Urotensin II receptor antagonist attenuates monocrotaline-induced cardiac hypertrophy in rats

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Departments of [1]Physiology and [2]Pathology, Diabetic Research Center, Chonbuk National University Medical School, Jeonju; and [3]Department of Physiology, College of Medicine, Yonsei University, Seoul, Korea

Gao S, Oh YB, Shah A, Park WH, Chung MJ, Lee YH, Kim SH. Urotensin II receptor antagonist attenuates monocrotaline-induced cardiac hypertrophy in rats. *Am J Physiol Heart Circ Physiol* 299: H1782–H1789, 2010. First published September 24, 2010; doi:10.1152/ajpheart.00438.2010.—Urotensin II (UII) is a vasoactive peptide with potent cardiovascular effects through a G protein-coupled receptor. Hypoxia stimulates the secretion of UII and atrial natriuretic peptide (ANP). However, the effect of UII on hypoxia-induced cardiac hypertrophy is still controversial. The present study was conducted to determine whether human UII (hUII)-mediated ANP secretion influences hypoxia-induced cardiac hypertrophy using in vitro and in vivo models. Hypoxia caused an increase in ANP secretion and a decrease in atrial contractility in isolated perfused beating rat atria. hUII (0.01 and 0.1 nM) attenuated hypoxia-induced ANP secretion without changing the atrial contractility, and the hUII effect was mediated by the UII receptor signaling involving phospholipase C, inositol 1,3,4 trisphosphate receptor, and protein kinase C. Rats treated with monocrotaline (MCT, 60 mg/kg) showed right ventricular hypertrophy with increases in pulmonary arterial pressure and its diameter and plasma levels of UII and ANP that were attenuated by the pretreatment with an UII receptor antagonist, urantide. An acute administration of hUII (5 μM injection plus 2.5 μM infusion for 15 min) decreased the plasma ANP level in MCT-treated rats but increased the plasma ANP level in MCT plus urantide-treated and sham-operated rats. These results suggest that hUII may deteriorate MCT-induced cardiac hypertrophy mainly through a vasoconstriction of the pulmonary artery and partly through the suppression of ANP secretion.

atrial natriuetic peptide; hypoxia; pulmonary hypertension

UROTENSIN II (UII) is a neuropeptide originally isolated from teleost urophysis (12) and has been cloned recently from humans (1, 13). UII has been identified as a selective ligand of G protein-coupled receptor termed UII receptor (1, 27, 29). The stimulation of the UII receptors by UII activates the $G\alpha_q$-phospholipase C_β pathway, generating diacylglycerol (DAG) and inositol 1,3,4-trisphosphate (IP_3) that stimulate protein kinase C (PKC) and Ca^{2+} release from intracellular stores, respectively (1, 29, 35). Human UII (hUII) is a potent vasoconstrictor in the artery, which is more potent than that of endothelin-1 (1, 27). hUII also causes positive inotropy in human and rat myocardium (21, 34) and mitogenesis in vascular smooth muscle cells (36, 40). However, negative inotropy in rabbit papillary muscle (19) and vasodilation by hUII have also been reported (17).

Although the expression of the UII receptor is relatively low to undetectable in normal myocardium, UII and its receptor are highly expressed in the cardiovascular system and upregulated in pathological conditions such as ischemic (43, 44) and chronic hypoxic myocardium (41). An upregulation of the UII receptor may worsen cardiac hypertrophy (3, 30) and cardiac injury under ischemia-reperfusion (43). In contrast, the beneficial effects of UII on decreasing coronary flow and cardiac contractility induced by ischemia-reperfusion have also been reported (32). Thus the effects of UII on cardiac function in pathological conditions are still controversial and remain to be clarified.

Recently, we have shown that hUII stimulates high-pacing frequency-induced atrial natriuretic peptide (ANP) secretion in isolated perfused beating atria of normal rats (20). ANP is a well-known cardiac hormone participating in the regulation of body fluid and blood pressure (BP) (28). ANP causes natriuresis, diuresis, and vasodilation, followed by a reduction in BP (15). In this study, we investigated the effects of UII on hypoxia-induced ANP secretion and cardiac hypertrophy using isolated perfused atria and rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, obtained from Orientbio (Seoungnam, Korea), were housed in a temperature-controlled room with a 12-h:12-h light-dark cycle. Animals were provided with free access to standard laboratory chow (5L79 Purina rat and mouse 18% chow, Charles River, Wilmington, MA) and water. All of the experimental protocols conformed to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, Revised 1996) and were approved by the Institutional Animal Care and Use Committee of the Chonbuk National University.

Preparation of isolated perfused rat atria. Isolated perfused beating atria were prepared using a method described previously (22). In brief, hearts were rapidly excised after decapitation, and left atria were dissected and inserted into a cannulus and ligated by a silk. Cannulated atria were kept in an organ chamber perfused with oxygenated HEPES-buffered saline at 37.0°C, and the atrium was then paced at 1.2 Hz (duration, 0.4 ms; and voltage, 30 V). The composition of HEPES-buffered saline was as follows: (in mM) 10 HEPES, 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, and 10 glucose and 0.1% bovine serum albumin (pH 7.4). Intra-atrial pressure was recorded using a power lab (ML-820, ADInstruments) via a pressure transducer (Statham P23Db, Oxnard, CA), and pulse pressure was obtained from the difference between systolic and diastolic pressure. The pericardial buffer solution, which contained [^{3}H]inulin (Amersham Biosciences) for measurement of extracellular fluid (ECF) translocation, was also oxygenated via silicone tube coils inside the organ chamber. The atrium was perfused for 70 min to stabilize ANP secretion and to maintain a steady-state [^{3}H]inulin level in the extracellular space. Atrial perfusate was collected five times at 2-min intervals as a control period, and perfusate was then changed to HEPES buffer, which was preexposed to N_2. Atrial perfusate was continuously collected for 40 min at 4°C. Partial pressure of O_2 (P_{O_2})

in HEPES buffer was measured during normoxia and hypoxia using an autoanalyzer (Roche Omni C, Roche Diagnostics).

Experiments were performed with three groups. *Group 1* included control atria exposed to N_2-containing HEPES buffer after the control period ($n = 10$). *Group 2* included atria exposed to N_2-containing HEPES buffer and hUII (0.01 nM, $n = 4$; 0.1 nM, $n = 8$) after the control period in the presence of hUII. *Group 3* included atria exposed to N_2-containing HEPES buffer with hUII (0.1 nM) in the presence of an UII receptor antagonist (urantide, 1 µM; $n = 4$), an inhibitor for phospholipase C (PLC; U-73122, 5 µM; $n = 5$), phosphoinositide 3-kinase (PI3K; wortmannin, 0.1 µM; $n = 6$), IP_3 receptor [2-aminoethyldiphenylborinate (2-APB), 10 µM; $n = 7$], or PKC (GF109203x, 1 µM; $n = 5$) during hypoxia. The antagonist or inhibitor was pretreated from 30 min before starting the sample collection, and N_2-containing HEPES buffer with hUII (0.1 nM) was then perfused after the control period. For an evaluation of the effects of inhibitor itself, urantide or the inhibitor was pretreated from 30 min before starting the perfusate collection and N_2-containing HEPES buffer was then perfused ($n = 4–7$).

Chronic infusion of urantide in monocrotaline-treated rats. Male rats weighing 160–180 g were anesthetized by an intraperitoneal injection of a mixture of ketamine and xylazine (9:1, 2 ml/kg). Before implantation, a mini-osmotic pump (Alzet 2002, Cupertino, CA) was

filled with urantide solution and kept in autoclaved saline (37°C) for 8 h. After a transverse incision in the scapular region (1.0–1.5 cm), the osmotic pump was implanted subcutaneously between the scapula and urantide was infused for 14 days at a dose of 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$. Sham-operated rats were anesthetized and operated with the same procedure, except for the implantation of osmotic pump (37). Two days after implantation, the rats were given a single subcutaneous injection of 60 mg/kg monocrotaline (MCT) or vehicle. The rats were euthanized at 2 wk by decapitation, and the blood was collected. Body weight and right ventricle, left ventricle plus septum, left atrium, and right atrium weights were measured. Tissue and plasma were stored at −70°C until assayed.

Acute intravenous infusion of hUII in MCT-treated rats. Male Sprague-Dawley rats were given a single subcutaneous injection of 60 mg/kg MCT in the absence and presence of urantide or vehicle (26). After 2 wk, the rats were anesthetized by an intraperitoneal injection of a mixture of ketamine and xylazine (9:1, 2 ml/kg). Body temperature was maintained at 36 to 37°C using a heating pad. After a midline incision in the neck, the jugular vein and carotid artery were carefully dissected, cannulated with polyethylene tube (PE-50), and secured with ligation. The cannula in the jugular vein was connected to a peristaltic pump (Minipuls 2 Gilson, Villiers le Bel, France) for infusion of vehicle (0.9% NaCl) and hUII in 0.9% NaCl at a constant rate of 60 µl/min and the cannula in the carotid artery to the pressure
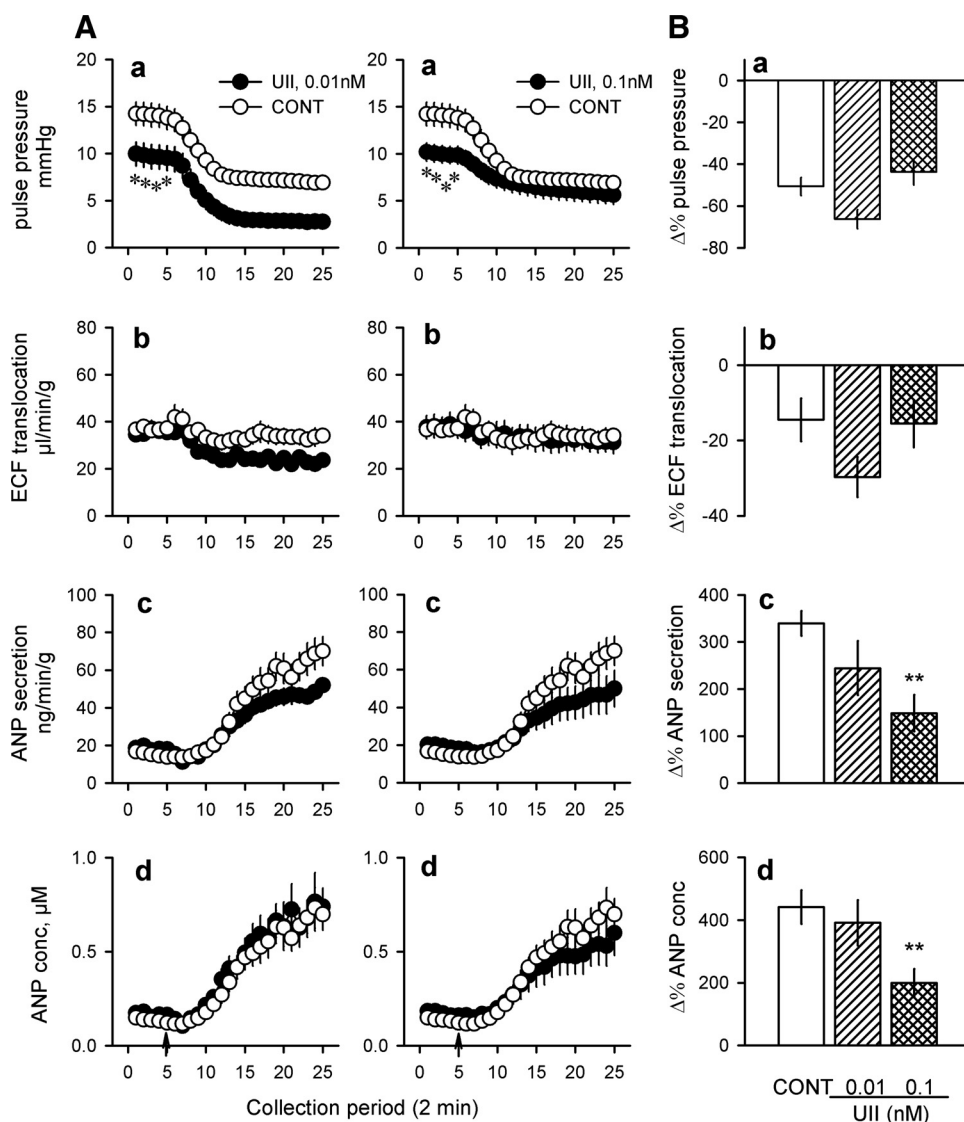

Fig. 1. *A*: effects of hypoxia on pulse pressure (*a*), extracellular fluid (ECF) translocation (*b*), atrial natriuretic peptide (ANP) secretion (*c*), and ANP concentration (Conc) (*d*) as a function of time in isolated perfused atria beating at 1.2 Hz in the absence and presence of human urotensin II (UII; 0.01 and 0.1 nM). Perfusate was collected every 2 min as a control at 1.2 Hz for 10 min, and then hypoxia was applied by changing oxygenated HEPES buffer to N_2-containing buffer. Perfusate was continuously collected for 40 min. Atria were exposed to UII simultaneously with hypoxia. Arrow indicates the exposure time to hypoxia. *B*: relative percent changes in pulse pressure (*a*), ECF translocation (*b*), ANP secretion (*c*), ANP Conc (*d*) by hypoxia in the absence and presence of different concentrations of UII. Values are expressed as percent changes of the mean of the last 5 experimental values (fraction no. 21–25) exposed to UII, compared with the mean of control values (fraction no. 1–5). Values are expressed as means ± SE. Cont, control hypoxia group. *$P < 0.05$ and **$P < 0.01$ vs. Cont.

transducer (Statham P23Db) (20). BP and heart rate (HR) were recorded using a power lab (ML-820, ADInstruments) via the pressure transducer. Animals were stabilized for 10 min with 0.9% saline infusion, and blood (800 μl) was collected via the carotid artery. hUII (5 μM) was injected followed by subsequent infusion (2.5 μM) through the jugular vein for 15 min. Blood was collected at 15 and 20 min after the start of hUII infusion. Blood was centrifuged at 10,000 g at 4°C for 10 min, and plasma was kept at −70°C until assay.

Measurement of right ventricular and pulmonary arterial pressure. Both right ventricular and pulmonary arterial pressures were measured by catheterization through right heart. In brief, rats were anesthetized by an intraperitoneal injection of a mixture of ketamine and xylazine (9:1, 2 ml/kg). The PE tube with a curl at the end of tube was cannulated into the jugular vein and reached the main pulmonary artery via the right ventricle while tracing pressure change onto a power lab (ML-820, ADInstruments) using pressure transducer (Statham P23Kb) connected with PE tube. After bleeding, the left ventricle and abdominal aorta were cut and 10 ml phosphate-buffered saline (0.01 M) was injected into the pulmonary artery through the cannula. Both lungs were isolated and stored in saline at 4°C for hematoxylin and eosin staining.

Lung histology and morphometric analysis. Lung sections (6 μm) were stained with hematoxylin and eosin and examined in a blind fashion using a light microscope. Images of arteries were captured with a microscope digital camera, and the internal and external diameter of the pulmonary arteries was measured. At least 30 arteries of comparable size (50–100 μm in diameter) per rat from each group were evaluated ($n = 3$). The percentage of medial thickness was determined by dividing the area occupied by the medial muscular layer by the total cross-sectional area of the artery (11).

Radioimmunoassay of ANP concentrations. The ANPs in plasma were extracted using a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) (7), and the concentration of ANP in perfusate and plasma was measured by a specific radioimmunoassay (RIA) as described previously (9, 10). The secreted amount of ANP in perfusate was expressed in nanograms per minute per gram of atrial tissue. We previously reported a two-step sequential mechanism of ANP secretion; first, the stored ANP is released from atrial myocytes into interstitial space by atrial distension, and second, the released ANP is secreted into the atrial lumen, concomitant with translocation of ECF by atrial contraction (6, 8). Therefore, the molar concentration of ANP release in the interstitium was calculated as follows:
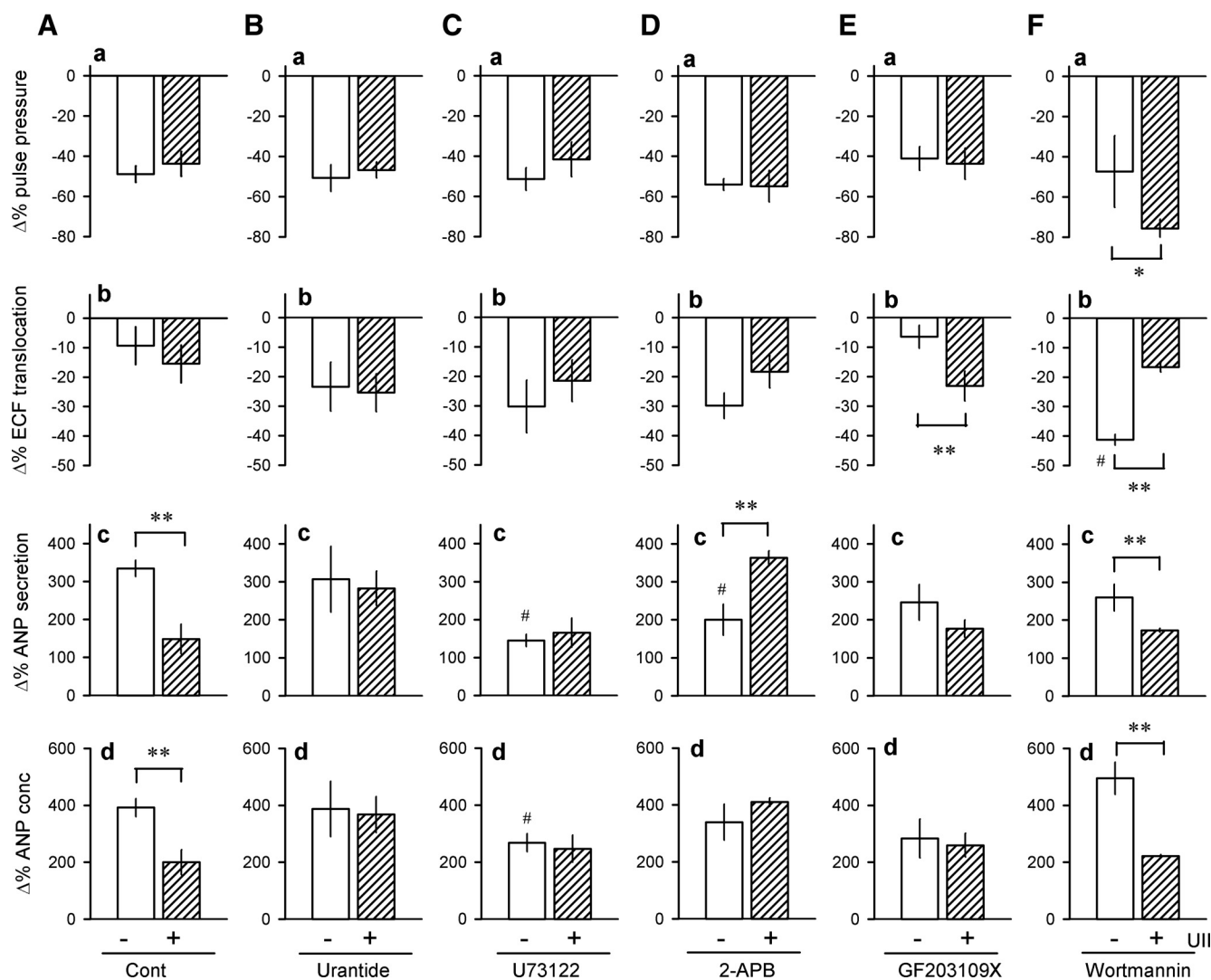

Fig. 2. *A*: modification of effects of UII on hypoxia-induced changes in pulse pressure (*a*), ECF translocation (*b*), ANP secretion (*c*), and ANP Conc (*d*) depicted as the relative percent changes in the presence of inhibitor for UII receptor and downstream signaling molecules. Antagonist or inhibitors [1 μM urantide (*B*), 5 μM U-73122 (*C*), 1 μM 2-aminoethyldiphenylborinate (2-APB; *D*), 1 μM GF-109203x (*E*), and 0.1 μM wortmannin (*F*)] was pretreated from 30 min before the start of sample collection. After 10-min control period, atria were exposed to N2-containing buffer and UII (0.1 nM). Cont, control group in the absence of inhibitor. *$P < 0.05$ and ** $P < 0.01$; #$P < 0.05$ vs. control group. Legend is the same as in Fig. 1.
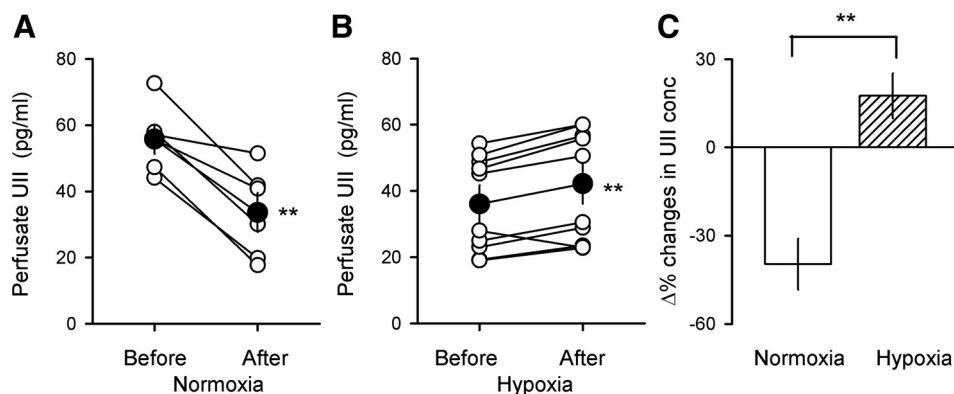
Fig. 3. Changes of UII level in perfusate in terms of time in control normoxic group (*A*) and in experimental hypoxic group (*B*). UII level was measured in perfusates collected from the 5 control periods (fraction no. 1–5) and last 5 experimental periods (fraction no. 21–25). *C*: values are expressed as the relative percent changes of the mean of experimental values, compared with means of control values. Values are means ± SE. **$P < 0.01$ vs. corresponding group.

ANP concentration (μM) =

$$\frac{\text{ANP} (\text{pg} \cdot \text{min}^{-1} \cdot \text{g}^{-1})}{\text{ECF translocation} (\mu\text{l} \cdot \text{min}^{-1} \cdot \text{g}^{-1}) \times 3{,}090}$$

The denominator 3,090 indicates the molecular mass of ANP_{1-28} (in Da) since the ANP secreted was found to be the processed ANP (6).

Measurement of ECF translocation. The radioactivity of [^{3}H]inulin in atrial perfusate was measured by a liquid scintillation counter (Tris-Carb 23-TR; A Packard Bioscience, Downers Grove, IL) (6, 8). The amount of ECF translocated (in μl·min^{-1}·g^{-1}) through the atrial wall was calculated as follows:

$$\frac{\text{Total radioactivity in perfusate (cpm/min)} \times 1{,}000}{\text{radioactivity in pericardial reservoir (cpm/}\mu\text{l)} \times \text{atrial wet weight (mg)}}$$

Measurement of UII concentration. UII in perfusate and plasma was extracted as the same method as ANP using a Sep-Pak C18 cartridge (Waters Associates) (7). The recovery rate was 81.5 ± 2.4% (*n* = 6). The UII concentration was measured using a rat UII RIA kit (Phoenix Pharmaceuticals, Bulingame, CA).

Real time-PCR. Total RNA was extracted from rat heart tissue using TRIzol reagent (Invitrogen, Carlsbad, CA), and reverse transcription was performed using Superscript II and 18-mers Oligo-dT (Invitrogen). Specific primers were designed using primer express software (Applied Biosystems, Carlsbad, CA), and their primer sequences were as follows: rat UII (accession no. NM_019160.1), 5'-GGCGAGAACCAGGAAA-CAAC-3' (forward) and 5'-CAAATGCAGTACTTCCAGAAGCA-3' (reverse); rat UII receptor (accession no. NM_020537.1), 5'-ACAGATCCCAGCTCCCTGAA-3' (forward) and 5'-GAGCACTGC-CCCGATGAC-3' (reverse); rat ANP (accession no. NM_012612.2), 5'-CCGGTACCGAAGATAACAGC-3' (forward) and 5'-CTCCAG-GAGGGTATTCACCA-3' (reverse); and rat actin (accession no. NM_031144.2), 5'-ACCAGTTCGCCATGGATGAC-3' (forward), and 5'-TGCCGGAGCCGTTGTC-3' (reverse).

Real-time PCR reaction contained in a final volume of 10 μl, 10 ng of reverse transcribed total RNA, 200 nM of forward and reverse primers, and 2 × PCR master mix. PCR reaction was carried out in
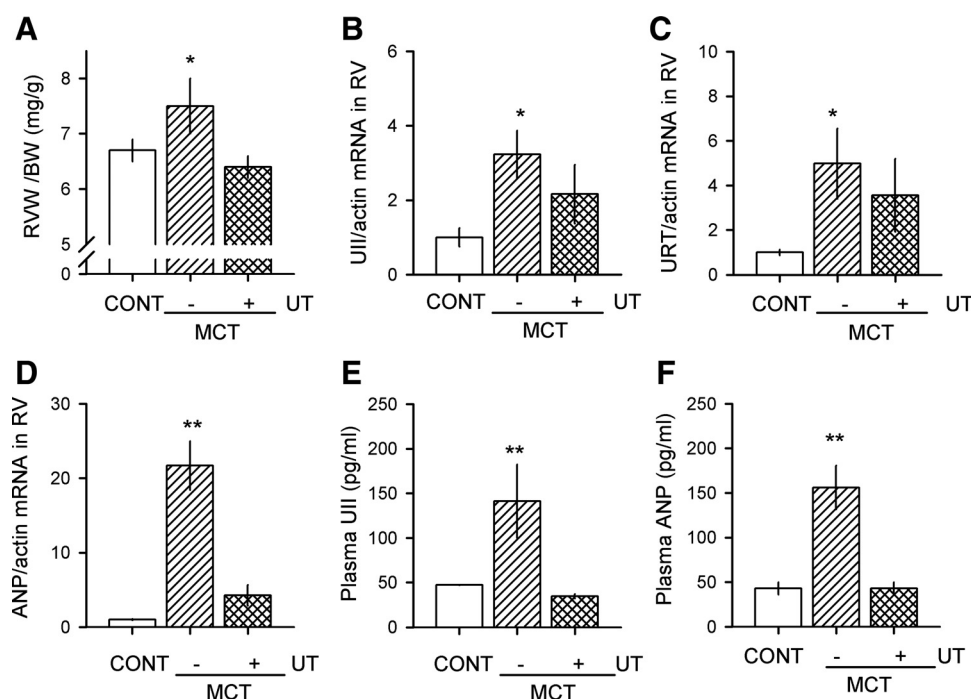

Fig. 4. Changes in right ventricular (RV) weight (RVW; *A*); expression of UII (*B*), UII receptor (URT; *C*), and ANP mRNA (*D*) in RV; and plasma concentrations of UII (*E*) and ANP (*F*) in monocrotaline-treated (MCT) rats in the absence and presence of urantide. Rats were given a single subcutaneous injection of 60 mg/kg MCT or vehicle. Two days before MCT injection, urantide or vehicle started to infuse using osmotic pump. Rats were euthanized 2 wk after MCT injection. UT, urantide-treated rats; RVW/BW, ratio of right ventricular weight to body weight; UII/actin, URT/actin, and ANP/actin mRNA, relative expression of UII, UII receptor, and ANP mRNA to actin mRNA in RV, respectively. *$P < 0.05$ and **$P < 0.01$ vs. control rats.

384-well plates using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). All reactions were done in triplicate.

Statistical analysis. Results are presented as means ± SE. Statistical significance of differences was assessed using ANOVA followed by Bonferroni multiple comparison test. Student's *t*-test was also used. The critical level of significance was set at $P < 0.05$.

RESULTS

Effects of hUII on hypoxia-induced atrial contractility and ANP secretion. After a collection of five samples as a control, the buffer was changed to N_2-preexposed HEPES buffer. Po_2 in control buffer was 181.7 ± 2.9 mmHg, and Po_2 during hypoxic condition was 49.83 ± 4.8 mmHg ($n = 6$). Hypoxia decreased atrial contractility (Fig. 1*A,a*), and ECF translocation remained unchanged (Fig. 1*A,b*). In contrast, hypoxia markedly increased ANP secretion and concentration (Fig. 1*A*, *c* and *d*). When atria were treated with hUII at a dose of 0.01 and 0.1 nM, the hypoxia-induced decrease of atrial contractility and ECF translocation were virtually unaffected. However, the hypoxia-induced ANP secretion and concentration were reduced. Figure 1*B* shows a relative percent change of the mean of the first five control values during normoxia and the last five experimental values during hypoxia in the absence or presence of hUII. Hypoxia increased ANP secretion by 320%. Pretreatment with hUII (0.1 nM) attenuated the hypoxia-induced ANP secretion by 130% (Fig. 1*B,c*).

Effects of hUII on hypoxia-induced ANP secretion in the presence of receptor antagonist or inhibitor of signaling modulators. Since it has been shown that the stimulation of the UII receptor by UII activates $G\alpha_q$-phospholipase C_β, producing two second messengers, DAG and IP_3, which stimulate

PKC and Ca^{2+} release from intracellular Ca^{2+} stores, respectively (1, 29, 35), we examined whether these signaling molecules regulate hypoxia-induced ANP secretion. Atria were pretreated with a UII receptor antagonist or inhibitors of these signaling molecules. As expected, an antagonist of UII receptor urantide (0.1 μM) blocked the suppressive effect of UII on the hypoxia-induced increase of ANP secretion and concentration (Fig. 2*B*, *c* and *d*) without affecting pulse pressure and ECF translocation. Pretreatment with U-73122 (PLC inhibitor, 5 μM) or 2-APB (IP_3 receptor inhibitor, 10 μM) attenuated the suppressive effect of hUII on hypoxia-induced ANP secretion and concentration (Fig. 2, *C* and *D*). The PKC inhibitor GF-109203x (1 μM) slightly attenuated the effect of hUII on the ANP secretion and concentration but without statistical significance (Fig. 1*E*). In addition, PI3K has been known to be activated in hypoxia and oxidative stress (18). An involvement of this kinase in hUII signaling was examined using wortmannin, an inhibitor of PI3K. Pretreatment with wortmannin (0.1 μM) did not block the hUII effect on hypoxia-induced ANP secretion (Fig. 2*F*, *c* and *d*), whereas the hUII/hypoxia-induced pulse pressure was decreased (Fig. 2*F,a*). U-73122 or 2-APB alone decreased hypoxia-induced ANP secretion and concentration, and wortmannin alone markedly decreased ECF translocation during hypoxia. Other inhibitors did not affect hypoxia-induced changes in these atrial parameters (Fig. 2).

To determine whether an acute exposure of atria to hypoxia increases the UII level, the UII level in perfusate was measured before and during hypoxia. The perfusate was collected during the first five control periods and the last five experimental periods, and UII was extracted and then assayed. The UII level in the perfusate of normoxic atria was decreased by 40% (Fig. 3, *A*
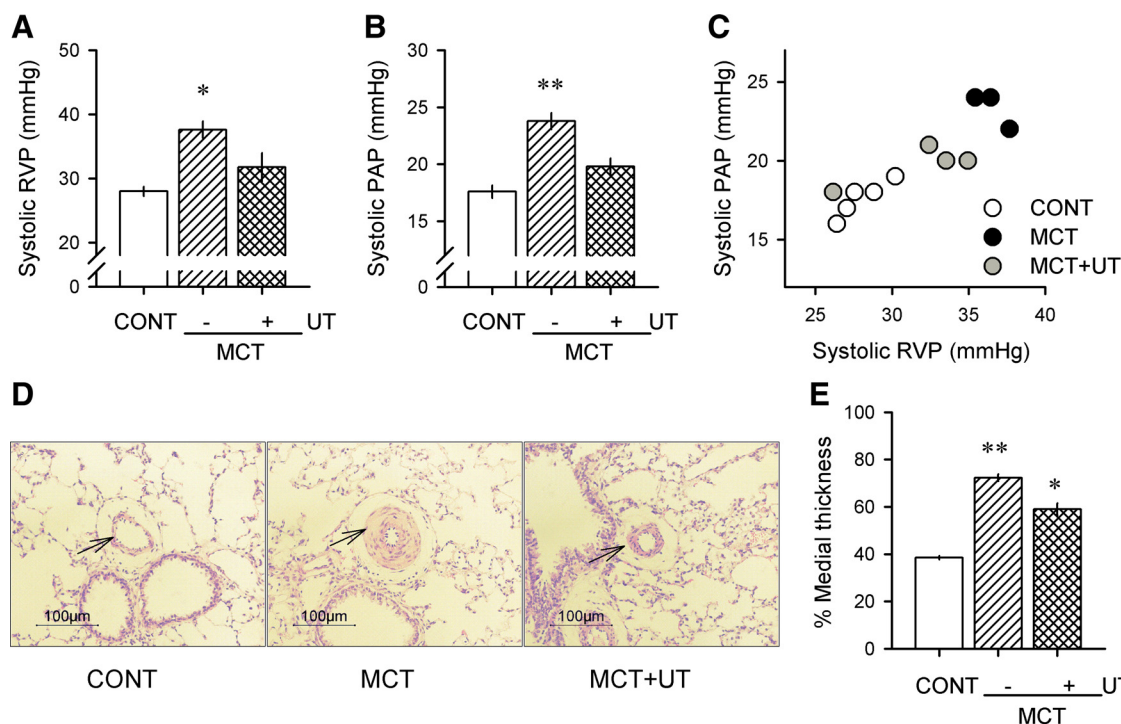

Fig. 5. *A* and *B*: changes in systolic RV pressure (RVP) and pulmonary arterial pressure (PAP) in control, MCT-treated, and MCT plus UT-treated rats. *C*: relationship between RVP and PAP. *D*: representative hematoxylin and eosin-stained peripheral lung sections from control and MCT-treated and MCT plus UT-treated rats. Pulmonary artery is indicated with arrows. Total magnification, ×400. *E*: quantitative morphological analysis of percentage of wall thickness of pulmonary artery. *$P < 0.05$ and **$P < 0.01$ vs. control group.

and *C*). In contrast, the UII level in perfusate of hypoxic atria was increased by 20% (Fig. 3, *B* and *C*).

Effects of urantide on MCT-induced cardiac hypertrophy. The above in vitro data indicated that UII suppresses hypoxia-induced ANP secretion, and ANP plays a critical role in regulating BP (15, 28). To examine the effects of hypoxia and UII on cardiac hypertrophy and ANP secretion, MCT was injected into rats to induce hypoxia in the absence or presence of urantide. The treatment of rats with MCT increased the ratio of right ventricle weight to body weight (Fig. 4*A*) and the expressions of UII (Fig. 4*B*), UII receptor (Fig. 4*C*), and ANP mRNAs (Fig. 4*D*) in the right ventricle compared with those in control rats. Plasma levels of UII (Fig. 4*E*) and ANP (Fig. 4*F*) were also increased substantially compared with the levels in control rats. The treatment of MCT-injected rats with urantide for 2 wk significantly attenuated ventricular hypertrophy and decreased the expression of ANP mRNA and the plasma UII and ANP levels. However, the expression of UII and URT mRNAs in the right ventricle was slightly decreased but without statistical significance (Fig. 4, *B* and *C*).

MCT-treated rats also showed higher right ventricular pressure (Fig. 5*A*) and pulmonary arterial pressure (Fig. 5*B*) than those in control rats. Pretreatment with urantide prevented the MCT-induced increase of right ventricular pressure and pulmonary arterial pressure. There was a positive correlation between right ventricular pressure and pulmonary arterial pressure (Fig. 5*C*) ($y = 0.56x + 2.32$, $r^2 = 0.85$, $P < 0.01$). Figure 5*D* shows the representative hematoxylin and eosin-stained peripheral lung sections from control normoxic, MCT-treated, and MCT plus urantide-treated rats. The percentage of wall thickness of the pulmonary arteries was greater in MCT-treated rats than that of control rats (Fig. 5*E*). Pretreatment with urantide attenuated wall thickening of the pulmonary arteries in MCT-treated rats.

Acute intravenous infusion of hUII in MCT-treated rats. To determine the acute effect of hUII on plasma ANP level and the hemodynamics in MCT-treated rats, 2.5 μM hUII was infused for 15 min after 5 μM bolus injection to anesthetized rats in the absence or presence of urantide. The infusion of hUII caused increases in mean arterial pressure, HR, and pulse pressure in MCT-treated rats, which were similar to control rats (Fig. 6*A*). With urantide-treated rats, the infusion of hUII caused increases in mean arterial pressure and pulse pressure but not HR (Fig. 6*B*). The infusion of hUII decreased the plasma concentration of ANP in MCT-treated rats (Fig. 6*A,d*). In contrast, hUII increased the plasma concentration of ANP in control and MCT plus urantide-treated rats (Fig. 6*B,d*).

DISCUSSION

In this study, we have examined the roles of UII in ANP secretion under hypoxic condition and in the pathogenesis of hypoxia-induced cardiac hypertrophy. The results revealed that UII suppresses ANP secretion from hypoxic atria via the UII receptor/PLC/IP$_3$ pathway. The administration of MCT caused right ventricular hypertrophy and increased pulmonary arterial pressure with an increased plasma UII and ANP levels that were attenuated by pretreatment with a UII receptor antagonist. The acute infusion of hUII also reduced the plasma ANP level in MCT-treated rats but not in MCT plus urantide rats. These results suggest that UII may deteriorate MCT-induced cardiac
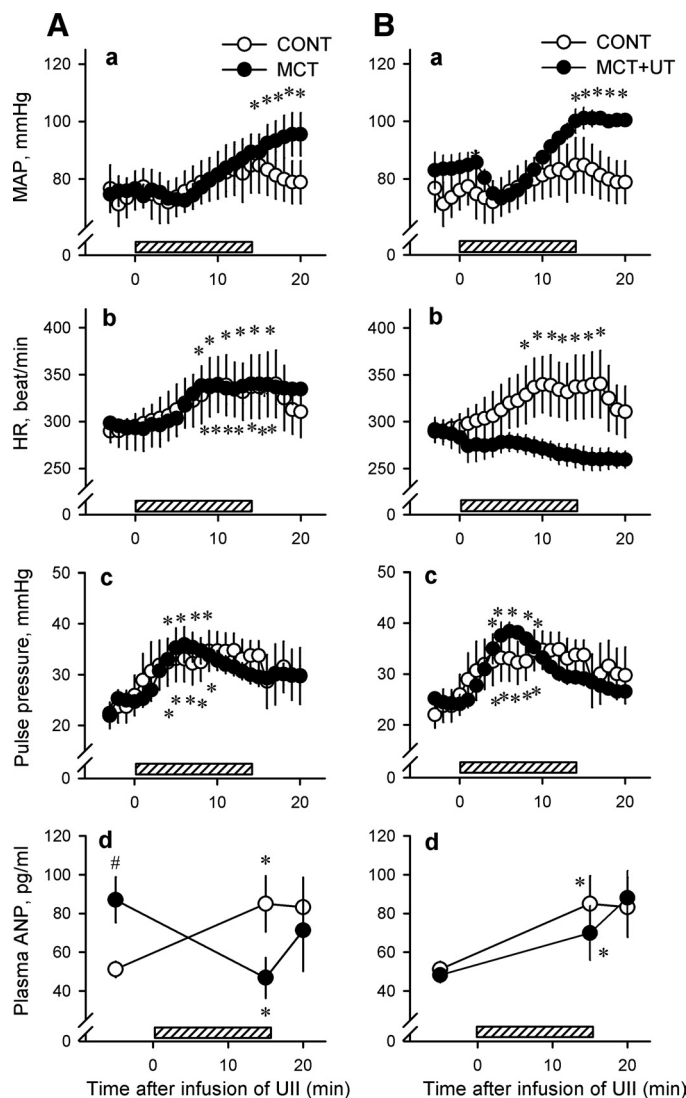

Fig. 6. Changes in mean arterial pressure (MAP; *a*), heart rate (HR; *b*), pulse pressure (*c*), and plasma ANP level (*d*) by acute infusion of UII in control, MCT-treated (*A*), and MCT plus UT-treated (*B*) rats. Human UII (5 μM) was injected intravenously and subsequently infused at a dose of 2.5 μM for 15 min (hatched bar). *$P < 0.05$ vs. control value; #$P < 0.05$ vs. control group.

hypertrophy mainly through the vasoconstriction of the pulmonary artery and partly through the suppression of ANP secretion.

Studies have shown that the plasma concentration of UII is elevated in various cardiovascular disorders (31, 39) and that the UII receptor is upregulated in ischemic (43) and chronic hypoxic myocardium (41). Hypoxia also stimulates UII secretion both in vitro and in vivo (32, 41). The present study showed that the exposure of isolated atria to hypoxia for 40 min stimulated UII secretion even though the amount of UII was relatively low. MCT-treated rats, an in vivo hypoxic model, showed an increased expression of UII and UII receptor mRNAs in the right ventricle and a high-plasma UII level compared with those in control rats. Thus our findings provide additional information on the regulation of UII and the expression of the UII receptor in hypoxia-induced cardiac hypertrophy.

ANP plays an important role in the normal adaptation to hypoxia and in the pathogenesis of acute and chronic pulmonary hypertension (5, 23). Hypoxia is a well-known stimulus for ANP secretion (5, 15, 23, 38). A hypoxia-induced increase of plasma ANP causes a vasodilation of pulmonary artery and attenuates hypoxia-induced pulmonary hypertension (2). In the present study, we have found that treatment of isolated atria with hUII inhibited hypoxia-induced ANP secretion and that the infusion of hUII into MCT-treated rats decreased the plasma ANP level. Our data are in good agreement with the report showing that rat UII inhibits ANP secretion induced by cardiac ischemia-reperfusion in Langendorff preparations (32). In contrast, we have previously reported that a low concentration of UII stimulates ANP secretion in normal atria paced with high frequency (20). Taken together, these observations suggest that UII may differently regulate ANP secretion in pathological versus physiological conditions. Since the mechanism of differential regulation of ANP secretion by UII is completely unknown, further studies are needed to address this issue.

Stimulation of UII receptors by UII activates $G\alpha_q$-phospholipase C_β, generating DAG and IP_3, which stimulate PKC and IP_3 receptors (1, 29, 35). Our results showed that hypoxia-induced ANP secretion is mediated by the stimulation of the UII receptor/PLC/IP_3 pathway. Thus urantide reversed UII-mediated inhibition of hypoxia-induced ANP secretion, and the treatment of perfused beating atria with a PLC inhibitor or an IP_3 receptor inhibitor alone decreased hypoxia-induced ANP secretion. However, the inhibitors for PI3K and PKC tended to decrease hypoxia-induced ANP secretion but without statistical significance. These results suggest that hypoxia induces the stimulation of PLC and IP_3 receptor, and these signaling molecules play a critical role in ANP secretion and concentration.

It is of interest to observe that hypoxia-induced ANP secretion in isolated beating atria was inhibited by a treatment with hUII but not by urantide, whereas a hypoxia-induced increase of the plasma ANP level as well as the expression in MCT-treated rats was inhibited by urantide. It has been suggested that a decreased plasma ANP level causes vasoconstriction of the pulmonary artery and worsens pulmonary hypertension (2). Therefore, it is possible that a high level of plasma UII observed in MCT-treated rats may suppress hypoxia-induced ANP secretion, followed by deteriorating cardiac hypertrophy. However, the plasma ANP level was higher in MCT-treated rats than that in urantide-treated rats. Ventricular ANP synthesis is reactivated by hypertrophy and then secreted constitutively into the circulation (15). Therefore, a high level of plasma ANP in MCT-treated rats may be due to the reactivation of ANP synthesis in hypertrophied ventricle. In fact, the level of ANP mRNA in the left ventricle of MCT-treated rats was increased, and the treatment with urantide reduced the levels of ANP mRNA and plasma ANP as well as ventricular hypertrophy, suggesting an important role of UII in the pathogenesis of the heart.

In addition, hUII has been shown to promote pulmonary vascular remodeling (16), and the vasoconstrictor response to UII is increased by pulmonary hypertension (14). Studies have also suggested that hypoxia causes pulmonary hypertension by UII-dependent activation of NADPH oxidase in the pulmonary vascular wall and that UII worsens the injury of the hearts under ischemia-reperfusion (4, 33, 43). MCT is known to cause an epithelial proliferation of small pulmonary arteries, followed by pulmonary hypertension and congestive heart failure (25). In our study, MCT-treated rats showed right ventricular hypertrophy, high pressure, and wall thickening of the pulmonary artery. These MCT-induced pathological features were attenuated by the treatment with urantide. Taken together, these findings demonstrate that hypoxia induces cardiac hypertrophy, at least in part, through wall thickening of the pulmonary artery and that the antagonists for the UII receptor may protect hypoxia-induced cardiac hypertrophy.

In conclusion, our results have shown that UII may deteriorate hypoxia-induced cardiac hypertrophy mainly through vasoconstriction of the pulmonary artery and partly through the suppression of ANP secretion. These findings suggesting that a blockade of the UT receptor improved hypoxia-induced cardiac hypertrophy.

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