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SPECIAL REPORT

# Cardiostimulant effects of urotensin-II in human heart *in vitro*

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The effects of the recently identified human peptide urotensin-II (hU-II) were investigated on human cardiac muscle contractility and coronary artery tone. In right atrial trabeculae from non-failing hearts, hU-II caused a concentration-dependent increase in contractile force ($pEC_{50} = 9.5 \pm 0.1$; $E_{max} = 31.3 \pm 4.8\%$ compared to 9.25 mM $Ca^{2+}$; $n = 9$) with no change in contraction duration. In right ventricular trabeculae from explanted hearts, 20 nM hU-II caused a small increase in contractile force ($7.8 \pm 1.4\%$ compared to 9.25 mM $Ca^{2+}$; $n = 3/6$ tissues from 2 out of 4 patients). The peptide caused arrhythmic contractions in 3/26 right atrial trabeculae from 3/9 patients in an experimental model of arrhythmia and therefore has less potential to cause arrhythmias than ET-1. hU-II (20 nM) increased tone (17.9% of the response to 90 mM KCl) in 7/7 tissues from 1 patient, with no response detected in 8/8 tissues from 2 patients. hU-II is a potent cardiac stimulant with low efficacy.

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**Keywords:** Human urotensin-II; human heart; coronary artery; atrium; ventricle

**Abbreviations:** HOCM, hypertrophic obstructive cardiomyopathy; hU-II, human urotensin-II; ICI 118,551 [*erythro*-D,L-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol]; IDCM, idiopathic dilated cardiomyopathy; IHD, ischaemic heart disease; U-II, urotensin-II homologues; UT-II, human urotensin-II receptor

**Introduction** Urotensin-II (U-II) is a vasoactive peptide, first isolated from the urophysis of teleost fish (Moore *et al*., 1975). Several species homologues have since been identified, including an 11 amino acid human isoform (hU-11) (Coulouarn *et al*., 1998). U-II was identified as the endogenous ligand for GPR14 (Ames *et al*., 1999; Liu *et al*., 1999; Mori *et al*., 1999), a G-protein-coupled receptor first cloned from rat (Marchese *et al*., 1995). A human receptor that has 75% homology with GPR14 (UT-II receptors) (Ames *et al*., 1999) and hU-II have been detected in human heart, with positive immunoreactive staining for U-II observed over cardiac myocytes and atherosclerotic plaques of coronary arteries. UT-II receptor mRNA expression was observed in both atrial and ventricular tissue (Ames *et al*., 1999; Liu *et al*., 1999). Taken together, these data raise the possibility that the peptide may be an endogenous modulator of cardiac function.

Systemic infusion of sub-lethal doses of U-II into anaesthetized cynomolgus monkeys caused increased vascular resistance, a moderate reduction in blood pressure, a fall in cardiac output and severely depressed myocardial contractility (Ames *et al*., 1999). It is not known whether the myocardial depression observed following administration of U-II was a result of direct effects on cardiac muscle or was secondary to increases in coronary artery tone or a combination of both effects.

Whilst the direct effects of U-II on cardiac muscle are currently unknown, it is known that the vascular effects of U-II are species-dependent, and contingent on the regional location of the vessel. For example, U-II mediates contraction of monkey, but not rat renal artery, and contraction of rat thoracic- but not rat abdominal aorta (Ames *et al*., 1999). A contractile response was observed to U-II in a subset of isolated human pulmonary arteries (i.d = 250 $\mu$m) when pretreated with the nitric oxide synthase inhibitor, $N^{\omega}$-nitro-L-arginine methylester (MacLean *et al*., 2000).

In view of the complex haemodynamic responses observed to U-II in monkeys, and the cardiovascular distribution of U-II and UT-II-receptors in humans, we sought to dissociate its effects on human cardiac muscle and coronary arteries. Therefore the aims of this study were to (i) determine the effects of hU-II on contractile force in human atrial and ventricular muscle, (ii) investigate its arrhythmogenic potential in a model of atrial arrhythmia and (iii) determine the effects of hU-II on endothelial-denuded coronary artery tone.

**Methods** *Patients* Human right atrial appendages were obtained from patients undergoing coronary artery bypass grafting at The Prince Charles Hospital. Human right ventricles and left circumflex- and left anterior descending coronary arteries were obtained from explanted hearts with the aetiologies of ischaemic heart disease (IHD, $n = 2$), idiopathic dilated cardiomyopathy (IDCM, $n = 1$) hypertrophic obstructive cardiomyopathy (HOCM, $n = 2$) and an unused donor heart ($n = 1$). The studies were approved by the ethics committees of The Prince Charles Hospital (EC9876) and the University of Queensland (H/29/Med/PCH/NHMRC/99).

*Preparation of tissues* After surgical removal, heart tissue was placed immediately into ice-cold, pre-oxygenated (95% $O_2$/5% $CO_2$) modified Krebs' solution (in mM: $Na^+$ 125, $K^+$ 5, $Ca^{2+}$ 2.25, $Mg^{2+}$ 0.5, $Cl^-$ 98.5, $SO_4^{2-}$ 0.5, $HCO_3^-$ 32, $HPO_4^{2-}$ 1, EDTA 0.04) and transported to the laboratory. Atrial and ventricular trabeculae or helicoidal strips of coronary arteries

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were prepared and set up in 50 ml tissue baths at 37°C as described previously (Burrell *et al*., 2000). Atrial and ventricular tissues were driven with square wave pulses (5 ms duration, just over threshold voltage, 1 Hz). Krebs' solution was supplemented with 15 mM $Na^+$, 5 mM fumerate, 5 mM pyruvate, 5 mM L-glutamate and 10 mM glucose.

Cardiac tissues were incubated with 200 nM (−)-propranolol for 60 min to block $\beta_1$- and $\beta_2$-adrenoceptors. Additionally, tissues were incubated with 6 $\mu$M cocaine to block neuronal uptake for experiments with 5-hydroxytryptamine (Kaumann *et al*., 1990). In other experiments with (−)-noradrenaline at $\beta_1$-adrenoceptors, tissues were incubated with 50 nM ICI 118,551 to block $\beta_2$-adrenoceptors, 1 $\mu$M prazosin to block $\alpha_1$-adrenoceptors and 6 $\mu$M cocaine.

*Effect of U-II on human myocardial contractility* The effects of U-II on myocardial contractility were established by carrying out cumulative concentration-effect curves or by using a single (20 nM, maximal) concentration. In other experiments concentration-effect curves were established to endothelin-1, 5-hydroxytryptamine or (−)-noradrenaline in the absence or presence of 20 nM U-II. All experiments were completed by the addition of a single concentration of (−)-isoprenaline (200 $\mu$M) to obtain a maximal effect caused by stimulation of both $\beta_1$- and $\beta_2$-adrenoceptors and then by raising the $Ca^{2+}$ concentration to 9.25 mM.

*Arrhythmogenicity of hU-II in right atrial trabeculae* The potential of hU-II to cause arrhythmic contractions in human right atrium was determined in a staircase model as described in detail for stimulation of human atrial $\beta_1$- and $\beta_2$-adrenoceptors (Kaumann & Sanders, 1993) and endothelin receptors (Burrell *et al*., 2000). Briefly, atrial tissues from right atrial appendages were set up as described above and incubated with 200 nM (−)-propranolol. The pacing frequency was then set at 0.1 Hz and reset at 0.2, 0.5, 1 and 2 Hz at 2 min intervals (forward staircase). The staircase was then run backwards (2–0.1 Hz), with 2 min intervals during which time the stimulator was turned off (rest periods) between each 2 min stimulation period. The pacing rate was then set at 1 Hz and upon stabilization hU-II was added to the tissue bath. After equilibration, the backward staircase (2–0.1 Hz) was established with 2 min rest periods between each frequency repeated in the presence of hU-II. One trabeculum from each patient was not incubated with hU-II and served as a time-matched control.

*Effect of U-II on human coronary artery tone* Left circumflex and left anterior descending coronary arteries were cut into helicoidal strips (Burrell *et al*., 2000). As it has been reported that U-II causes the release of endothelium-derived vasodilators which can mask contractile responses (MacLean *et al*., 2000; Katano *et al*., 2000) vessels were denuded of endothelium by rubbing the luminal surface with coarse-grade paper towel. Resting tension was adjusted to 20 mN, and endothelial denudation was confirmed by the absence of relaxation to 1 $\mu$M carbachol. Tissues were allowed to stabilise for 3 h before addition of 90 mM KCl followed by washout, and re-addition of KCl 2 h later. After washout, vessels were exposed to 20 nM hU-II, followed by 20 nM endothelin-1.

*Analysis and statistics* In cardiac preparations, the effects of U-II were corrected for time-dependent reductions in contractile force using tissues not exposed to agonists. Increases in contractile force were expressed as a percentage of the response to 9.25 mM $Ca^{2+}$. The potency of U-II was determined using tissues that responded to cumulative concentrations of the human (9/18 tissues), goby (7/27 tissues), mouse (6/18 tissues), rat (8/14 tissues), porcine A (7/10 tissues) and porcine B (7/15 tissues) homologues. Values are expressed as mean ± s.e.mean. Statistical comparisons were made using the Students' unpaired two-sided *t*-test. Differences were considered significant when $P<0.05$.

**Results** *Effect of U-II on human myocardial contractility* hU-II (20 nM) caused an increase in contractile force in human right atrial trabeculae with no change in the time to reach peak force of contraction (basal tpf = 120.4 ± 5.7 ms, hU-II tpf = 124.0 ± 5.4 ms; $n=12$ tissues/8 patients, $P=0.7$) or time to reach 50% relaxation (basal $t_{50}=88.7\pm4.8$ ms, hU-II $t_{50}=88.3\pm4.6$ ms, $n=12$ tissues/8 patients, $P=0.9$) (Figure 1a). In right ventricle, 20 nM, hU-II increased the force of contraction in three out of six tissues from two out of four patients (effect = 7.8 ± 1.4% with respect to $Ca^{2+}$, responders: IDCM, HOCM; non-responders: donor, HOCM; Figure 1b). hU-II caused potent ($pEC_{50}=9.5\pm0.1$, Table 1), concentration-dependent positive inotropic effects in human right atrial trabeculae with a maximal effect of 31.3 ± 4.8% relative to 9.25 mM $Ca^{2+}$. For comparison, the subsequent addition of (−)-isoprenaline (200 $\mu$M) increased contractile force to 96.3 ± 2.3% ($n=9$) of that of 9.25 mM $Ca^{2+}$.

Species holologues of hU-II also increased right atrial force of contraction (Figure 2). Human, goby, porcine A and porcine B isoforms were slightly, but significantly more

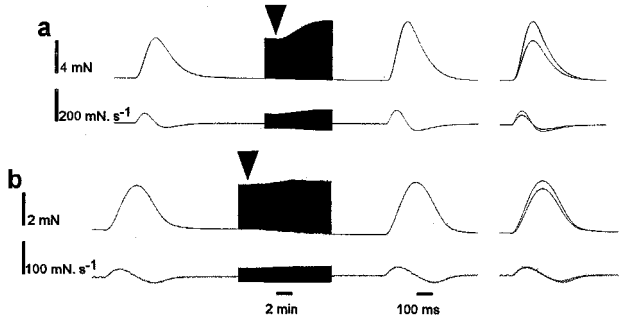


**Figure 1** Original traces showing the increase in force of contraction caused by hU-II in right atrial- (a) and right ventricular trabeculae (b). Atrial and ventricular tissues were obtained from a 74-year-old female patient undergoing coronary artery bypass surgery, and a male patient undergoing cardiac transplantation for IDCM, respectively. Upper panels in (a) and (b) show contractile force and bottom panels show the differentiated signal. Fast speed recordings show individual contractions. The increase in force of contraction is observed in the slow speed recording following addition of 20 nM hU-II (arrow heads). Single contractions before and after addition of hU-II are superimposed on the right hand side of the diagram to allow visual comparisons of contractile force (f) and times to reach peak force of contraction (tpf) and 50% relaxation ($t_{50}$). Right atrial trabeculum: (basal f = 5.0 mN, hU-II f = 7.4 mN; basal tpf = 135 ms, hU-II tpf = 132.5 ms; basal $t_{50}=107.5$ ms, hU-II $t_{50}=107.5$ ms). Right ventricular trabeculum: (basal f = 2.7 mN, hU-II f = 3.25 mN; basal tpf = 200 ms; hU-II tpf = 210 ms; basal $t_{50}=120$ ms, hU-II $t_{50}=130$ ms).

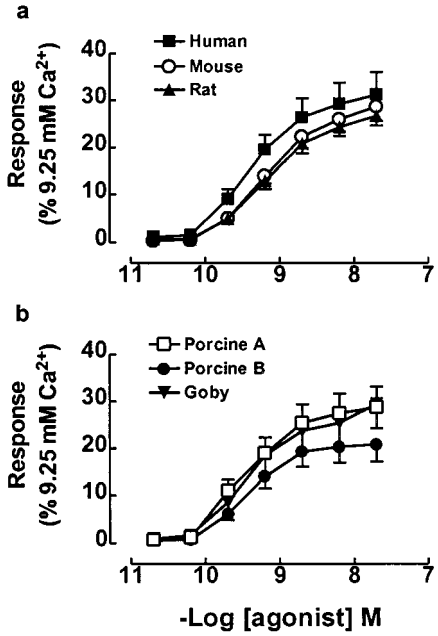


**Figure 2** Concentration response curves to (a) human-, mouse-, rat- and (b) porcine A-, porcine B- and goby U-II in human right atrial trabeculae. Number of tissues/patients for species homologues were: human, 9/9; mouse, 6/6; rat, 8/8; porcine A, 7/7; porcine B, 7/6; goby, 7/6. Positive inotropic effects are mean ± s.e.mean, expressed as a percentage of responses to 9.25 mM $Ca^{2+}$.

potent than rat ($P=0.01$, 0.02, 0.02 and 0.01, respectively) and mouse isoforms ($P=0.01$, 0.04, 0.04 and 0.01, respectively) (Table 1). There was no difference in the potency of hU-II and goby, porcine A or porcine B homologues in human right atrium ($P>0.05$).

Concentration-effect curves to endothelin-1, 5-hydroxytryptamine and (−)-noradrenaline were carried out in the absence and presence of 20 nM hU-II to determine the specificity of hU-II. hU-II did not affect responses to endothelin-1 at endothelin receptors (control $pEC_{50}=8.5\pm0.1$, $n=5$ tissues/5 patients, plus hU-II $=8.6\pm0.1$, $n=5$ tissues/5 patients; $P=0.4$), 5-hydroxytryptamine at 5-$HT_4$ receptors (control $pEC_{50}=7.4\pm0.1$, $n=8$ tissues/7 patients, plus hU-II $=7.3\pm0.1$, $n=5$ tissues/5 patients; $P=0.9$) or (−)-noradrenaline at $\beta_1$-adrenoceptors ($pEC_{50}=6.9\pm0.1$, $n=8$ tissues/3 patients, plus hU-II $=7.2\pm0.1$, $n=5$ tissues/3 patients; $P=0.1$). hU-II was 11-, 143- and 363-fold more potent than endothelin-1, 5-hydroxytryptamine and (−)-noradrenaline, respectively in mediating positive inotropic responses.

*Evaluation of the arrhythmogenic potential of hU-II in right atrial trabeculae* hU-II (20 nM) caused arrhythmias in three tissues (one, two and 16 ectopic contractions) from three patients out of a total of 26 tissues from nine patients (Figure 3). No arrhythmic contractions were observed in the backward staircase carried out prior to addition of hU-II, or in untreated, time-dependent control tissues (nine tissues/nine patients).

*Effect of U-II on human coronary artery tone* hU-II (20 nM) caused an increase in tone in 7/7 preparations of left circumflex coronary artery obtained from one patient with HOCM (17.9% of the response to 90 mM KCl; range = 8.9–23.0%) (Figure 4). No response was observed in 3/3 left circumflex coronary arteries obtained from a donor heart, or 3/3 left circumflex- and 2/2 left anterior descending coronary arteries obtained from a patient with IDCM. All preparations responded to 90 mM KCl and 20 nM endothelin-1.

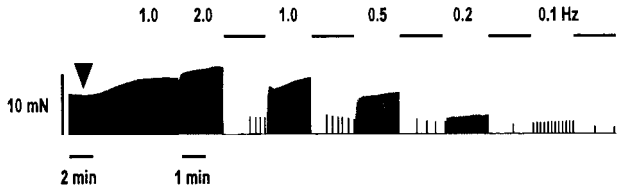


**Figure 3** Spontaneous contractions induced by 20 nM hU-II in a right atrial trabeculum obtained from a 58-year-old male patient undergoing coronary artery bypass surgery. Tissues were paced using forward and backward staircases, as described in the Methods. No spontaneous contractions were observed during this procedure (not shown). The tissue was then paced at 1 Hz and 20 nM hU-II was added to the bath (arrow head). Upon equilibration, the tissue was stimulated at 2, 1, 0.5, 0.2 and 0.1 Hz, with a 2 min quiescent period after each change of frequency (upper horizontal lines). Note the spontaneous contractions that occurred during quiescent periods.

**Table 1** Potency of U-II species homologues in human right atrial trabeculae

| *U-II* | *Peptide sequence* | $pEC_{50}$ (M) | n *tissues /patients* |
|---|---|---|---|
| Human | ETPD **CFWKYC** V[1] | 9.5±0.1 | 9/9 |
| Porcine A | GPTSE **CFWKYC** V[2] | 9.6±0.1 | 7/7 |
| Porcine B | GPPSE **CFWKYC** V[2] | 9.5±0.1 | 7/6 |
| Goby | AGTADV **CFWKYC** V[3] | 9.6±0.2 | 7/6 |
| Rat | EHGTAPE **CFWKYC** I[1] | 9.1±0.1 | 8/8 |
| Mouse | QHGAAPE **CFWKYC** I[1] | 9.2±0.1 | 6/6 |

Bold lettering indicates conserved sequences across species. [1]Coulouarn *et al*., 1999; [2]Mori *et al*., 1999; [3]Clark *et al*., 1982.

**Discussion** We have shown that U-II can increase contractile force in human atrium and ventricle. This finding was not predicted from experiments carried out in monkeys, where systemic administration of the peptide caused marked myocardial contractile dysfunction (decreased d$P$/d$t$ and stroke volume) (Ames *et al*., 1999). In fact U-II is the most potent inotropic agent identified to date (see Table 1), with 11, 143 and 363 fold higher potency than endothelin-1 at endothelin receptors, 5-hydroxytryptamine at 5-$HT_4$ receptors and (−)-noradrenaline at $\beta_1$ adrenoceptors, respectively. The $pEC_{50}$ value determined for hU-II in right atrial strips (9.5±0.1) was similar to that reported in non-human primate left circumflex coronary artery (9.56±0.05) and left anterior descending coronary artery (9.39±0.40) (Ames *et al*., 1999). The high potency of U-II indicates that only small changes in peptide production or receptor expression may be required to alter cardiac function. However, it is not yet known whether endogenous U-II can modulate cardiac output or whether over-production of U-II has a pathophysiological role in human myocardial diseases. The positive inotropic effects of hU-II appeared to be greater in right atrium compared to right ventricle, possibly due to differences in receptor density, efficiency of G-protein coupling or pathology of hearts.

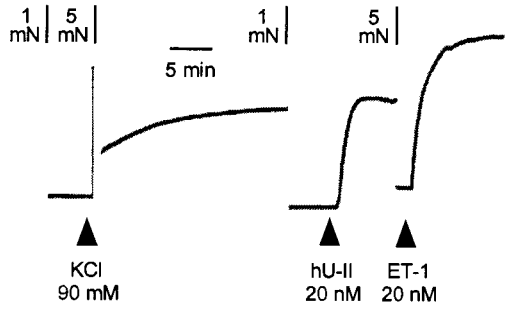


**Figure 4** Response of an endothelium-denuded human left circumflex coronary artery to 20 nM hU-II. Tissue was obtained from a male patient undergoing cardiac transplantation for HOCM. The tissue was prepared as described in the Methods. hU-II (20 nM) increased contractile force by 21.5% of the response to 90 mM KCl. Endothelin-1 (20 nM) produced an additional increase in contractile force. Note the increase in sensitivity during incubation of hU-II.

The effect of U-II species homologues on myocardial contractility was examined to investigate structure-activity relationships. Previously it has been shown that an amino acid sequence, conserved throughout species homologues of U-II, is important for functional responsiveness (Itoh *et al*., 1988). In right atrial trabeculae we observed a slightly but significantly more potent contractile response to human, goby, porcine A and porcine B homologues than to rat and mouse isoforms (Table 1). The carboxy-terminal amino acid that follows the conserved sequence is valine in human, goby, porcine A and porcine B peptides and isoleucine in the rat and mouse peptides (Table 1), which may account for the different potencies. In support of the importance of the carboxy-terminal amino acid to functional responsiveness, goby U-II-(6-11) was reportedly much less potent than goby U-II-(6-12) in producing contraction of rat isolated thoracic aorta (Itoh *et al*., 1988). Whilst the amino acids at the amino-terminal side of the conserved sequence are more variable between peptide homologues, we cannot rule out a contributing role of these to the observed differences in functional responsiveness.

The mechanism for the cardiac effects of U-II is at present unknown. Several important differences in myocardial responses to stimulation of the U-II, endothelin and $\beta$-adrenoceptor systems have been observed. Whereas hU-II did not alter the time to reach 50% relaxation ($t_{50}$), endothelin-1 caused prolongation of relaxation (Burrell *et al*., 2000) and the $\beta$-adrenoceptor agonist (−)-isoprenaline caused hastening of relaxation (not shown). The arrhythmogenic effects of hU-II and endothelin-1 also differed. hU-II, at a concentration (20 nM) that increased force of contraction in right atrial trabeculae to 30% of responses to 9.25 mM $Ca^{2+}$, caused spontaneous contractions in only 12% of tissues. In contrast, endothelin-1 at a concentration (6 nM) which produced a similar increase in force of contraction as hU-II, caused spontaneous contractions in 70% of tissues (Burrell *et al*., 2000). Finally, whilst both U-II and endothelin-1 have been reported to increase $\alpha_1$(I) procollagen gene expression in neonatal rat cardiac fibroblasts, endothelin-1 but not U-II caused cardiac myocyte hypertrophy (Tzanidis *et al*., 2000). Further studies are required to delineate the signalling pathway(s) coupled to UT-II receptors.

hU-II caused intense contractile effects in monkey coronary arteries. We therefore examined the effect of hU-II on human coronary artery tone *in vitro*. hU-II caused a small increase in tone (17.9% of 90 mM KCl; 1/3 patients), in line with that observed by Maguire *et al*., 2000 (~15% of 100 mM KCl; 6/9 patients). The process of endothelium denudation did not damage smooth muscle cells since the vessels were responsive to KCl and endothelin-1.

In summary, we have revealed the direct effects of hU-II and species homologues of the peptide on human cardiac muscle. We provide evidence to suggest that U-II is the most potent positive inotropic agent to be identified, mediating its effects independent of endothelin-1 or 5-$HT_4$ receptors and $\beta_1$-adrenoceptors and with less arrhythmogenic potential than endothelin-1. hU-II can cause an increase in tone in some isolated, endothelin-denuded human coronary arteries.

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