Urotensin II causes fatal circulatory collapse in anesthetized monkeys in vivo: a “vasoconstrictor” with a unique hemodynamic profile

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Urotensin II (UII) is a cyclic peptide originally isolated from the urophysis of the teleost fish gillichthys mirabilis (30). Several species homologues have since been identified, including the human isopeptide (11). UII isopeptides vary in length from 11–14 amino acids (AA) with human UII being an 11 AA peptide. Whereas the NH₂ terminal AA are variable across the different species, the COOH terminal cyclic hexapeptide sequence has been highly conserved during evolution from fish to mammals, suggesting that its function is not redundant. UII indeed shows biological activity in a variety of mammalian and nonmammalian species. It is particularly well known for its ability to regulate smooth muscle tone in vascular (1, 6, 13, 21, 23) and nonvascular preparations (2, 8–10, 15, 16, 34), but its effects exhibit marked species differences and are contingent on the regional location of the vessel. In the rat, vasoconstrictor responses are observed in the thoracic aorta (5) but are less evident in the abdominal aorta (24). In contrast, UII acts as a vasodilator in rat coronary (25) and mesenteric vessels (4, 14). Maguire et al. (28) reported UII to contract human coronary, mammary, and radial arteries, and saphenous and umbilical veins. In contrast, Camarda et al. (6) did not observe vasoconstriction in saphenous veins, nor in mesenteric arteries, nor did Hillier et al. (22) observe contraction of human small subcutaneous resistance arteries, internal mammary arteries, saphenous veins, and small subcutaneous veins. These anatomic and species variations are further contrasted by the observation that UII is a coronary selective spasmsogen in the dog (13) but a potent vasodilator of porcine coronary arteries (W. Linz, unpublished observation). In cynomolgus monkeys in vitro, human UII caused concentration-dependent contraction in all arterial vessels studied, including both elastic and muscular arteries (thoracic and abdominal aorta, coronary, basilar, ca-
rotid, pulmonary, mesenteric, renal, and mammary arteries). It was also found to be more potent and more efficacious than endothelin-1 (13). Unlike endothelin-1, however, the contractile actions were restricted to the arterial side of the vasculature, consistent with the differential expression of human GPR 14 determined by PCR. The only vessel of venous origin that responded to human UII was isolated from pulmonary vasculature. Whereas several reports are available on the in vitro effects of UII, much less is known about its effects in vivo. In anesthetized rats, UII causes systemic vasodepression (17, 19, 20). In conscious rats, the predominant cardiovascular action of UII is vasodilation accompanied by tachycardia (14). Only one report is available on the in vivo effects of UII in anesthetized cynomolgus monkeys (1). On application of UII, total peripheral resistance was increased associated with profound contractile dysfunction ultimately leading to total circulatory collapse and death. So far, the nature of death remained elusive and it is rather hypothetical to speculate (based on the existing data) about different factors contributing to the fatal event. To shed more light on the mechanism underlying the fatal circulatory collapse, we sought to carefully characterize the hemodynamic profile of UII in vivo in anesthetized cynomolgus monkeys. Because the observed sudden drop of various cardiovascular parameters is reminiscent of an anaphylactic shock, we explored the effects of higher doses of UII on the plasma histamine levels of the monkeys via RIA.

MATERIALS AND METHODS

A blood flowmeter (model TT206; Transonic Systems), flowmeter probes (Transonic Systems), computer software (Windaq), cannula (20 G), blood pressure (BP) monitor (Press-Mate-BP-8800, Colin; Komaki, Japan), and human UII (Bachem; Basel, Switzerland) were used in the study. UII was dissolved in double-distilled water, which was used as vehicle. We observed that the application of vehicle did not have any significant effects on hemodynamic parameters.

Animals. Four adult male cynomolgus monkeys (5 to 6 kg, from Indonesia) were studied. The animals used in the present study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals. The animals were fasted for 24 h before anesthesia was given. Water was available at all times before anesthesia. None of the animals had been used for any previous experimental studies. The study received approval from the Ethics Committee of National University of Singapore.

The stability of the preparation and adequacy of the ventilation is reliable. A mechanical ventilator (Titus, Dräger) for monkeys was used, which monitored the oxygen consumption with an oximeter (model N-185, Nellcor). Continuous measurement of BP and periodic measurement of arterial PCO 2 , arterial PO 2 , and arterial pH was performed. Continuous measurement of BP and periodic measurement of arterial PCO 2 , arterial PO 2 , and arterial pH was performed. A mechanical ventilator (Titus, Draeger) was inserted in the femoral artery. Another incision was made at the left lower cervical area, and a carotid flowmeter probe was held in the carotid artery to measure carotid blood flow. An incision was made in the fourth left intercostal space. A laser Doppler probe was used to measure coronary blood flow at the left anterior descending coronary artery. A cannula (20G) was inserted into the left ventricle (LV) through the apex. A pulmonary pressure transducer probe (Transonic Systems) inserted via a Swan-Ganz catheter (Biosensort, Singapore) was implanted in the root of the pulmonary artery. All incisions and catheterizations were made sterile surgical material.

Baseline hemodynamic parameters were digitally recorded before intravenous administration of human UII (Table 1). Hemodynamic parameters were monitored and recorded during the whole period of the experiments. Accumulative administration of human UII was given intravenously in 30-min intervals. The doses of human UII used in our present study were 0.03, 0.3, and 3 mmol/kg, because the dose of 0.003 mmol/kg was found not to change any hemodynamic parameters in our previous study.

Hemodynamic changes were captured by the flow probes or pressure probes and translated into analog readings on the blood flowmeter (model TT206, Transonic Systems). From here, the readings were converted to digital signals and fed into a computer system. This allowed changes in BP or blood flow to be reflected as a series of oscillating waves on the computer screen. A computer program, Windaq browser software, is used “online” to detect the maximum and minimum of the wave to calculate the average value. It was also used to record the number of wave cycles per second, which reflected the number of heartbeats.

Heart rate (HR), mean arterial BP, coronary flow, carotid flow, pulmonary pressure, LV end-diastolic pressure (LVEDP), first-order derivative of LV pressure at 30 mmHg (dP/dt 30 mmHg), and maximum dP/dt (dP/dt max) were measured as a series of oscillating waves on the computer screen. A computer program, Windaq browser software, was used “online” to detect the maximum and minimum of blood flow, summed up the number of maximums captured for every minute (60 s) and heart rate was calculated as number of maximums captured divided by 60 s.

The dP/dt was calculated according to the measurement of LV pressure. On the other hand, coronary flow and carotid flow were measured with Transonic flow probes connected to a blood flowmeter.

| Blood sample for histamine release collection. Blood samples (2 ml) were withdrawn after 5, 10, and 30 min after every dose of human UII administration and after the breakdown (acute reduction in recorded BP or the absence of pulsatile pressure) of the cardiovascular system or a sudden change in the recorded parameters. Blood volume was replaced with the use of Ringer’s lactate solution.

Table 1. Baseline of hemodynamic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (± SE)</th>
<th>Units</th>
<th>Value (± SE)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, Heart/min</td>
<td>90.9 ± 5.6</td>
<td>75.3 ± 3.8</td>
<td>57.8 ± 3.2</td>
<td>LV dP/dt max, 1,000 mmHg/s</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVP, left ventricular pressure; HR, heart rate; BP, blood pressure; CoBF, coronary blood flow; CaBF, carotid blood flow; PP, pulmonary pressure; dP/dt max, maximum first-order derivative of LVP.
Fig. 1. Human urotensin II (UII) produces a dose-dependent cardiovascular dysfunction in anesthetized cynomolgus monkeys. Changes of hemodynamic parameters after systemic administration of 0.03 nmol/kg (†) and 0.3 nmol/kg (‡) human UII (n = 4). Bolus intravenous applications of UII are indicated by the arrows. Heart rate (HR) (A), mean arterial blood pressure (MBP) (B), maximum first derivative of left ventricular pressure (dP/dt max) (C), pulmonary pressure (D), coronary blood flow (E), and carotid blood flow (F). *P < 0.05 and **P < 0.001, relative to baseline measurement before human UII administration (n = 4). Values are means and vertical bars show standard deviation.

Quantitation of histamine in monkey plasma. Determination of histamine in monkey plasma was performed via a RIA kit (IM1659, Immunotech; Beckman, France). The RIA is based on the competition between histamine in the sample and the tracer for the binding to an antibody-coated tube. The kit provides antiacylated histamine antibody-coated tube. The kit provides antiacylated histamine antibody-coated tube. The kit provides antiacylated histamine antibody-coated tube. The kit provides antiacylated histamine antibody-coated tube. The kit provides antiacylated histamine antibody-coated tube.

Statistical analysis. Data are represented as means ± SE. All data were analyzed by a one-way ANOVA for independent evaluations over all groups. If P < 0.05, differences between individual groups were analyzed by the use of Student’s t-test. Significance was accepted when the P value was <0.05.

RESULTS

Systemic administration of 0.03 nmol/kg bolus intravenous dose of human UII in the cynomolgus monkeys reduced heart rate by 13% (Fig. 1A) (P < 0.05) and mean arterial BP by 13% (Fig. 1B) (P < 0.05) compared with baseline values before human UII administration. The dP/dt was reduced by 11% (Fig. 1C). Pulmonary pressure (Fig. 1D) was not affected by this dose of human UII. Regional blood flows (coronary flow and carotid flow) were slightly reduced after 0.03 nmol/kg by 7% (Fig. 1E) and 9% (Fig. 1F), respectively. Similarly, a dose-dependent reduction of LV systolic pressure, LVEDP, and dP/dt at a LV pressure of 30 mmHg was observed, as shown in Table 2.

Bolus intravenous application of 0.3 nmol/kg human UII significantly decreased dP/dt by 45% (Fig. 1C) (P < 0.05), heart rate by 50.3% (P < 0.001) (Fig. 1A), and mean arterial BP by 65% (Fig. 1B) (P < 0.001). A drop in regional blood flow was observed, with a profound reduction of 30% (P < 0.05) in coronary blood flow (Fig. 1E) and 38% (P < 0.05) in carotid blood flow (Fig. 1F). Pulmonary pressure was increased by 30% after application of 0.3 nmol/kg UII (Fig. 1D).

Interestingly, a transient (2 to 3 min) increase in heart rate (Fig. 1A) and mean arterial BP (Fig. 1B) was observed after bolus intravenous injection of 0.03 and 0.3 nmol/kg UII. The

### Table 2. LVSP, LVEDP, and LV dP/dt 30 mmHg after administration of UII

<table>
<thead>
<tr>
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<th>LVSP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>LV dP/dt, 30 mmHg and 1,000 (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>65.2±5.6</td>
<td>19.7±2.8</td>
<td>0.90±0.04</td>
</tr>
<tr>
<td>0.03 nmol/kg UII</td>
<td>45.1±4.1</td>
<td>11.6±3.1</td>
<td>0.78±0.03</td>
</tr>
<tr>
<td>0.3 nmol/kg UII</td>
<td>32.2±3.2</td>
<td>6.7±1.8</td>
<td>0.48±0.02</td>
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</table>

Values are means ± SE. UII, urotensin II; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; LV dP/dt 30 mmHg, dP/dt at a LVP of 30 mmHg; N/A, not applicable.
effect was significant at a dose of 0.03 nmol/kg. All of the monkeys died after systemic administration of the highest applied dose of 3 nmol/kg human UII. Death was characterized by a profound increase in pulmonary pressure, depressed \( \frac{dP}{dt} \), and reduced regional blood flow (carotid and coronary) (see Fig. 1).

To determine whether the breakdown of the cardiovascular system may in part be mediated by an anaphylactic reaction, plasma histamine levels in monkeys were determined before and 5, 10, and 30 min after application of each UII dose and additionally after a sudden change of hemodynamic parameters via a RIA as outlined in detail in the experimental section. Normal plasma histamine levels are in the range of 0.02–0.6 ng/ml blood. The RIA determines plasma histamine concentrations in the range of 0.111–400 ng/ml blood. The minimum quantitation limit of the RIA was 0.111 ng histamine/ml blood. Histamine plasma levels were at all time points below the minimum quantitation limit of 0.111 ng/ml blood (data not shown).

Only one monkey showed histamine plasma levels ranging from 0.5 to 1.3 ng/ml, which are still being considered as normal and far from plasma levels reported during an anaphylactic shock (3, 7).

**DISCUSSION**

The cloning of the cDNA encoding human UII, and the identification of this peptide as the endogenous ligand for the orphan receptor, GPR14, have stimulated interest in the potential physiological and pathological roles of human UII (1, 11, 23, 26, 29). The present study was set out to characterize the cardiovascular effects of systemic administration of human UII in anesthetized cynomolgus monkeys and to elucidate the mechanism underlying cardiovascular breakdown and death. Ames et al. (1) have shown that infusion of UII into nonhuman primates leads to extraordinary increases in systemic vascular resistance and a profound reduction of cardiac output, ultimately causing fatal cardiovascular collapse. Although Ames and colleagues recorded several different cardiovascular parameters (cardiac output, heart rate, stroke volume, myocardial contractility, mean arterial pressure, total peripheral resistance, and LVEDP), several questions remained unanswered: 1) how does UII influence coronary flow, 2) how does UII influence pulmonary pressure, and 3) does the rapid circulatory collapse observed after the highest-applied concentration of UII have an anaphylactic component. The determination of these additional parameters was thought to help understand the mechanism underlying the fatal cardiovascular effects of UII in cynomolgus monkeys.

**Dosage of UII.** At doses of 0.03 and 0.3 nmol/kg, a transient tachycardia was observed accompanied by a transient increase in mean arterial BP, probably reflecting an immediate early response to an increase in total peripheral resistance. These transient adaptive responses were rapidly followed by a decrease in heart rate and mean arterial BP (Fig. 1, A and B). In addition, UII decreased myocardial contractility (Fig. 1C), coronary flow (Fig. 1E), carotid blood flow (Fig. 1F), and LVEDP (Table 2). Hassan and colleagues (19) reported similar findings, showing that an intravenous bolus injection of human UII resulted in a dose-dependent decrease in BP and cardiac contractility (dP/dt) in rats (19), although they did not observe a concomitant change in HR. In cynomolgus monkeys, however, HR decreased by 13% and 50.3% after UII doses of 0.03 and 0.3 nmol/kg iv, respectively. Species differences may account for these discrepant observations.

**Systemic and peripheral vascular effect.** Contrary to the present study, UII has been reported to be a potent positive inotropic agent in human right atrial and right ventricular trabeculae from explanted hearts (31). Direct effects of UII on myocardial trabeculae from cynomolgus monkeys have not been determined yet, but given that myocardial preparations of humans and nonhuman primates respond equally, direct cardio depressive actions of UII in monkeys may be ruled out as a cause for decreased cardiac output. Nevertheless, the conclusive experiment to elucidate the reason for decreased cardiac output would be to test UII effects on monkey myocardial preparations.

As shown in the Table 3, which represents a theoretical analysis of the putative effects of UII on the heart and total peripheral resistance based on the hemodynamic parameters such as HR, BP, and dP/dt 30 mmHg, a decrease of dP/dt 30 mmHg and HR suggests a direct inhibitory effect of UII on the heart, thus leading to three potential explanations raised in conjecture 1–3 (Table 3). However, the direct action of UII on peripheral resistance is debatable. If UII extraordinarily increased total peripheral resistance as shown by Ames et al. (1) in nonhuman primates, the BP value would be a result of counter balance decreased cardiac output and increased total peripheral resistance and may not show a significant change (conjecture 2 in Table 3). Moreover, if UII increases or has no effect on total peripheral resistance, as speculated in Table 3 (conjectures 2 and 3), UII-induced inhibition of cardiac pumping capacity would lead to a reduction of cardiac stroke volume and an increase of LV end-diastolic volume and preload, which may result in an increase of LVEDP. Nevertheless, in the present study, UII administration significantly lowered LVEDP during an inhibition of LV pumping capacity, suggesting an extraordinary inhibitory effect of this peptide on total peripheral resistance that might in turn reduce LV workload and subsequently cause a decrease of LVEDP (conjecture 1 in

<table>
<thead>
<tr>
<th>Conjectures</th>
<th>Heart (negative or positive inotropic and chronotropic effects)</th>
<th>Total peripheral resistance (vasoconstriction or vasoconstriction of peripheral resistance vessel beds)</th>
<th>Possible Hemodynamic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative</td>
<td>Decrease</td>
<td>HR decrease</td>
</tr>
<tr>
<td>2</td>
<td>Negative*</td>
<td>Increase</td>
<td>BP decrease</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>No change</td>
<td>dP/dt decrease</td>
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*Negative inotropic and chronotropic effects (decreased heart rate and myocardial contractility).
Table 3). Therefore, the hemodynamic parameters finally elucidated the cardiovascular effects of UII in cynomolgus monkeys: a negative inotropic and chronotropic effect on the heart is combined with a reduction of total peripheral resistance. We are aware that our observations are in contrast to the recordings reported by Ames and colleagues (1) and can only speculate that the origin of the animals may play a role for the discrepant observations in both studies.

Coronary and pulmonary vascular effect. It has been just shown that UII is a unique vasoconstrictor: it is a potent and efficacious spasmogen in a series of arterial vessels from cynomolgus monkeys, such as coronary and pulmonary arteries, renal, femoral, mammary, basilar, carotid, and mesenteric arteries while having no effect on venous vessels (12). However, the variable vasoactive profile of UII from a potent vessel constrictor to a vessel dilator, which is species- and anatomically dependent, distinguishes UII from other known vasoconstrictors, such as endothelin and angiotensin II (1, 5, 6, 12, 21, 22, 28, 32, 33). The latter two simultaneously increase afterload (arterial vasoconstriction) and preload (venous vasoconstriction) in addition to stimulating their cardiac receptors to increase contractility, which overall can be regarded as physiological response of an intact circulation. In contrast, UII selectively constricts or dilates arterial vessels in a species- and anatomically dependent pattern and “forgets” to increase preload and myocardial contractility.

As we observed from the original data (Fig. 2), the reduction in coronary blood flow lagged behind the changes of ventricular myocardial contractility and heart rate suggesting that the UII-induced vasoconstriction of coronary artery is a metabolic adaption secondary to the negative inotropic and chronotropic effect of UII but not a direct action of UII on coronary arteries.

An additional important parameter that has not been experimentally addressed yet is the influence of UII on pulmonary pressure. As shown in Fig. 1D, UII potently increases pulmonary pressure. These in vivo observations are supported by the in vitro findings of Douglas et al. (13) and Hay et al. (21), who reported UII to potently and efficaciously constrict monkey pulmonary arteries. Thus the combination of an inhibition of cardiac pumping capacity, regionally selective (arterial) vasoconstriction (1, 13) and vasodilatation, decrease of total peripheral resistance, and increase in pulmonary pressure may be collectively regarded as factors causing fatal circulatory collapse.

Is UII a vasoconstrictor? It should be of high interest to determine whether the maladaptive response in monkeys on UII application resembled that in humans. UII is assumed as most potent vasoconstrictor in arterial vessels of cynomolgus monkeys (13). All vessels tested responded to UII being up to 50 times more potent than ET1 constricting coronary and mammary arteries, although 30% of the vessels did not respond to UII; furthermore, despite the high potency, efficacy was so low as to be questionable in terms of biological significance. Sturrat et al. (32) characterized UII as a very potent dilator in human pulmonary and abdominal resistance arteries. Hillier et al. (22) questioned the vasoactive role of UII in humans because they did not detect either constrictor or dilator effects on human arteries and veins of different sizes and vascular beds (small subcutaneous resistance arteries, internal mammary arteries, saphenous veins, and small subcutaneous veins). Camarda et al. (6) reported UII to constrict some human vessels (umbilical artery, umbilical vein, and epigastric vein) and to be without effect on others (splanic and mesenteric inferior artery, renal, and saphenous vein) with UII effects being characterized as highly potent but of very low efficacy. On the basis of these contradictory findings, it was difficult to predict effects of UII in humans in vivo. Few reports on the in vivo effects of UII in human are available so far: Böhm and Pernow (4) infused UII into the brachial artery of nine healthy volunteers and recorded changes in forearm bloodflow by venous occlusion plethysmography, identifying UII as a potent vasoconstrictor in humans in vivo. In contrast, Wilkinson et al. (33) failed to observe any vasoconstriction using a similar, if not identical study protocol. Apparently, conflicting human in vitro data resembled the in vivo situation and the precise role of UII in human cardiovascular homeostasis still awaits clarification. Clearly, development of selective UII receptor antagonists would assist greatly in defining whether the UII/UII receptor system is of clinical significance in human cardiovascular pathophysiology. On the other hand, in vitro data on vasoactive properties of UII in monkeys (13, 21) are in close agreement with the in vivo data of two independent groups (Ref. 3 and present study), demonstrating that UII physiology shows less variability in nonhuman primates. Given the high conservatism of the cyclic hexapeptide sequence of UII during evolution from fish to mammals, it can been inferred that UII and its G protein coupled receptor play a seminal role in the physiological regulation of major mammalian organ systems, most notably within the cardiovascular. However, the intriguing questions remain in attempts to understand the mechanisms that underlie such intraspecies disparities.

Possible presence of shock? Besides the characterization of hemodynamic properties of UII in anesthetized monkeys, our goal was to determine whether anaphylactic shock might have occurred on intravenous bolus application of UII, particularly after high doses. Toward that goal, blood samples were withdrawn before and at 5, 10, and 30 min after application of each
UII dose and additionally after a sudden change of recorded parameters and plasma histamine levels were determined with RIA. Our assay allowed us to quantify histamine levels from 0.111–400 ng/ml blood. Normal plasma histamine levels range from 0.02 to 0.6 ng/ml in blood; however, we were unable to detect any significant change in plasma histamine levels. Only one monkey allowed detecting histamine levels of 0.5–1.3 ng/ml blood, but these concentrations are still regarded as normal and far from plasma levels reported for an anaphylactic situation (3, 7).

In summary, the present study demonstrates that systemic administration of human UII into anesthetized cynomolgus monkeys exhibits a complex hemodynamic profile and culminated in severe pulmonary hypertension, myocardial depression, and fatal circulatory collapse. Our data, together with literature findings (1, 12), suggest that death occurs due to a combination of several different effects of UII: 1) negative inotropic and chortropic effects on the heart; 2) decreases afterload due to potent arterial vasodilatation; and 3) increase in pulmonary pressure in agreement with UII potently constricting pulmonary vessels from cynomolgus monkeys, supporting the development of failure of the right ventricle. It should be emphasized that the observed responses, i.e., the pharmacological effects are the results of bolus administration of UII. The true contribution of UII to control of cardiovascular homeostasis and pathophysiology awaits development of UII receptor antagonists.

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GRANTS

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