Urotensin II Acts Centrally to Increase Epinephrine and ACTH Release and Cause Potent Inotropic and Chronotropic Actions

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Abstract—Urotensin II is a small peptide whose receptor was recently identified in mammals as the orphan G protein–coupled receptor-14. The reported cardiovascular responses to systemic urotensin II administration are variable, and there is little detailed information on its central cardiovascular actions. We examined the cardiovascular and humoral actions of intracerebroventricular urotensin II (0.02 and 0.2 nmol/kg and vehicle) and intravenous urotensin II (2, 20, and 40 nmol/kg and vehicle) in conscious ewes previously surgically implanted with flow probes and intracerebroventricular guide tubes. Two hours after intracerebroventricular infusion of urotensin II (0.2 nmol/kg over 1 hour; n=5), heart rate (+56±13 beats per minute [bpm]), dF/dt (an index of cardiac contractility; +533±128 L·min⁻¹·s⁻¹), and cardiac output (+3.4±0.4 L/min) increased significantly compared with vehicle, as did renal, mesenteric, and iliac blood flows and conductances. Plasma epinephrine, adrenocorticotropic hormone, and glucose levels also increased dramatically (+753±166 pg/mL, +14.3±3.5 pmol/L, and +7.0±1.4 mmol/L, respectively). All of these variables remained elevated for up to 4 hours after infusion. In contrast, 1 hour after intravenous urotensin II (40 nmol/kg bolus; n=6), a sustained tachycardia (+25±8 bpm) ensued, but cardiac output, cardiac contractility, total peripheral conductance, and plasma glucose levels did not change significantly. In summary, this is the first study to show that urotensin II acts centrally to stimulate sympathoadrenal and pituitary-adrenal pathways, resulting in increased adrenocorticotropic hormone and epinephrine release and potent chronotropic and inotropic actions. In contrast, tachycardia was the only major response to intravenous urotensin II. These findings suggest that urotensin II is a novel stimulator of central pathways that mediate responses to alerting stimuli or stress. (Hypertension. 2003;42:373-379.)

Key Words: cardiac output ■ adrenocorticotropic hormone ■ glucose ■ hemodynamics ■ urotensin ■ sheep

Urotensin II (U-II) is a small peptide that was originally isolated and cloned from the fish urophysis, a small, neurosecretory organ located in the caudal area of the spinal cord.¹ Subsequently, U-II isopeptides have been found in a wide range of species, including frogs, rats, and humans.²,³ The human isoform of U-II was cloned and its receptor found to be the orphan G protein–coupled receptor-14,⁴ also known as sensory epithelium neuropeptide–like receptor.⁴ The U-II isopeptides are 11- to 15-amino acid structures consisting of a conserved C' ring that is identical in all species studied, and a species-variable N' tail. Numerous species isoforms of U-II have been shown to bind, with similar affinities, to cloned mouse and monkey U-II receptors, suggesting that the conserved C' cyclic ring is the major biologically active portion of the peptide.⁵

Interest in the cardiovascular actions of U-II has been stimulated by the finding that in the cynomolgus monkey, U-II is a more potent vasoconstrictor than is endothelin. U-II causes vasoconstriction in isolated rat,⁶ dog,⁷ cynomolgus monkey,³ and human⁸ vessels. The effects of U-II on the cardiovascular system have, however, been found to vary, depending on the vascular bed and species studied.⁹ Human U-II caused constriction of rat thoracic aorta but not rat abdominal aorta,³ and studies in vivo have also given inconsistent results. For example, peripheral infusion of U-II in humans caused vasoconstriction,⁹ whereas in other studies, it was found to have no effect.¹⁰,¹¹ A pathophysiologic role for U-II is suggested by the observation that its plasma levels¹²,¹³ and myocardial expression are increased in patients with heart failure,¹⁴ although the increase in plasma levels was not confirmed by a recent study.¹⁵ In addition, a higher urinary excretion of U-II has been shown in patients with essential hypertension.¹⁶

A central role for U-II is suggested by the findings that U-II and G protein–coupled receptor 14 occur in various regions in the brain of rats, cattle, and humans,²,⁴,¹⁷–¹⁹ but the detailed cardiovascular and humoral actions of central U-II have not been studied. In recent studies, increases in mean arterial pressure (MAP) and heart rate (HR) were shown after microinjections of U-II into the paraventricular nucleus...
(PVN) of the hypothalamus and the arcuate nucleus in anesthetized rats\textsuperscript{20} and after intracerebroventricular (ICV) administration of U-II in conscious rats.\textsuperscript{21}

The aim of this study was to determine the cardiovascular effects of central and systemic administration of U-II in conscious, unstressed sheep. To determine the hemodynamic mechanisms of any changes in arterial pressure and to determine whether the responses to intravenous (IV) U-II are regionally specific, we examined regional as well as systemic hemodynamic actions. Because there is evidence that U-II has endocrine and metabolic actions in fish,\textsuperscript{22} we measured the effects of U-II on plasma levels of adrenocorticotropic hormone (ACTH), catecholamines, and glucose to determine whether these actions are conserved in mammals.

### Methods

#### Animals and Surgery

Experiments were completed on 11 adult Merino ewes, weighing 33 to 46 kg, while standing in individual metabolism cages. Experiments were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute under guidelines laid down by the National Health and Medical Research Council of Australia.

Before the studies, sheep underwent 4 aseptic surgical procedures, each separated by 2 weeks. Anesthesia was induced with IV sodium thiopental (15 mg/kg) and after intubation was maintained with 1.5% isoflurane/O\textsubscript{2}. In the first stage, sheep were oophorectomized and prepared with bilateral carotid arterial loops. In the second stage, guide tubes were placed above the lateral cerebral ventricles. In 2 further operations, sheep were implanted with flow probes as previously described.\textsuperscript{23} Transit-time flow probes (Transonic Systems Inc) were implanted on the ascending aorta (20 mm) and on the left circumflex coronary artery (3 mm) and 2 weeks later around the cranial mesenteric (6 mm), left renal (4 mm), and left external iliac (6 mm) arteries. Antibiotic (900 mg procaine penicillin, Troy Laboratories) was administered prophylactically for 3 days after surgery. Postsurgical analgesia was maintained with intramuscular (6 mm) arteries. Antibiotic (900 mg procaine penicillin, Troy Laboratories) was administered prophylactically for 3 days after surgery. Postsurgical analgesia was maintained with intramuscular injections of flunixin meglumine (1 mg/kg, Mavlab) at the end of surgery and then 4 and 16 hours after surgery. Experiments commenced at least 2 weeks after surgery.

On the day before experiments, with the use of aseptic techniques, a cannula was inserted into the carotid artery for measurement of arterial pressure and blood sampling.\textsuperscript{23} Two additional cannulas were inserted into a jugular vein for measurement of central venous pressure (CVP) and for infusion. Data from flow probes were collected by flowmeters (Transonic Systems). A separate circuit measured the first differential of the upstream of aortic flow (\(df/dt\)) at each beat from the cardiac output (CO) signal. After analog-to-digital conversion, data were collected on a computer at 100 Hz for 10 seconds at 5-minute intervals with custom-written software.

#### Synthesis of Human U-II

Human U-II (ETPDCEWFKWYCVC) was synthesized by standard Fmoc-solid-phase synthesis.\textsuperscript{24} The crude 5-reduced peptide was subjected to cystine formation by treatment with aqueous dimethyl sulfoxide. Subsequent characterization and purification of the crude peptide were accomplished by analytical and preparative reverse-phase high-performance liquid chromatography on a C\textsubscript{18} column (Vydac, Alltech) with a gradient of acetonitrile in 0.1% aqueous trifluoroacetic acid. The peptide was eluted as a single peak at 3.8 minutes, resulting in a 99% pure peptide. Chemical characterization by analytical reverse-phase high-performance liquid chromatography and matrix-assisted laser desorption/ionization-time of flight (MALDITOF) mass spectrometry confirmed its high purity (theoretical MH\textsuperscript{+} = 1389.6; found MH\textsuperscript{+} = 1390.1).

#### ICV Administration of U-II

After a 1-hour control period, ICV infusion of vehicle (artificial cerebrospinal fluid [aCSF], 1 mL/h) or U-II (0.2 or 0.02 nmol/kg) was given for 1 hour. Cardiovascular variables were recorded for a further 4.5 hours. Arterial blood was collected before the U-II infusion (time 0) and at 0.5, 1, 2, 3, 4, and 5 hours after the beginning of the infusion. Experiments were conducted on alternate days.

#### IV Administration of U-II

After a 1-hour control period, a bolus of vehicle (1 mL 0.9% saline) or U-II (2, 20, or 40 nmol/kg) was given into the jugular vein, followed by 4 mL saline. Cardiovascular variables were continuously monitored for a further 6 hours. Arterial blood (13 mL) was collected before (time 0) and at 0.5 and 1 hour after the U-II bolus for measurement of plasma glucose and ACTH.

#### Plasma Glucose, ACTH, and Catecholamine Assays

Blood samples were taken for measurement of plasma glucose (2 mL in fluoride/heparin tubes), ACTH (5 mL in heparinized tubes), and norepinephrine and epinephrine (5 mL in ice-cold tubes containing EGTA and reduced glutathione). Blood samples were kept on ice and centrifuged at 4°C within 0.5 hour of collection. Plasma samples were stored at -20°C, except those for measurement of catecholamines, which were stored at -80°C.

Plasma glucose levels were measured with a glucose hexokinase reaction on a commercially available clinical system (Beckman Synchron CX5). Plasma ACTH was measured by radioimmunoassay (ACTH DYNO test, Brahms). Plasma concentrations of norepinephrine and epinephrine were determined by high-performance liquid chromatography with electrochemical detection.\textsuperscript{25}

#### Data Analysis

Two groups of 6 ewes were used: one group for the IV and the other for the ICV studies (including one animal from the first group). (See figure legends for the numbers used in individual treatments.) Means of cardiovascular data for the first hour (control) and then every half hour, or individual assay time points, were analyzed by repeated-measures 2-way ANOVA. When this test produced a significant result (\(P<0.05\)), a pairwise multiple comparison was performed for individual time-point values with the Bonferroni \(t\) test. For CVP, the mean of the control period (time 0) was subtracted from all other values to obtain \(\Delta\)CVP, and the effects of treatment with U-II compared with vehicle were analyzed by comparing the area under the curves by the Wilcoxon signed rank test. Analyses were performed with available software (SigmaStat, version 2.03, Access Softek Inc). Values presented in the text represent mean±SEM, calculated as the difference between the 1-hour control values and the experimental values 2 hours after the beginning of the ICV infusion, or 1 hour after the IV bolus, unless another time period is stated.

### Results

#### Cardiovascular Effects of ICV Infusion of U-II

In conscious sheep, ICV infusion of U-II (0.2 nmol/kg) caused large, sustained increases in HR (+55.2±13.5 beats per minute [bpm]; \(P<0.001\)) as well as indices of ventricular contractility, \(dF/dt\) (+533±128 L·min\textsuperscript{-1}·s\textsuperscript{-1}; \(P<0.003\)) and maximum aortic flow (Fmax, +7.9±1.0; \(P<0.001\)) at 2 hours (Figure 1). These changes resulted in a prolonged increase in CO (+3.4±0.43 L/min; \(P<0.001\); Figure 1). These variables remained significantly elevated for the 4.5 hours during which data were collected after infusion. These cardiac effects were accompanied by peripheral vasodilatation, as shown by the substantial increase in total peripheral conductance (TPC, +34.5±3.6 mL·min\textsuperscript{-1}·mm Hg\textsuperscript{-1}; \(P<0.001\)).
There was a prolonged increase in MAP (9.7±2.3 mm Hg; P<0.003), but CVP was unchanged (Figure 1).

The increase in TPC depended on increases in mesenteric, renal, and iliac conductances, which together with the increase in MAP resulted in increased flow in these vascular beds (Figure 2). Mesenteric flow (MF) remained significantly elevated for 2 hours after infusion (+236.3±74.8 mL/min; P=0.008) but had returned to baseline by 4.5 hours after the end of the infusion. Iliac flow (IF) and iliac conductance (IC) remained elevated throughout the 4.5-hour postinfusion period, whereas renal flow (RF) and renal conductance (RC) were still increasing 4.5 hours after the end of the infusion (Figure 2). Coronary conductance (CC) tended to increase, but this did not reach significance, and there was a transient but significant increase in coronary flow.

The responses to a 10-fold lower dose of ICV U-II (0.02 nmol/kg) were substantially lower than the responses to the higher dose. By 1.5 hours after the start of the ICV infusion of U-II (0.02 nmol/kg), there were significant increases in CO (+0.64±0.31 L/min; P=0.018) and Fmax (+2.35±0.43 L/min; P=0.002; Figure 3). Although the changes in TPC, HR, and dF/dt did not reach significance, IF (83.3±31.3 mL/min; P=0.002) and IC (1.17±0.28 mL·min⁻¹·mm Hg⁻¹; P<0.001) were increased. By 2 hours after ICV U-II (0.02 nmol/kg), there were significant increases in RF (51.0±17.2 mL/min; P=0.007) and RC (1.01±0.27 mL·min⁻¹·mm Hg⁻¹; P<0.001), but coronary and mesenteric flows and conductances were unchanged.

Curiously, it was noted that approximately half an hour into the ICV infusion of 0.2 nmol/kg U-II, animals began to scratch, mainly around the head, and became restless. This condition generally lasted for <1 hour after onset. The sheep did not appear anxious or show increased responses to external stimuli, such as touch or noise. Scratching also occurred with 0.02 nmol/kg ICV U-II, but to a much lesser extent.

**Cardiovascular Effects of IV Administration of U-II**

IV administration of U-II (40 nmol/kg) to conscious sheep caused a transient increase in MAP (+7.7±3.2 mm Hg at 0.5 hour; P<0.001), which started within 5 minutes of the bolus, peaked by 30 minutes, and returned to control levels by 1 hour. MAP remained below control levels from 3 to 6 hours after the administration of U-II (Figure 4). This dose of U-II caused a sustained increase in HR (+22±5 bpm; P=0.029 at 1 hour), but CO was unchanged, and cardiac stroke volume (SV) decreased (−21.7±7.9 mL/beat; P=0.035). There were small reductions in indices of ventricular contractility, dF/dt (−191.9±72.4 L·min⁻¹·s⁻¹ at 3.5 hours; P=0.03) and Fmax (−5.23±2.2 L/min at 3.0 hours; P=0.049; Figure 4).

In the regional vasculature, there were differential changes in individual vascular beds. There was a prolonged reduction in MF, falling by a maximum of 117.5±24.6 mL/min after 3.5
Humoral Effects of U-II

ICV infusion of U-II (0.02 and 0.2 nmol/kg) significantly increased plasma levels of epinephrine, which reached a maximum 1 hour after the end of the infusion (+164±49 and +727±165 pg/mL; P<0.001 and P<0.001, respectively) and remained elevated at this level for a further 3 hours (Figure 6). After IV U-II (0.02 and 0.2 nmol/kg), norepinephrine levels were higher in all sheep (+344±16 and 471±271 pg/mL, respectively, at 1 hour after the end of the infusion), but these changes were not consistent during the infusion, varied between animals, and did not reach significance. IV U-II (0.02 and 0.2 nmol/kg) induced dose-related increases in plasma ACTH (+5.6±2.5 and +14.3±3.5 pmol/L; P=0.002 and P<0.001, respectively; Figure 6). Plasma cortisol levels were measured in 1 of the 5 animals after IV U-II and were found to have increased in a dose-dependent manner compared with vehicle, with a 3-fold increase after the highest dose of U-II. Plasma glucose rose only with the higher dose (+7.0±1.4 mmol/L; P<0.001) but did so dramatically, more than tripling.

After IV administration of U-II (2, 20, and 40 nmol/kg), there were no changes in plasma glucose. Plasma ACTH tended to increase after IV U-II (20 nmol/kg), changing from 5.5±2.2 to 66.7±33.4 pmol/L at 30 minutes and to 47.4±25.5 pmol/L at 60 minutes. After IV U-II (40 nmol/kg), ACTH changed from 2.4±0.9 to 35.3±18.8 pmol/L at 30 minutes and to 17.2±6.7 pmol/L at 60 minutes. These changes did not reach significance owing to the large inter-animal variability.
Discussion

This study is the first to demonstrate that in a conscious mammal, U-II acts centrally to stimulate the sympathoadrenal medullary and pituitary-adrenal cortex axes, resulting in secretion of ACTH and epinephrine, actions which are widely considered specific markers of stress. These effects were accompanied by sustained cardiovascular and metabolic changes, including potent inotropic and chronotropic actions, increased CO, increased arterial pressure, peripheral vasodilation, and hyperglycemia. In contrast, a positive chronotropic effect was the only major action after IV administration of U-II.

Although there is evidence that both U-II and its receptor are localized in the brain,\textsuperscript{3,4,8,17,18,26} the detailed cardiovascular, humoral, and metabolic responses to central U-II have not been studied. In a behavioral study in rats, ICV human U-II was found to increase motor activity.\textsuperscript{18} Microinjection of U-II into the PVN and the arcuate nucleus of anesthetized rats increased MAP and HR, but ICV U-II in conscious rats did not.\textsuperscript{21} These effects of ICV U-II were blocked by ganglion blockade, suggesting that they were mediated by the autonomic nervous system.\textsuperscript{21}

In our studies in conscious sheep, we found similarly that ICV U-II increased MAP and HR. Interestingly, however, ICV U-II was significantly more potent in sheep than in rats, the highest dose in sheep being 150-fold less (on a per kilogram basis) than that used in rats.\textsuperscript{21} In addition to a positive chronotropic action, ICV U-II caused a positive inotropic action, and together these effects resulted in sustained increases in SV and CO. These changes were accompanied by peripheral vasodilation, as shown by an increase in TPC, demonstrating that the increase in arterial pressure depended entirely on the increase in CO. The increased levels of epinephrine are probably the main cause of these striking cardiovascular responses to central administration of U-II. The large and prolonged increase in plasma epinephrine, with no significant increase in plasma norepinephrine, suggests that ICV U-II increased sympathetic nerve activity to the adrenal medulla but did not cause generalized sympathetic activation. The increased circulating epinephrine would increase HR and cardiac contractility via its action on cardiac β-receptors. However, further studies are required to confirm that cardiac sympathetic activity is not also increased.

The increase in TPC seen after ICV U-II resulted from vasodilatation in the major vascular beds monitored, which was probably partly mediated by baroreflex-mediated withdrawal of sympathetic vasomotor tone in response to the increase in arterial pressure. Other humoral changes induced by ICV U-II would also have influenced the peripheral vasculature. Skeletal muscle vasodilatation is a characteristic action of epinephrine, indicating that the increased circulating levels could account for the increases in IF and IC. Increased circulating levels of cortisol have a direct renal action, inducing progressive renal vasodilatation over many hours.\textsuperscript{27} The increase in plasma ACTH and subsequent stimulation of cortisol release might therefore account for the prolonged increases in RF and RC after ICV U-II. The hyperglycemia after ICV U-II could also be accounted for by the actions of increased plasma levels of epinephrine and cortisol.

U-II binding has been localized in numerous regions in the brain,\textsuperscript{18,26} but the sites at which U-II acts to stimulate increased adrenal medullary secretion of epinephrine and pituitary secretion of ACTH cannot be determined from the present study. The U-II–induced stimulation of sympathetic outflow to the adrenal medulla might result from stimulation of specific nuclei in the medulla oblongata, which has been shown to contain U-II receptors in the rat.\textsuperscript{18} To our knowledge, binding sites for U-II have not been located in the rat PVN, this being the classic site where stimulation of
corticotrophin-releasing hormone–containing neurones leads to pituitary ACTH release.28 In rats, ICV U-II did not increase plasma corticosterone levels,18 suggesting that in this species U-II might not stimulate ACTH secretion, although the high baseline levels of corticosterone noted by the authors might have masked an increase. Further studies are required to confirm the hypothalamicadrenal responses to U-II in rats and to localize brain U-II receptors in sheep.

The responses to peripheral administration of U-II in sheep contrast sharply with those after central administration. The only major responses to IV U-II were a sustained tachycardia and a fall in SV. There was a small reduction in cardiac contractility, CO did not significantly change, and after a transient increase, arterial pressure remained moderately below control levels. IV U-II did not change TPC, and in the regional vasculature, there were no effects on the coronary, renal, or iliac vascular beds, but MF decreased. These effects were clearly different from those of ICV U-II, indicating that the changes seen after centrally administered U-II (a 200-fold lower dose than that given IV) did not result from spillover of U-II into the circulation.

These responses to IV U-II in sheep contrast strongly with the report that U-II is a more potent vasoconstrictor than endothelin in the cynomolgus monkey.3 In humans, U-II has been found to have no effect on systemic or local hemodynamics10,11 or to cause local vasoconstriction.9 In conscious rats, IV U-II caused tachycardia and a fall in arterial pressure,21,29 mesenteric and hindquarter vasodilatation being the cause of this hypotensive response.29 These responses are similar to the tachycardia, fall in arterial pressure, and fall in MF that we found in sheep after IV U-II, suggesting that these effects are consistent across these species.

The sustained tachycardia that we observed in response to IV U-II in sheep occurred rapidly and concurrently with a transient elevation in arterial pressure, demonstrating that this response was not baroreceptor mediated. In anesthetized rats, β-blockade prevented the tachycardia after IV U-II,30 suggesting that this response results from epinephrine release or increased cardiac sympathetic nerve activity. Receptors for U-II are, however, abundantly expressed in the human heart,31 so it is possible that U-II could have caused this effect by a direct action on the heart.

After IV U-II, there was no increase in plasma glucose, suggesting that there was no increase in plasma epinephrine. ACTH levels were 12- to 14-fold higher after U-II, but this did not reach significance owing to the high interanimal variability. It is possible that with a different protocol, circulating U-II might have significantly stimulated ACTH release from the pituitary. To our knowledge, circulating U-II has not been shown to stimulate ACTH excretion in mammals, although in flounder, in vivo micromolar interarterial infusion of U-II resulted in increases in plasma cortisol levels.32

There is substantial evidence that the responses to different isopeptides of U-II are independent of the species in which they are studied. Human and rat U-II isopeptides have almost identical actions on blood pressure and regional blood flows in conscious rats,29 and competition binding analysis demonstrated equipotent, high-affinity binding of numerous mamalian, amphibian, and piscine U-II isopeptides to cloned mouse and monkey U-II receptors.7 Additionally, a peptide consisting only of the C’ cyclic octapeptide core from goby U-II retained full biologic activity in rat aorta.33 These consistent responses across species are thought to result from the conserved C’ ring of U-II being the main biologically active part of the peptide. These studies support the supposition that responses to human U-II in sheep probably closely reflect the responses to endogenous ovine U-II isopeptide, the structure of which has not been determined.

Perspectives

The present findings that U-II acts centrally to stimulate pituitary-adrenal and sympathoadrenal medullary pathways suggest that U-II might be involved in mediating the endocrine and cardiovascular responses to alerting stimuli or stress. Further studies are required to determine the central sites at which U-II acts to modulate these central neuroendocrine and autonomic systems. Although it has been proposed that circulating U-II is a potent vasconstrictor,3 this is not supported by our findings or recent reports that it is a vasodilator in rats and has no action in humans,10,11,29 although vasconstrictor actions in specific vessels in these species have been reported.3,9 Interestingly, there is evidence that circulating levels of U-II are increased in heart failure,12,13 but its role in the pathology of this disease remains to be established. The use and development of U-II antagonists should help determine the physiologic role of U-II and its role in pathologic conditions, such as heart failure.

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