). Anaesthetic reversal and the provision of analgesia was achieved using atipamezole and nalbuphine, respectively (1 $mg\,kg^{-1}$ of each s.c.). In the first stage, miniaturised 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, under anaesthesia (as above), catheters were implanted in the distal abdominal aorta (*via* the ventral caudal artery), for monitoring mean arterial blood pressure (BP) and heart rate (HR), and in the right jugular vein for the administration of substances. Three separate catheters were placed in the jugular vein for independent administration of substances. The fitness of the animals between surgical stages was certified by the Named Veterinary Surgeon.

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*.

Cardiovascular recordings

Continuous recordings of cardiovascular variables (HR, arterial blood pressure, renal, mesenteric and hindquarters Doppler shifts (flow)) were made using a customized, computer-based system (Haemodynamics Data Acquisition System (HDAS), University of Limburg, Maastricht, The Netherlands) connected to a transducer amplifier (Gould model 13-4615-50) and a Doppler flowmeter (Crystal Biotech VF-1 mainframe (pulse repetition frequency 125 kHz) fitted with high velocity (HVPD-20) modules). Raw data were sampled by HDAS every 2 ms, averaged every cardiac cycle, and stored to disc at 5 s intervals. Data were analysed offline using software (Datview, University of Limburg, Maastricht, The Netherlands), which interfaced with HDAS.

Experimental protocols

Experiment 1: effects of 6 h infusions of different doses of hUII Rats were randomised to receive a 6 h i.v. infusion of saline (0.4 $ml\,h^{-1}$, $n=8$) or hUII (0.4 $ml\,h^{-1}$) at a dose of 30 ($n=9$), 300 ($n=9$) or 3000 ($n=8$) $pmol\,kg^{-1}\,h^{-1}$.

Experiment 2: effects of indomethacin on responses to hUII infusion On the first experimental day, rats ($n=8$) were given indomethacin vehicle (10 mM Na_2CO_3) as a continuous infusion (0.4 $ml\,h^{-1}$) starting 90 min before the onset of a 6 h infusion of hUII (300 $pmol\,kg^{-1}\,h^{-1}$; 0.4 $ml\,h^{-1}$). This dose of hUII was chosen on the basis of the results of Experiment 1 which showed it to be effective, but submaximal. After a 24 h wash-out period, on the third experimental day, the same animals were given a continuous infusion of indomethacin (5 $mg\,kg^{-1}\,h^{-1}$) starting 90 min before infusion of hUII (300 $pmol\,kg^{-1}\,h^{-1}$). The dose of indomethacin has been shown to be effective elsewhere (Gardiner *et al*., 1990b; 2004).

As time controls, rats ($n=4$) were given 6 h infusions of saline (0.4 $ml\,h^{-1}$) in the presence of vehicle (Day 1) and in the presence of indomethacin (Day 3).

Experiment 3: effects of L-NAME on responses to hUII infusion On the first experimental day, rats ($n=8$) were given the vehicle for L-NAME (saline) as a continuous infusion (0.4 $ml\,h^{-1}$) starting 90 min before the onset of a 6 h infusion of hUII (300 $pmol\,kg^{-1}\,h^{-1}$, 0.4 $ml\,h^{-1}$). After a 24 h wash-out period, on the third experimental day, the same animals were given a continuous infusion of L-NAME (3 $mg\,kg^{-1}\,h^{-1}$) starting 90 min before infusion of hUII (300 $pmol\,kg^{-1}\,h^{-1}$). The dose of L-NAME has been shown to inhibit vasodilator responses to acetylcholine *in vivo* in Sprague–Dawley rats (Wakefield *et al*., 2003).

As time controls, rats ($n=6$) were given 6 h infusions of saline in the presence of vehicle (Day 1) and in the presence of L-NAME (Day 3).

Experiment 4: effects of propranolol on responses to hUII infusion Separate groups of rats were given a 6 h infusion of hUII (300 $pmol\,kg^{-1}\,h^{-1}$, 0.4 $ml\,h^{-1}$) starting 30 min after the onset of treatment with propranolol (1 $mg\,kg^{-1}$ bolus, 0.5 $mg\,kg^{-1}\,h^{-1}$ infusion; $n=6$) or saline (0.1 ml bolus, 0.4 $ml\,h^{-1}$ infusion; $n=4$). The dose of propranolol has been shown to effectively antagonise responses to isoprenaline (Woolard *et al*., 2004).

Cardiovascular data analysis

We have previously applied tests of normality to data of this sort (Kolmogorov–Smirnov and Shapiro–Wilk; SPSS version 11.0 for Windows) and the only cardiovascular variable which usually shows a Gaussian distribution is BP. Therefore, we have used nonparametric statistics, since this is a more cautious approach when the majority of the data are not normally distributed. However, the graphical presentation is given in a form (mean values with s.e.m.), which is the most commonly used and, therefore, most easily understood by the readership. Within-group analyses were carried out using a nonparametric equivalent of ANOVA (Friedman's test) with correction for multiple comparisons (Theodorsson-Norheim, 1987), or Wilcoxon's test, as appropriate. Between-group (unpaired) analyses were performed using the Mann–Whitney *U*-test (two groups) or Kruskal–Wallis test (more than two groups). Significance was accepted if $P<0.05$. The primary analysis was performed on the integrated areas under/over the curves (0–360 min) relative to the appropriate time control, that is, in the absence of hUII. If a significant difference was found, differences from baseline were tested to determine the time course of changes.

Immunocytochemistry

Male Sprague–Dawley rats (350–400 g, $n=4$) were rendered unconscious with CO_2, and killed by exsanguination. Based on

the cardiovascular responses obtained in Experiment 1, relevant organs, that is, hindquarters skeletal muscle (gastrocnemius), heart, kidney and mesentery were removed, frozen, and cryostat sections (30 μm) were cut onto poly-L-lysine-coated microscope slides. These were air-dried and fixed by immersion in ice-cold acetone for 10 min. Tissue sections were incubated for 2 h, at 23°C, in 5% swine serum in phosphate-buffered saline (PBS), followed by incubation at 4°C with rabbit-anti-UT receptor primary antiserum raised against a sequence in the 3rd cytoplasmic domain of the human UT receptor (Lifespan Biosciences Inc., Suffolk, U.K.), at 1 : 100 (for 72 h) and 1 : 50 (for 48 h) dilutions in PBS containing 3% swine serum and 0.1% Tween-20. Adjacent sections were incubated without primary antiserum as negative controls. Tissue sections were washed, and specific staining revealed using the peroxidase antiperoxidase method, with diaminobenzidine as the chromogenic substrate (Kuc, 2002). Following alcoholic dehydration, slides were cleared in xylene, mounted using a coverslip and permanent-mounting medium and examined using a standard bright field microscope. Images were captured using a U-TV1-X digital camera (Olympus U.K. Ltd, London, U.K.).

Drugs and reagents

Fentanyl citrate was from Janssen-Cilag (High Wycombe, U.K.); medetomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were from Pfizer (Sandwich, Kent, U.K.); nalbuphine hydrochloride (Nubain) was from Bristol Myers Squibb (Hounslow, U.K.). Human UII was purchased from Peptide Institute Inc. (Scientific Marketing Associates, Barnet, U.K.). N^G-nitro-L-NAME was purchased from Sigma (Dorset, U.K.). Indomethacin was purchased from Merck Biosciences Ltd (Nottingham, U.K.). Propranolol ((*RS*)-1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol) hydrochloride was purchased from Tocris (Avonmouth, U.K.). All drugs and peptides were dissolved in sterile saline with the exception of indomethacin, which was dissolved in 10 mM Na_2CO_3. Bolus injections were in a volume of 0.1 ml and infusions were at a rate of 0.4 ml h^{-1}. Rabbit-anti-human UT receptor primary antiserum was from Lifespan Biosciences Inc. (Suffolk, U.K.) and was reconstituted in PBS. Other reagents for immunocytochemistry were from DakoCytomation (Ely, U.K.), Sigma-Aldrich Ltd (Dorset, U.K.) or VWR International Ltd (Poole, U.K.).

Results

Cardiovascular studies

Experiment 1: effects of 6 h infusions of different doses of hUII Resting cardiovascular variables in the four groups of rats prior to the onset of infusions of saline or hUII are shown in Table 1. There were no significant differences (Kruskal–Wallis test).

In rats infused with the lowest dose of hUII (30 pmol kg^{-1} h^{-1}), the integrated (0–360 min) changes in all cardiovascular variables were not different from those in the group infused with saline, whereas in rats infused with hUII at 300 and 3000 pmol kg^{-1} h^{-1}, the integrated (0–360 min) changes in HR, BP, renal Doppler shift and hindquarters Doppler shift and vascular conductance were significantly ($P<0.05$, Kruskal–Wallis test) different from the saline control. Within-group analyses (Friedman's test) showed that with the 300 pmol kg^{-1} h^{-1} dose, the haemodynamic changes were slow to develop but not sustained, whereas with the 3000 pmol kg^{-1} h^{-1} dose, the tachycardia and increase in hindquarters vascular conductance were sustained and the pressor effect was biphasic (Figure 1).

The integrated (0–360 min) tachycardia (24,114 $\pm$ 2480 beats) and increases in hindquarters Doppler shift (24,075 $\pm$ 3515% min) and vascular conductance (20,203 $\pm$ 2805% min) in response to hUII at 3000 pmol kg^{-1} h^{-1} were significantly ($P<0.05$ Kruskal–Wallis test) greater than the corresponding changes following hUII at 300 pmol kg^{-1} h^{-1} (11,586 $\pm$ 2221 beats, 9608 $\pm$ 2603% min and 6843 $\pm$ 2310% min, respectively). Although the time course of BP change differed (see above), there was no difference between the integrated pressor responses to hUII at 300 and 3000 pmol kg^{-1} h^{-1} (2873 $\pm$ 307 and 2818 $\pm$ 489 mmHg min, respectively).

Experiment 2: effects of indomethacin on responses to hUII infusion Resting cardiovascular variables prior to administration of hUII in the presence of vehicle or indomethacin are shown in Table 2. There were no significant differences (Wilcoxon's test). In animals receiving indomethacin or its vehicle in the presence of a 6 h saline infusion, there were no consistent cardiovascular changes (data not shown).

In the presence of vehicle, infusion of hUII (300 pmol kg^{-1} h^{-1}) caused effects as described above, that is, integrated (0–360 min) rises in HR, BP, renal Doppler shift

Table 1 Resting cardiovascular variables (Experiment 1)

	Saline (n = 8)	*hUII 30 (n = 9)*	*hUII 300 (n = 9)*	*hUII 3000 (n = 8)*
Heart rate (beats min^{-1})	349 $\pm$ 13	333 $\pm$ 8	339 $\pm$ 7	338 $\pm$ 8
Mean BP (mmHg)	105 $\pm$ 1	107 $\pm$ 7	105 $\pm$ 2	110 $\pm$ 2
Renal DS (kHz)	10.5 $\pm$ 0.7	8.8 $\pm$ 0.4	9.2 $\pm$ 0.7	8.8 $\pm$ 0.9
Mesenteric DS (kHz)	10.8 $\pm$ 0.8	10.3 $\pm$ 0.7	10.5 $\pm$ 0.8	11.0 $\pm$ 0.8
Hindquarters DS (kHz)	4.5 $\pm$ 0.4	3.3 $\pm$ 0.4	4.0 $\pm$ 0.6	3.4 $\pm$ 0.4
Renal VC (units)	99 $\pm$ 6	85 $\pm$ 6	87 $\pm$ 7	81 $\pm$ 8
Mesenteric VC (units)	103 $\pm$ 8	99 $\pm$ 9	100 $\pm$ 8	100 $\pm$ 7
Hindquarters VC (units)	43 $\pm$ 4	32 $\pm$ 4	38 $\pm$ 5	31 $\pm$ 4

DS = Doppler shift; VC = vascular conductance.
Units for VC are (kHz $mmHg^{-1}$) 10^3.
Values are mean $\pm$ s.e.m.

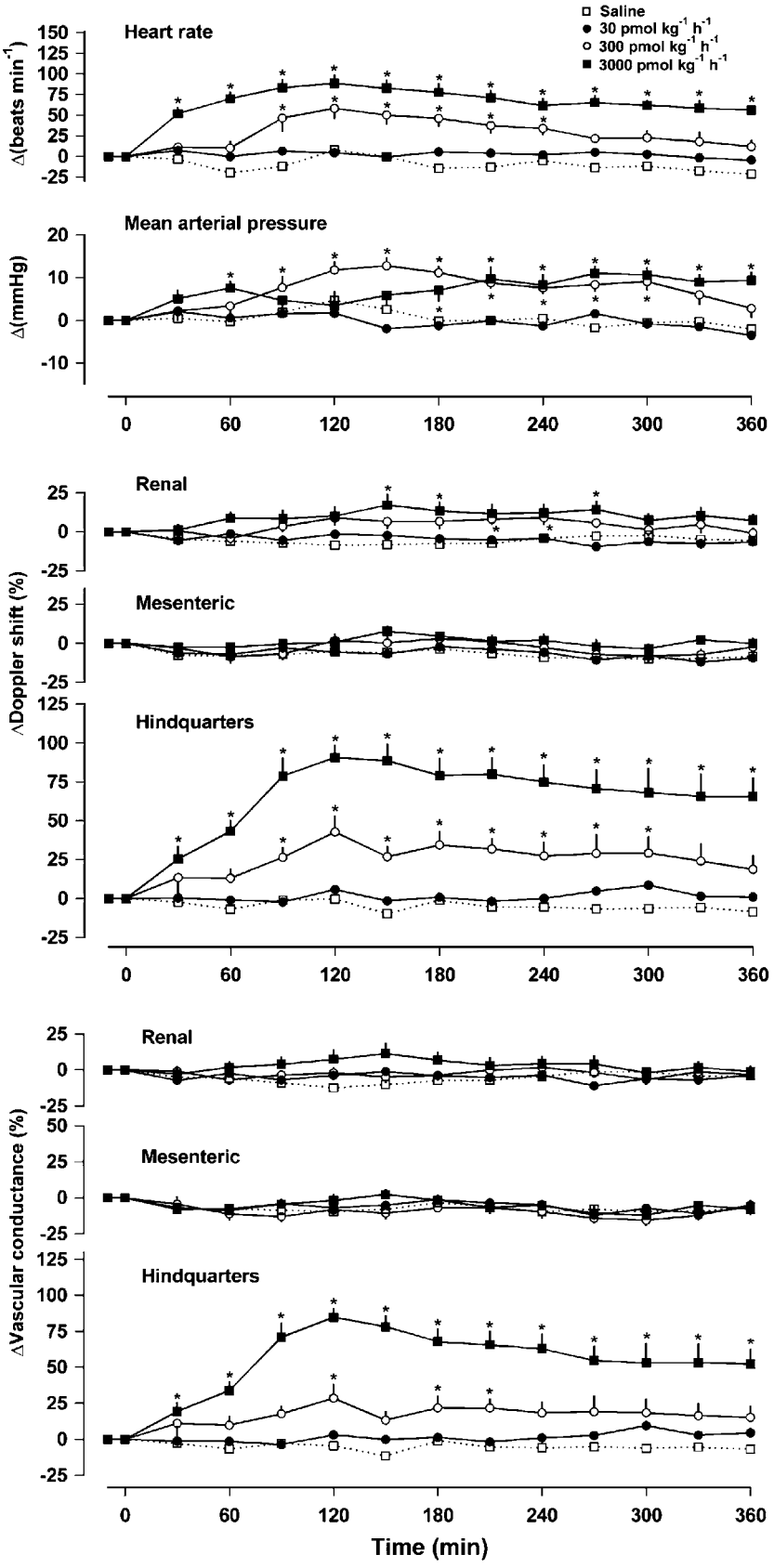

Figure 1 Haemodynamic responses to i.v. infusions of saline (dotted line, $n=8$) or human UII at 30 (filled circles, $n=9$), 300 (open circles, $n=9$) and 3000 (filled squares, $n=8$) pmol kg^{-1} h^{-1} for 6 h in conscious rats. Values are mean and vertical bars represent s.e.m. For clarity, data points have been omitted from the saline group, and error bars omitted from the saline and 30 pmol kg^{-1} h^{-1} groups. *$P<0.05$ vs baseline (Friedman's test).

Table 2 Cardiovascular variables prior to hUII infusion in Experiments 2, 3 and 4

	Expt 2 (n=8) Vehicle	*Expt 2 (n=8) Indomethacin*	*Expt 3 (n=8) Saline*	*Expt 3 (n=8) L-NAME*	*Expt 4 (n=6) Saline*	*Expt 4 (n=6) Propranolol*
Heart rate (beats min^{-1})	327±5	308±7	322±6	285±7[a]	328±9	333±8
Mean BP (mm Hg)	106±2	102±2	104±2	125±3[a]	105±5	109±4
Renal DS (kHz)	8.4±0.7	8.1±0.8	7.2±1.2	6.7±1.0	8.8±1.1	10.0±0.8
Mesenteric DS (kHz)	6.6±0.5	6.1±0.6	6.7±0.7	5.1±0.7[a]	6.2±0.6	7.0±0.6
Hindquarters DS (kHz)	4.0±0.3	3.6±0.4	4.7±0.5	3.1±0.3[a]	4.4±0.6	4.4±0.4
Renal VC (units)	79±7	80±8	69±11	53±8[a]	84±10	91±9
Mesenteric VC (units)	61±4	60±7	65±7	41±6[a]	59±7	64±6
Hindquarters VC (units)	38±3	37±4	46±6	25±3[a]	42±4	40±4

All values are mean±s.e.m. for measurements 90 min after the onset of treatment, immediately prior to administration of hUII. DS = Doppler shift; VC = vascular conductance. Units for VC are (kHz mmHg^{-1})10^3.
Rats were given vehicle on Day 1 and indomethacin (Experiment 2), L-NAME (Experiment 3) or propranolol (Experiment 4) on Day 3.
[a]$P \leqslant 0.05$ vs Day 1 (Wilcoxon's test).

and hindquarters Doppler shift and vascular conductance which were significantly ($P<0.05$, Mann–Whitney test) different from the time control (i.e. indomethacin vehicle in the absence of hUII). As before, the effects on HR and BP were not sustained, but in this group of animals, the hindquarters vasodilatation was still significant at the end of the 6 h period of infusion. Indomethacin had no significant effect on resting cardiovascular variables (Table 2), but inhibited all the cardiovascular effects of hUII infusion (Figure 2).

Experiment 3: effects of L-NAME on responses to hUII infusion Resting cardiovascular variables prior to administration of hUII in the presence of saline or L-NAME are shown in Table 2. Administration of L-NAME caused bradycardia, a rise in BP and vasoconstriction in all three vascular beds (Table 2).

In the presence of saline, hUII (300 pmol kg^{-1} h^{-1}) caused tachycardia, a rise in BP, an increase in renal Doppler shift and hyperaemic hindquarters vasodilatation, all of which differed from the saline-infused control group (Mann–Whitney test) and were generally sustained in this group of rats (Figure 3). In the presence of L-NAME, the integrated (0–360 min) tachycardic (11,002±2508 beats) and pressor responses (4413±1106 mmHg min) to hUII were smaller ($P<0.05$) than in the presence of saline (18,869±3676 beats, 5675±931 mmHg) and the hindquarters vasodilatation was abolished (Figure 3).

In animals receiving a 6 h infusion of saline in the presence of L-NAME, cardiovascular variables at the start and end of the saline infusion were (HR 274±9 and 298±6 beats min^{-1}, BP 122±4 and 125±3 mmHg, renal vascular conductance 36±5 and 43±5, mesenteric vascular conductance 30±2 and 31±4, hindquarters vascular conductance 23±2 and 18±2 (kHz mmHg^{-1})10^3, respectively). Only the small further reduction in hindquarters vascular conductance was significant.

Experiment 4: effects of propranolol on responses to hUII infusion Resting cardiovascular variables prior to administration of hUII in the presence of saline or propranolol are shown in Table 2. Propranolol had no significant effect on resting cardiovascular variables (Table 2). The effects of hUII infusion in the animals infused with saline in this experiment were similar to those described above. In the presence of propranolol vs saline, the integrated (0–360 min) increases in HR (9374±2174 vs 18,810±5585 beats) BP (2656±979 vs 4986±706 mmHg min) and hindquarters Doppler shift (5890±1656 vs 16,181±2615% min) and vascular conductance (4704±1884 vs 11,627±2349% min) were significantly ($P<0.05$, Mann–Whitney test) reduced (Figure 4).

Immunocytochemistry

In rat tissues, UT receptor-like immunoreactivity was localised to skeletal muscle fibres (Figure 5a). Positive immunoreactivity was also present on vascular smooth muscle, for example of artery and vein associated with skeletal muscle (Figure 5a).

Discussion

The present experiments provide the first description of the regional haemodynamic effects of continuous, 6 h infusions of hUII in conscious, chronically instrumented rats. The results show that, during continuous infusion of the peptide, there was dose-related tachycardia and an increase blood flow velocity (Doppler shift) which was slow to develop and, of the vascular beds monitored, was confined to the hindquarters. In addition, there was a modest pressor effect which also developed slowly (after 90–120 min infusion) in the rats receiving the middle dose of hUII, and even more slowly (after about 180 min) in the rats receiving the high dose infusion. Changes in blood flow velocity are a good index of changes in blood flow, providing that the vessel diameter underneath the flow probe does not change, as is the case under the conditions of chronic implantation in our experimental paradigm. Using the change in Doppler shift as an index of blood flow, there was shown to be a calculated, selective, increase in hindquarters vascular conductance, that is, vasodilatation. Since others have reported hUII-induced thoracic aortic constriction *in vitro* in rats (Douglas *et al*., 2000), an hypothetical possibility is that constriction of the thoracic aorta occurred in our experiments, and this produced an increase in blood flow velocity immediately downstream of the site of constriction. However, we believe it is most unlikely that thoracic aortic constriction could explain the selective increase in hindquarters Doppler shift seen with hUII infusion since (i) such an effect would not be localised to one vascular bed and (ii) it would be difficult to explain the inhibitory effects of propranolol, L-NAME and indomethacin (see below).

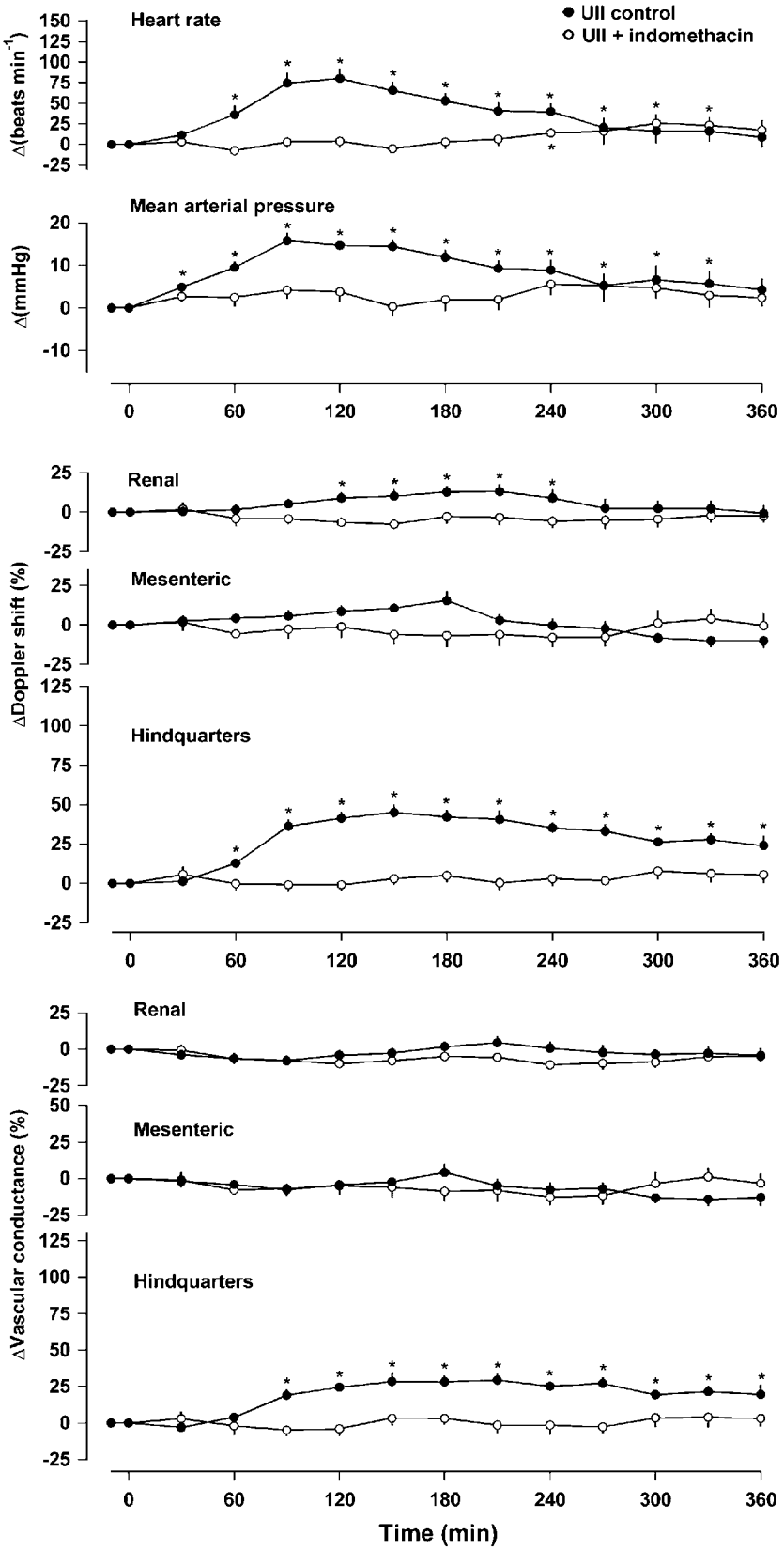

Figure 2 Haemodynamic responses to i.v. infusion of human UII (300 pmol kg^{-1} h^{-1}) in the presence of indomethacin (5 mg kg^{-1} h^{-1}, open circles) or its vehicle (10 nM Na_2CO_3 at 0.4 ml h^{-1}, filled circles) in conscious rats ($n=8$). Values are mean and vertical bars represent s.e.m. *$P<0.05$ vs baseline (Friedman's test).

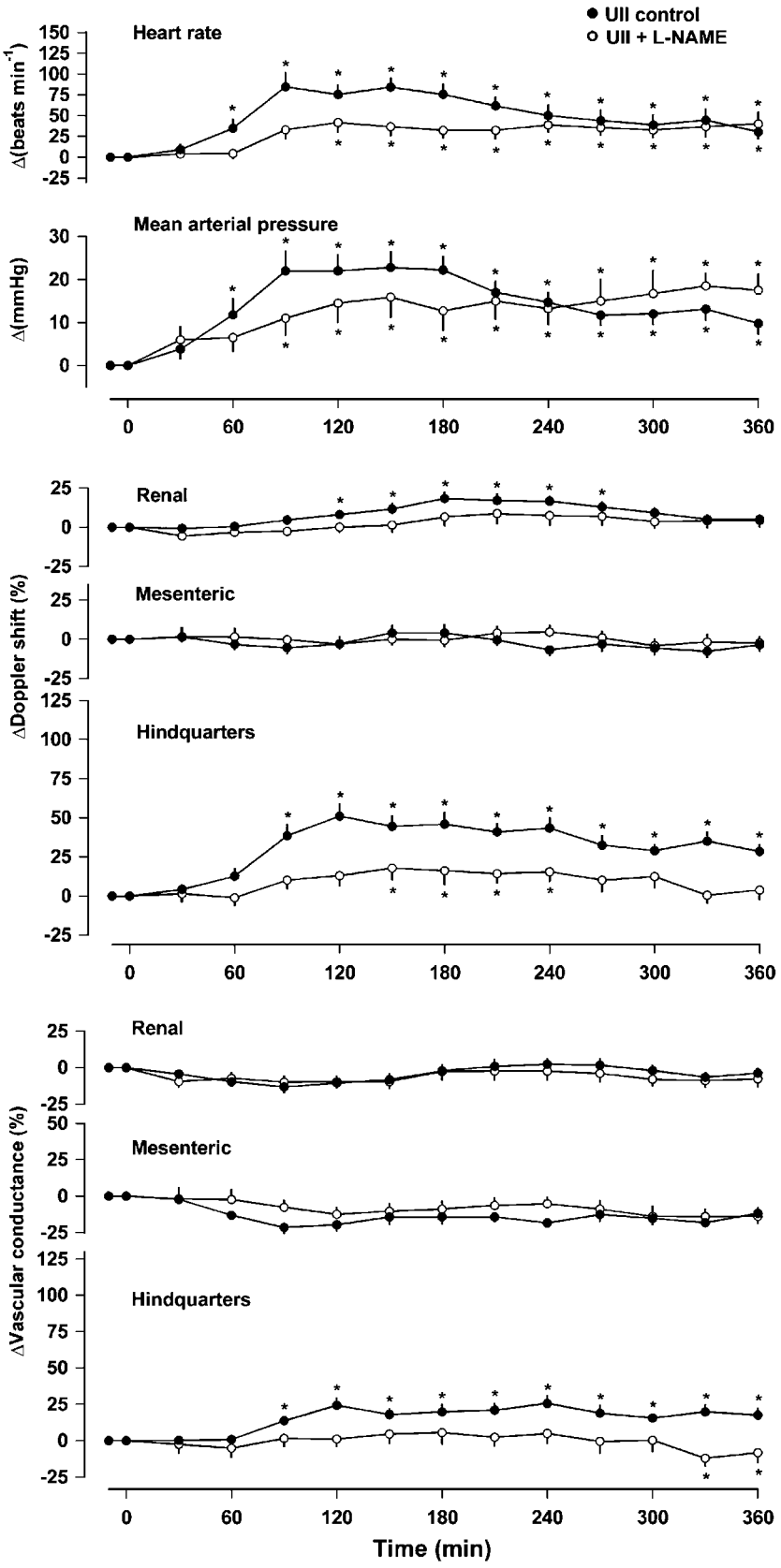

Figure 3 Haemodynamic responses to i.v. infusion of human UII (300 pmol kg^{-1} h^{-1}) in the presence of L-NAME (3 mg kg^{-1} h^{-1}, open circles) or its vehicle (saline at 0.4 ml h^{-1}, filled circles) in conscious rats ($n=8$). Values are mean and vertical bars represent s.e.m. *$P<0.05$ vs baseline (Friedman's test).

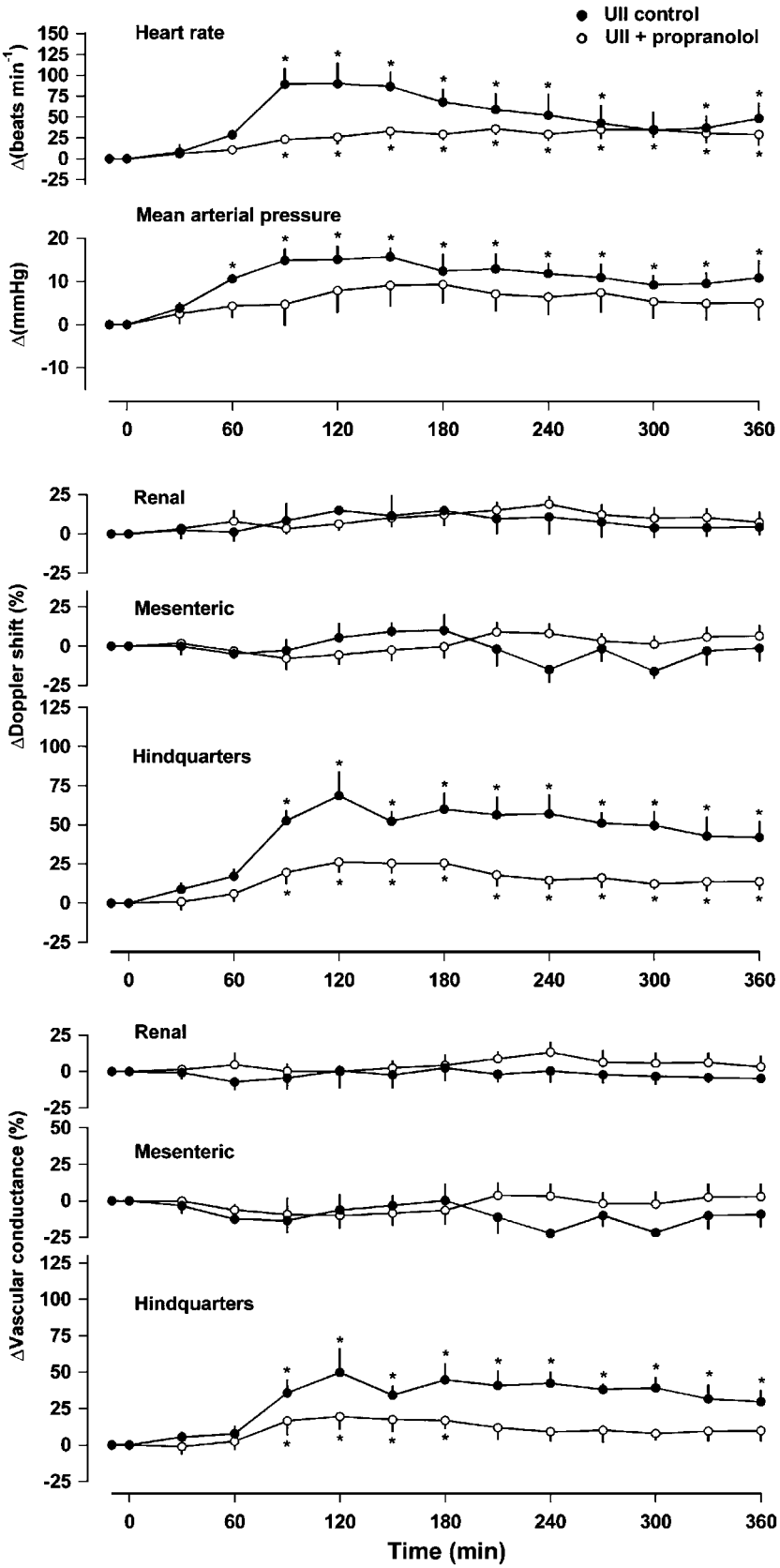

Figure 4 Haemodynamic responses to i.v. infusion of human UII (300 pmol kg^{-1} h^{-1}) in the presence of propranolol (1 mg kg^{-1}, 0.5 mg kg^{-1} h^{-1}, open circles; $n=6$) or its vehicle (saline, 0.1 ml, 0.4 ml h^{-1}, filled circles; $n=4$) in conscious rats. Values are mean and vertical bars represent s.e.m. *$P<0.05$ vs baseline (Friedman's test).

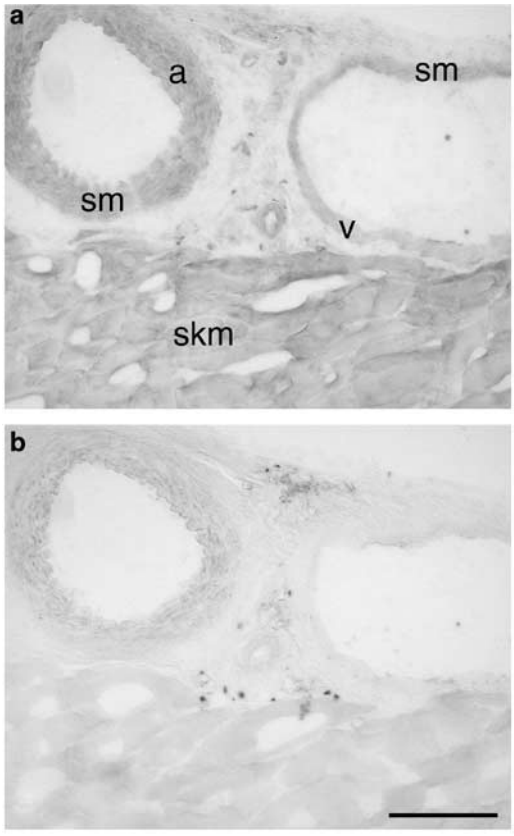

Figure 5 Light photomicrographs showing localisation of UT receptor-like immunoreactivity to (a) a transverse section through rat skeletal muscle (skm) and to the smooth muscle (sm) layer of an associated artery (a) and vein (v). (b) Negative control in which the UT receptor antiserum was omitted in an adjacent section of skeletal muscle. Scale bar = 200 μm.

The relationship between the hindquarters vascular conductance changes and the effects of hUII on BP is consistent with the rise in BP being due to an increase in cardiac output, and the hindquarters vasodilatation opposing this effect. Thus, it is feasible that, with the middle dose infusion of hUII, the smaller rise in hindquarters vascular conductance allowed a cardiac output-dependent pressor effect to occur sooner than with the higher dose because the latter caused a much greater increase in hindquarters vascular conductance, and, therefore, a rise in BP was apparent only as the hindquarters vasodilatation waned. Although we did not measure cardiac output directly, it was notable that the pressor effect was more apparent in systolic than in diastolic blood pressure (S.M. Gardiner, J.E. March, P.A. Kemp & T. Bennett, unpublished observations). A similar observation was made in earlier experiments with hUII given as a bolus dose (Gardiner *et al*., 2004), and is consistent with a positive inotropic effect of the peptide reported by others *in vitro* (Russell *et al*., 2001).

Watson *et al*. (2003) showed that central administration of hUII in conscious sheep caused activation of sympathoadrenal and pituitary–adrenal pathways. They observed marked adrenaline release, and suggested this accounted for the significant increase in cardiac output, resulting in the rise in BP, because there was concurrent dilatation in renal, mesenteric, and iliac vascular beds, which they suggested were in part due to baroreflex-mediated withdrawal of vasoconstrictor tone, with a possible contribution from adrenaline to the iliac vasodilatation. Whether or not peripherally administered hUII activates the central nervous system in rats is not known, but clearly, the time taken for the effect of the infusion of hUII to become apparent is not inconsistent with this being secondary to some other peripheral event and/or an action within the central nervous system. Furthermore, the ability of propranolol to attenuate the cardiovascular responses is consistent with an involvement of sympathoadrenal activation in the effects we observed.

Indomethacin largely abolished the significant effects of hUII infusion, consistent with these involving cyclooxygenase-dependent processes. However, L-NAME also markedly attenuated the cardiovascular effects of hUII infusion, suggesting an involvement of NO-mediated effects. This situation is analogous to our previous study (Gardiner *et al*., 2004) where we showed effects of either indomethacin or L-NAME on the delayed phase of the cardiovascular response to a bolus injection of hUII, and is consistent with interactions between prostanoid production and NO release. Since central sympathoadrenal activity can be modulated by arachidonic acid metabolites (Yokotani *et al*., 2001), the finding that propranolol also attenuated that cardiovascular responses to hUII infusion is not inconsistent with the possibility that the initiating process involves stimulation of cyclooxygenase, with activation of NO and sympathoadrenal systems occurring secondarily.

Some vasodilator mechanisms in skeletal muscle are thought to involve the local release of substances from skeletal muscle fibres and endothelial cells, in addition to those released from red blood cells (Stamler & Meissner, 2001; Clifford & Hellsten, 2004). The presence of UT receptor-like immunoreactivity in the skeletal muscle, consistent with the recent report of functional UT receptors in human skeletal muscle myoblasts (Qi *et al*., 2005), suggests that the marked hindquarters vasodilator response to infused hUII may be due, at least in part, to activation of UT receptors on skeletal muscle fibres.

The absence of a mesenteric hyperaemic vasodilator response to infusion of hUII, in the present experiments, is notable in the context of the ability of this peptide to cause rapid-onset and substantial increases in mesenteric flow and vascular conductance when it is administered as a bolus (see Gardiner *et al*., 2001; 2004). Such a phenomenon is, to some extent, reminiscent of the effects of endothelin on the hindquarters vasculature, inasmuch as a rapid bolus injection of endothelin causes vasodilatation, whereas infusion produces vasoconstriction (Gardiner *et al*., 1990a). However, in the case of endothelin, the initial hindquarters vasodilator response to bolus injection is followed by a developing vasoconstriction, whereas bolus injection of hUII does not cause any delayed mesenteric vasoconstriction (Gardiner *et al*., 2001; 2004). Furthermore, it was notable that when the vasodilator effects of hUII infusion were pharmacologically inhibited, there was no vasoconstriction unmasked in any vascular bed. Indeed, in a small group of animals ($n = 4$) we assessed the effects of the highest dose of hUII infusion (3000 pmol kg^{-1} h^{-1}) for 6 h in the presence of indomethacin, and still there was no sign of vasoconstriction (S.M. Gardiner, J.E. March, P.A. Kemp, T. Bennett unpublished data). Thus, while it is tempting to speculate that some of the apparent discrepancies in the literature regarding vasoconstrictor vs vasodilator effects of hUII may be due to differences in the functional state of the

endothelium, it seems clear that, in normal rats, systemic administration of hUII does not cause vasoconstriction in the vascular beds we monitored, despite the demonstrated presence of UT receptor-like immunoreactivity localised to the smooth muscle of skeletal muscle blood vessels.

In conclusion, these *in vivo* experiments which failed to uncover any peripheral vasoconstrictor action of hUII, are consistent with the literature on *in vitro* effects of hUII in rats which, for the most part, have only shown constriction in the thoracic aorta, with endothelium-dependent vasodilatation in some small resistance arteries (Bottrill *et al*., 2000; Douglas *et al*., 2000).

This work was funded by the British Heart Foundation.

References

ABDELRAHMAN, A.M. & PANG, C.C.Y. (2002). Involvement of the nitric oxide/L-arginine and sympathetic nervous systems on the vasodepressor action of human urotensin II in anesthetized rats. *Life Sci.,* **71,** 819–825.

AFFOLTER, J.T., NEWBY, D.E., WILKINSON, I.B., WINTER, M.J., BALMENT, R.J. & WEBB, D.J. (2002). No effect on central or peripheral blood pressure of systemic urotensin II infusion in humans. *Br. J. Clin. Pharmacol.,* **54,** 617–621.

AMES, R.S., SARAU, H.M., CHAMBERS, J.K., WILLETTE, R.N., AIYAR, N.V., ROMANIC, A.M., LOUDEN, C.S., FOLEY, J.J., SAUERMEICH, C.F., COATNEY, R.W., AO, Z., DISA, J., HOLMES, S.D., STADEL, J.M., MARTIN, J.D., LIU, W.-S., GLOVER, G.I., WILSON, S., MCNULTY, D.E., ELLIS, C.E., ELSHOURBAGY, N.A., SHABON, U., TRILL, J.J., HAY, D.W.P., OHLSTEIN, E.H., BERGSMA, D.J. & DOUGLAS, S.A. (1999). Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. *Nature,* **401,** 282–286.

BOTTRILL, F.E., DOUGLAS, S.A., HILEY, C.R. & WHITE, R. (2000). Human urotensin-II is an endothelium-dependent vasodilator in rat small arteries. *Br. J. Pharmacol.,* **130,** 1865–1870.

CLIFFORD, P.S. & HELLSTEN, Y. (2004). Vasodilatory mechanisms in contracting skeletal muscle. *J. Appl. Physiol.,* **97,** 393–403.

CLOZEL, M., BINKERT, C., BIRKER-ROBACZEWSKA, M., BOUKHADRA, C., DING, S.-S., FISCHLI, W., HESS, P., MATHYS, B., MORRISON, K., MÜLLER, C., MÜLLER, C., NAYLER, O., QUI, C., REY, M., SCHERZ, M.W., VELKER, J., WELLER, T., XI, J.-F. & ZILTENER, P. (2004). Pharmacology of the urotensin-II receptor antagonist palosuran (ACT-058362: 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulphate salt): first demonstration of a pathophysiological role of the urotensin system. *J. Pharmacol. Exp. Ther.,* **311,** 204–212.

DOUGLAS, S.A. (2003). Human urotensin-II as a novel cardiovascular target: 'heart' of the matter or simply a fishy 'tail'? *Curr. Opin. Pharmacol.,* **3,** 159–167.

DOUGLAS, S.A., SULPIZIO, A.C., PIERCY, V., SARAU, H.M., AMES, R.S., AIYAR, N.V., OHLSTEIN, E.H. & WILLETTE, R.N. (2000). Differential vasoconstrictor activity of human urotensin-II in vascular tissue isolated from the rat, mouse, dog, pig, marmoset and cynomolgus monkey. *Br. J. Pharmacol.,* **131,** 1262–1274.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990a). Regional haemodynamic effects of endothelin-1 and endothelin-3 in conscious Long Evans and Brattleboro rats. *Br. J. Pharmacol.,* **99,** 107–112.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990b). Effect of indomethacin on the regional haemodynamic responses to low doses of endothelins and sarafotoxin. *Br. J. Pharmacol.,* **100,** 158–162.

GARDINER, S.M., MARCH, J.E., KEMP, P.A., DAVENPORT, A.P. & BENNETT, T. (2001). Depressor and regionally-selective vasodilator effects of human and rat urotensin II in conscious rats. *Br. J. Pharmacol.,* **132,** 1625–1629.

GARDINER, S.M., MARCH, J.E., KEMP, P.A. & BENNETT, T. (2004). Bolus injection of human UII in conscious rats evokes a biphasic haemodynamic response. *Br. J. Pharmacol.,* **143,** 422–430.

HASSAN, G.S., CHOUIALI, F., SAITO, T., HU, F., DOUGLAS, S.A., AO, Z., AO, Z., WILLETTE, R.N., OHLSTEIN, E.H. & GIAID, A. (2003). Effect of human urotensin-II infusion on haemodynamics and cardiac function. *Can. J. Physiol. Pharmacol.,* **81,** 125–128.

KOMPA, A.R., THOMAS, W.G., SEE, F., TZANIDIS, A., HANNAN, R.D. & KRUM, H. (2004). Cardiovascular role of urotensin II: effect of chronic infusion in the rat. *Peptides,* **25,** 1783–1788.

KUC, R.E. (2002). Immunocytochemical localisation of endothelin peptides, precursors, and endothelin-converting enzymes. *Methods Mol. Biol.,* **206,** 3–19.

MAGUIRE, J.J. & DAVENPORT, A.P. (2002). Is urotensin-II the new endothelin? *Br. J. Pharmacol.,* **137,** 579–588.

QI, J.S., MINOR, L.K., SMITH, C., HU, B., YANG, J., ANDRADE-GORDON, P. & DAMIANO, B. (2005). Characterisation of functional urotensin II receptors in human skeletal muscle myoblasts: comparison with angiotensin II receptors. *Peptides,* **26,** 683–690.

RUSSELL, F.D., MOLENAAR, P. & O-BRIEN, D.M. (2001). Cardiostimulant effects of urotensin-II in human heart *in vitro*. *Br. J. Pharmacol.,* **132,** 5–9.

STAMLER, J.S. & MEISSNER, G. (2001). Physiology of nitric oxide in skeletal muscle. *Physiol. Rev.,* **81,** 209–237.

THANASSOULIS, G., HUYHN, T. & GIAID, A. (2004). Urotensin II and cardiovascular diseases. *Peptides,* **25,** 1789–1794.

THEODORSSON-NORHEIM, E. (1987). Friedman and Quade tests: BASIC computer program to perform nonparametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput. Biol. Med.,* **17,** 85–99.

WAKEFIELD, I.D., MARCH, J.E., KEMP, P.A., VALENTIN, J.-P., BENNETT, T. & GARDINER, S.M. (2003). Comparative regional haemodynamic effects of the nitric oxide synthase inhibitors, *S*-methyl-L-thiocitrulline and L-NAME, in conscious rats. *Br. J. Pharmacol.,* **139,** 1235–1243.

WATSON, A.M.D., LAMBERT, G.W., SMITH, K.J. & MAY, C.N. (2003). Urotensin II acts centrally to increase epinephrine and ACTH release and cause potent inotropic and chronotropic actions. *Hypertension,* **42,** 373–379.

WOOLARD, J., BENNETT, T., DUNN, W.R., HEAL, D.J., ASPLEY, S. & GARDINER, S.M. (2004). Acute cardiovascular effects of sibutramine in conscious rats. *J. Pharmacol. Exp. Ther.,* **308,** 1102–1110.

YOKOTANI, K., MURAKAMI, Y., OKADA, S. & HIRATA, M. (2001). Role of brain arachidonic acid cascade on central CRF_1 receptor-mediated activation of sympatho-adrenomedullary outflow in rats. *Eur. J. Pharmacol.,* **419,** 183–189.

ZHU, Y.Z., WANG, Z.J., ZHU, Y.C., ZHANG, L., OAKLEY, R.M.E., CHUNG, C.W., LIM, K.W., LEE, H.S., OZOUX, M.L., LINZ, W., BÖHM, M & KOSTENIS, E. (2004). Urotensin II causes fatal circulatory collapse in anesthetised monkeys *in vivo*: a 'vasoconstrictor' with a unique hemodynamic profile. *Am. J. Physiol.,* **286,** H830–H836.

(Received September 9, 2005
Revised October 20, 2005
Accepted October 25, 2005
Published online 28 November 2005)