Chronic urotensin-II infusion induces diastolic dysfunction and enhances collagen production in rats

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1Department of Epidemiology and Preventive Medicine, National Health Medical Research Council Centre of Cardiovascular Research and Education in Therapeutics, Monash University, Melbourne; 2Department of Medicine, University of Melbourne, St. Vincent’s Hospital, Melbourne; 3Alfred Hospital Gastroenterology and Hepatology Department, Melbourne, Australia; and 4Faculty of Medicine, Department of Internal Medicine, Chiang Mai University, Chiang Mai, Thailand

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Tran L, Kompa AR, Kemp W, Phrommintikul A, Wang BH, Krum H. Chronic urotensin-II infusion induces diastolic dysfunction and enhances collagen production in rats. Am J Physiol Heart Circ Physiol 298: H608–H613, 2010. First published December 11, 2009; doi:10.1152/ajpheart.00942.2009.—The vasoactive peptide urotensin-II (U-II) is likely to play a key causal role in cardiac remodeling that ultimately leads to heart failure. Its contribution, specifically to the development of diastolic dysfunction and the downstream intracellular signaling, however, remains unresolved. This study interrogates the effect of chronic U-II infusion in normal rats on cardiac structure and function. The contribution of Rho kinase (ROCK) signaling to these pathophysiological changes is evaluated in cell culture studies. Chronic high-dose U-II infusion over 4 wk significantly impaired diastolic function in rats on echocardiography-derived Doppler indexes, including E-wave deceleration time (vehicle 56.7 ± 3.3 ms, U-II 118.0 ± 21.5 ms; P < 0.01) and mitral valve annulus peak early/late diastolic tissue velocity (vehicle 2.01 ± 0.19 ms, U-II 1.04 ± 0.25 ms; P < 0.01). A lower dose of U-II infusion (1 nmol·kg⁻¹·h⁻¹) yielded comparable changes. Diastolic dysfunction was accompanied by molecular [significant increases in procollagen-I (P1) gene expression on real-time PCR] and morphological (increases in total collagen, P < 0.05, and collagen type-I protein deposition, P < 0.001) evidence of left ventricular (LV) fibrosis following high-dose U-II infusion. The ROCK inhibitor GSK-576371 (10⁻⁷ to 10⁻⁵ M) elicited concentration-dependent inhibition of U-II (10⁻⁷ M)-stimulated cardiac fibroblast collagen synthesis and cardiac myocyte protein synthesis. Chronic U-II infusion causes diastolic dysfunction, caused by fibrosis of the LV. The in vitro data suggest that this may be in part occurring via a ROCK-dependent pathway. Chronic U-II infusion on cardiac structure and function.

urotensin-II (U-II) is a potent vasoactive “somatostatin-like” cyclic peptide that first originated from the spinal cord of the Goby fish (Gillichthys mirabilis) (2) and now identified as the most potent vasoconstrictor in humans (1). Its receptor, the human orphan receptor GPR14 (now known as the UT receptor) is expressed in the brain, heart, kidney, adrenal glands, placenta, and colonic mucosa (26).

Plasma U-II levels are increased in patients with hypertension and other disease states such as chronic heart failure, atherosclerosis, renal failure, diabetes mellitus, cirrhosis, and portal hypertension (7, 25, 26). Although many studies have demonstrated that U-II is a vasoconstrictive, fibrotic, and hypertrophic modulator, the intracellular mechanism of action of U-II in cardiac cells has not been explored extensively (9, 13, 15, 24, 27, 29).

Although the involvement of Rho kinase (ROCK) in vascular pathophysiology has been explored, the mechanism and intracellular regulation in the heart per se has not been examined extensively. Furthermore, the direct effects of exogenous U-II on the heart have not been thoroughly investigated. The present study therefore sought to examine the effect of chronic U-II infusion on cardiac structure and function.

The RhoA-ROCK system is a key intracellular signaling pathway. ROCK is an intracellular serine-threonine kinase involved in the mediation of intracellular processes having several effects on cellular function, including smooth muscle contraction, cellular cytoskeletal formations and regulation of proinflammatory cytokines (8, 14, 16). It has also been found to mediate a number of actions of UT on functional activity of various cell types (8, 17). Interestingly, ROCK inhibition has been previously demonstrated by us to have beneficial effects on diastolic function in an animal model of pressure overload-induced hypertrophy with associated diastolic dysfunction (14). We were therefore interested in whether U-II-mediated effects on the heart may be occurring via a ROCK-dependent mechanism, at least in vitro.

MATERIALS AND METHODS:

In Vivo

Animals. Twenty male Sprague-Dawley rats (200–250 g) were randomized following baseline echocardiography (24 h before commencement of study) into one of three treatment groups [vehicle (saline); low-dose U-II 2.04 µg·kg⁻¹·h⁻¹; high-dose U-II 5.62 µg·kg⁻¹·h⁻¹]. Saline or rat U-II (GlyxoSmithKline, King of Prussia, PA) was continuously administered intravenously into the animals via an osmotic mini-pump (Alzet mini-osmotic pump, 0.25 µl/h; Durect) for 28 days. Animals were maintained on standard rat chow with water ad libitum.

Animal experiments were conducted in accordance with the protocols of and approved by the Alfred Medical Research and Education Precinct (AMREP) Animal Ethics Committee and conformed to the requirements of the National Health and Medical Research Council: Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004).

Surgery. Before osmotic mini-pump insertion (48 h), the pumps (200 µl) were filled with saline or U-II containing 10⁶ cpm of ¹²⁵I-labeled U-II and incubated at 37°C. Radioactivity (cpm) in the mini-pumps were measured before and after 28 days (adjusted for the rate of decay) to accurately determine the dose of U-II administered (10).
Animals were anesthetized with ketamine/xylazine (80 and 10 mg/kg, respectively, ip), and the mini-pumps were inserted subcutaneously over the right shoulder with the infusion catheter secured in the right internal jugular vein (10). The wound was closed, and animals were administered Temgesic (0.03 mg/kg sc buprenorphine) to reduce postoperative pain.

Echocardiography and hemodynamics. Echocardiography was performed on all rats under light anesthesia with ketamine/xylazine (40 and 5 mg/kg, respectively) 24 h before commencement of the study to obtain baseline measurements and on day 28 (end of study). Two-dimensional and m-mode images at the level of the mid-papillary muscle of the heart were obtained with a HP Sonos 5500 and a 12-MHz phased array probe (Agilent Technologies, Palo Alto, CA) to determine relative wall thickness (RWT) using a standard formula (18). Doppler images were obtained from the apical four-chamber view; early and late transmitral peak diastolic flow velocity (E and A waves), mitral valve (MV) inflow E wave deceleration time (DT), and isovolumic relaxation time (IVRT) were measured. Tissue Doppler imaging was performed and used to assess peak early (E') and late (A') diastolic tissue velocity. These procedures and measurements were performed as previously described (14).

Central aortic pressure (CAP) and heart rate (HR) were obtained at the end of study under anesthesia with pentobarbitone sodium (50 mg/kg ip). A precalibrated saline-filled catheter attached to a pressure transducer (model 105G; UFI) was inserted in the right carotid artery and then advanced in the aortic arch for measurement of arterial pressure. Traces were recorded on a MacLab/2e system (ADInstruments) and analyzed using Chart v3.6.8 (ADInstruments) to determine CAP and HR. Animals were then killed, and the heart was harvested as previously described (14).

Histology. Paraffin embedded left ventricular (LV) tissue sections (4 μm) were cut and mounted on silane-coated slides.

DETECTION OF COLLAGEN I. Slides were dewaxed, and antigen retrieval was performed in boiling 0.01 M citrate buffer for 15 min and then left to cool. Endogenous peroxidase was quenched in 3% H2O2 for 15 min followed by three washes with PBS. Sections were then blocked with 10% normal swine serum (Dako, Carpinteria, CA) followed by overnight incubation with goat anti-collagen I primary antibody at 4°C (Southern Biotech, Birmingham, AL). The next day, slides were washed with PBS, and biotinylated rabbit anti-goat was applied for 1 h at room temperature (Dako). Collagen I immunostaining was detected with avidin-biotin complex (Vectastain; Vector Laboratories, Burlingame, CA) followed by diaminobenzidine (Dako) staining. Slides were washed in distilled water, counterstained with hematoxylin, and dehydrated through graded ethanols before clearing in histolene and being covered with a cover slip.

DETECTION OF TOTAL COLLAGEN. Serial sections were stained for total collagen using picrosirius red. Briefly, after slides were dewaxed, 0.1% picrosirius red solution was applied to the slides for 1 h before a quick wash with 1% acidified water. The slides were dehydrated through graded ethanol before clearing in histolene and being covered with a cover slip.

Measurement of myocyte cross-sectional area. Tissue-Tek optimum cutting temperature-embedded tissues were stained with hematoxylin-eosin. The perimeters of 50 myocytes from each animal were determined using the Analytical Imaging Station (28) to determine the average percentage area of 10 random fields in the subendocardium of each LV was calculated using the Analytical Imaging Station software (AIS version 6; Imaging Research, St. Catherine’s, ON, Canada).

Quantitative mRNA expression in LV. Total RNA was extracted from frozen LV tissue using RNeasy columns (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Total RNA (2 ng) was reverse transcribed into cDNA and amplified in triplicate with sequence-specific primers (Geneworks, Adelaide, SA, Australia) and a TaqMan fluorogenic probe (Applied Biosystems, Foster City, CA). Real-time PCR was performed and quantified for mRNA expression of procollagen-α1(I), transforming growth factor-β1, (TGF-β1), and connective tissue growth factor (CTGF) using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The primer pairs and probes were designed using Primer Express 2.0 software (Applied Biosystems) based on published sequences (http://www.ncbi.nlm.nih.gov) as previously described (11). 18S rRNA was quantified and used as an endogenous control in all experiments.

In Vitro Studies

NEONATAL RAT CARDIOMYOCYTE AND FIBROBLAST CULTURES. Neonatal rat cardiac myocyte (NCM) and fibroblasts (NCF) were isolated from 1- to 2-day-old Sprague-Dawley pups with enzymatic digestion as described previously (14). NCM were seeded in gelatin-coated 12-well plates and maintained in serum-free DMEM (Invitrogen, Mountain Waverly, Vic, Australia) supplemented with insulin and transferrin. Bromodeoxyuridine was included for the first 3 days. KCI (50 mmol/l) was added to the medium to prevent spontaneous contraction, a characteristic of the plated NCM. NCF were used at passage 2 and were seeded and maintained in high-glucose (25 mM) DMEM in the presence of 1% antibiotic/antimycotic and 10% FBS (JRH Biosciences). Angiotensin II (ANG II) was used as a positive comparator in all in vitro experiments.

Transfection of NCM and NCF with U-II receptor. Recombinant adenovirus directing the expression of the rat UT receptor was generated by bacterial homologous recombination as described (3, 6). After plating (24 h), NCM and NCF in serum-free media were infected with UT (multiplicity of infection of 12.5) as previously described (27). After 48 h, the medium was replaced, and the cells were treated accordingly as described below.

Cardiomyocyte hypertrophy. NCM were seeded at 400,000 cells/well in 12-well plates. NCM hypertrophy was determined by [3H]leucine incorporation. After pretreatment (2 h) with the ROCK inhibitor GSK-576371 (GSK, 10−7 to 10−3 M), U-II (10−7 M) or ANG II (10−7 M) were used to stimulate hypertrophy, together with 1 μCi of [3H]leucine added to each well. After 60 h of stimulation, cells were harvested as previously described (14), and levels of incorporated [3H]leucine were counted. Control cells were treated accordingly as described below.

Cardiac fibroblast collagen synthesis. NCF were harvested at 50,000 cells/well in 12-well plates. NCF collagen synthesis levels were determined using [3H]proline incorporation as previously described (14). NCF were serum starved for 48 h in high-glucose medium before treatment. The cells were pretreated with GSK (10−7 to 10−5 M) for 2 h before stimulation with U-II (10−7 M) or ANG II (10−7 M) in the presence of 1 μCi of [3H]proline in each well. The cells were harvested for a further 48 h before harvest as previously described (14).

Cell viability assay. MTT assay was used to measure GSK-induced cytotoxicity in NCF and cell viability following treatment. Cells were treated as described above in 96-well plates including a high dose of GSK alone (10−5 M) and in the presence of ANG II (100 nM). After 48 h incubation, the MTT assay was performed, and absorbance was read at 595/655 nm using a standard protocol (22).

Statistical Analyses

Data are reported as means ± SE. Continuous data were analyzed using one-way ANOVA followed by Bonferroni or Newman-Keuls post hoc test where appropriate. A two-sided P value <0.05 was considered to be statistically significant.
RESULTS

Organ Weights and Survival

The heart weight (HW)-to-body weight (BW) ratio was unchanged between groups (vehicle 3.10 ± 0.13, low-dose U-II 2.89 ± 0.06, high-dose U-II 3.05 ± 0.09). The lung weight (LW)-to-BW ratio was also not different between groups at the end of treatment (vehicle 4.50 ± 0.08, low-dose U-II 4.61 ± 0.09, high-dose U-II 4.37 ± 0.17). There was no difference in LW between groups at the end of study (vehicle 1.62 ± 0.03 g, low-dose U-II 1.63 ± 0.04 g, and high-dose U-II 1.70 ± 0.03 g). However, high-dose U-II animals had significantly higher HW compared with the low-dose U-II group at the end of the study (P < 0.05) (vehicle 1.12 ± 0.05, low-dose U-II 1.02 ± 0.02, high-dose U-II 1.19 ± 0.04).

Survival rate in this study was 100% in all groups. Allowing for differences in radioactivity and death before and after treatment, the average dose of U-II administered in the low- and high-dose groups was 1.23 ± 0.04 and 3.38 ± 0.08 nmol·kg⁻¹·h⁻¹ (equivalent to 2.04 ± 0.06 and 5.62 ± 0.14 μg·kg⁻¹·h⁻¹, respectively).

Echocardiographic and Hemodynamic Measurements

Baseline echocardiography measurements were not different among the three groups (Table 1). After 4 wk of U-II infusion, the diastolic filling pattern was significantly impaired in high-dose U-II-treated animals as characterized by prolonged DT and reduced E'/A' ratio in this group compared with vehicle animals (Table 2). Furthermore, MV E/A was significantly reduced in the low-dose U-II-treated group. LV IVRT, ejection fraction (EF), and RWT were not different between treatment groups. U-II infusion had no effect on CAP or HR (Table 2).

Histology

Collagen deposition as determined by picrosirius red staining was significantly increased by 106.4% in the high-dose U-II group (P < 0.05, Fig. 1, A–D). Immunohistochemical detection of collagen I deposition in the LV was dose-dependently increased. Low-dose U-II increased collagen I deposition by 72.4% (P < 0.05) and high dose by 208.1% (P < 0.001) (Fig. 2).

Table 1. Baseline echocardiographic parameters

<table>
<thead>
<tr>
<th>Echocardiographic Parameters</th>
<th>Vehicle</th>
<th>Low-dose U-II</th>
<th>High-dose U-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVa E′/A′</td>
<td>1.78±0.33</td>
<td>1.72±0.60</td>
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<td>MV E/A</td>
<td>1.90±0.40</td>
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<td>DT, ms</td>
<td>47.1±2.9</td>
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<td>IVRT, ms</td>
<td>15.7±2.0</td>
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<tr>
<td>EF, %</td>
<td>87.3±1.0</td>
<td>83.7±3.0</td>
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<tr>
<td>LVIdD, cm</td>
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<tr>
<td>AWT, cm</td>
<td>0.139±0.003</td>
<td>0.129±0.005</td>
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</tr>
<tr>
<td>PWT, cm</td>
<td>0.116±0.01</td>
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<td>0.114±0.01</td>
</tr>
<tr>
<td>MV E′, cm/s</td>
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<td>3.76±2.83*</td>
<td>3.82±1.71*</td>
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<tr>
<td>MVaE′/A′</td>
<td>2.01±0.19</td>
<td>1.04±0.05†</td>
<td>1.04±0.25†</td>
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<tr>
<td>MV E/A</td>
<td>2.42±0.43</td>
<td>1.23±0.08**</td>
<td>1.56±0.28</td>
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<tr>
<td>DT, ms</td>
<td>57±3</td>
<td>93±5</td>
<td>118±22†</td>
</tr>
<tr>
<td>LV IVRT, ms</td>
<td>16.7±2.1</td>
<td>22.5±1.6</td>
<td>21.0±1.0</td>
</tr>
<tr>
<td>EF, %</td>
<td>84.4±1.2</td>
<td>81.3±1.2</td>
<td>80.4±1.5</td>
</tr>
<tr>
<td>LVIdD, cm</td>
<td>0.831±0.03</td>
<td>0.756±0.02</td>
<td>0.838±0.004</td>
</tr>
<tr>
<td>AWT, cm</td>
<td>0.156±0.004</td>
<td>0.144±0.008</td>
<td>0.148±0.005</td>
</tr>
<tr>
<td>PWT, cm</td>
<td>0.143±0.01</td>
<td>0.136±0.01</td>
<td>0.152±0.01</td>
</tr>
<tr>
<td>RWT, cm</td>
<td>0.36±0.02</td>
<td>0.37±0.02</td>
<td>0.36±0.01</td>
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<tr>
<td>CAP, mmHg</td>
<td>117±9</td>
<td>112±3</td>
<td>123±8</td>
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<td>HR, beats/min</td>
<td>330±22</td>
<td>311±6</td>
<td>323±20</td>
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</table>

Data are presented as means ± SE. MVa E′/A′, the ratio of peak early (E′) and late (A′) diastolic tissue velocity; MV E/A, the ratio of early and late transmitral peak diastolic flow velocity; DT, deceleration time; IVRT, isovolumetric relaxation time; EF, ejection fraction; LVIdD, left ventricular internal diameter at diastole; AWT, anterior wall thickness; PWT, posterior wall thickness; RWT, relative wall thickness.

Table 2. Echocardiographic parameters at 4 wk

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</tbody>
</table>

Data are presented in means ± SE. MV E′, peak early diastolic tissue velocity; LV, left ventricular; CAP, central aortic pressure; HR, heart rate. *P < 0.05 vs. vehicle. †P < 0.01 vs. vehicle.

Measurement of Myocyte Cross-Sectional Area

LV myocyte cross-sectional area (MCSA) was dose-dependently increased with U-II infusion; low-dose U-II increased MCSA by 33.0% (P < 0.001) and high-dose U-II by 60.6% (P < 0.001) compared with vehicle.

Real-Time PCR

U-II infusion dose-dependently increased LV procollagen-α(I) mRNA expression; low-dose U-II increased procollagen-α(I) gene expression by 58.6% (P < 0.05) and high-dose U-II by 89.2% (P < 0.01) compared with vehicle (Fig. 3). TGF-β1 level of gene expression remained unchanged in all groups [expressed over 18S (vehicle 1.03 ± 0.08, low-dose U-II: 1.01 ± 0.05, and high-dose U-II 1.13 ± 0.04)]. CTGF gene expression was also unchanged (vehicle 1.88 ± 0.19, low-dose U-II 1.53 ± 0.18, and high-dose U-II 1.70 ± 0.66).

Cardiomyocyte Hypertrophy

ANG II and U-II stimulation caused a 22.5 and 50.0% increase in hypertrophy, respectively, compared with unstimulated cells (P < 0.05). This was reduced with the ROCK inhibitor, GSK, at all concentrations to control levels (Fig. 4, A and B).

Cardiac Fibroblasts Collagen Synthesis

ANG II and U-II significantly increased collagen synthesis compared with control by 59.2 and 35.2%, respectively. ROCK inhibition dose-dependently inhibited collagen synthesis stimulated by ANG II and U-II (Fig. 5, A and B).

Cell viability. MTX assay demonstrated that, under all conditions (control, GSK 10⁻⁵ M alone, ANG II alone, and ANG II + GSK at all concentrations), absorbance did not fall below the control level of optical density = 0.169 ± 0.003, indicating no loss of cell viability.

DISCUSSION

Previous studies have demonstrated upregulation of both U-II and the UT receptor in the myocardium of heart failure
patients, suggesting that U-II may play a significant role in the cardiac dysfunction and remodeling associated with this condition (4, 12). Our group has demonstrated that U-II infusion in healthy rats can increase portal pressure and induce an increase in hepatic fibrosis (9). However, the direct effects of U-II on the myocardium have not been evaluated extensively.

The present study suggests that activation of the U-II system may be a major contributor to pathophysiological processes involved in the progression of diastolic dysfunction in vivo. Chronic U-II infusion (4 wk) in healthy animals significantly impaired diastolic function, as assessed by echocardiographic parameters, specifically increased DT, reduced E/A, and was associated with a dose-dependent reduction in MV E’/A’.

These effects were independent of changes in blood pressure levels. This lack of effect on blood pressure is consistent with findings from an earlier study by our group examining 14 days of chronic U-II infusion that further demonstrated a reduction in LV contractility and relaxation as determined from the rate of pressure rise and fall (10). Furthermore, a previous study by Hassan et al. (5) demonstrated that a single bolus dose of U-II infusion acutely decreased blood pressure, which then returned to baseline levels after 15 min.

The impairment of diastolic function observed in vivo in this study was associated with an upregulation of collagen protein deposition and gene expression in the LV. Total collagen, and specifically type-I collagen deposition, was increased threefold in the LV following high-dose U-II treatment, demonstrating fibrosis in the LV. In parallel, procollagen-α(I) mRNA expression was also upregulated in a dose-dependent manner. Additionally, MCSA was dose-dependently increased with U-II infusion, which may suggest that hypertrophy occurrence in vivo can contribute to diastolic dysfunction of the LV. Taken together, the significant increase in collagen expression and deposition in the LV may contribute to pathological reactive fibrosis in the LV, resulting in the observed diastolic dysfunction.

**Fig. 1.** Left ventricular total collagen. A–C: representative images of picrosirius red staining in the left ventricle for vehicle, low-dose urotensin-II (U-II), and high-dose U-II. D: total collagen in the left ventricle was increased significantly by 106.4% in the high-dose U-II group compared with the vehicle group. *P < 0.05 vs. vehicle.

**Fig. 2.** Left ventricular collagen type-I. Collagen type-I was significantly increased by 72.4% in the low-dose U-II group and 208.1% in the high-dose U-II group compared with vehicle. *P < 0.05 and ***P < 0.001 vs. vehicle.

**Fig. 3.** Left ventricular procollagen-α(I) mRNA expression. Low-dose U-II increased procollagen-α(I) mRNA expression by 58.6% (P < 0.05) and high dose by 89.2% (P < 0.01). *P < 0.05 and **P < 0.01 vs. vehicle.
impairment seen in this study. The increased MCSA without change in HW/BW or RWT may suggest the loss of cardiac cells in the U-II-infused animals. This finding is similar to a previously reported study of \( /H9252 \)2-transgenic animals with significantly higher MCSA, but the HW/BW ratio was not different when compared with nontransgenic animals (19).

Fibroblasts play a critical role in the development of cardiac fibrosis because of their ability to increase collagen protein synthesis and secretion in the extracellular matrix. Collagen synthesis by these cells is therefore of particular interest and importance. In this study, we demonstrate that collagen production by NCF is increased significantly upon stimulation with U-II. We also sought to determine whether this profibrotic effect may be mediated via the intracellular signaling kinase ROCK, as had been suggested in other settings (21, 23).

Dose-dependent reduction in collagen protein synthesis was mediated by the ROCK inhibitor GSK. The present study therefore demonstrated that ROCK may be a potentially important regulator of U-II-induced NCF collagen synthesis.

Additionally, cardiomyocyte hypertrophy occurred in vitro upon U-II stimulation, which was also dose-dependently reduced with ROCK inhibition, demonstrating the potential importance of ROCK in mediating induction of cardiomyocyte hypertrophy.

Our group has previously demonstrated that the ROCK signaling pathway is involved in diastolic dysfunction; inhibition of this pathway with the selective ROCK inhibitor GSK improved diastolic function accompanied by a reduction in LV collagen deposition (14). In the present study, we have shown, using primary cell culture, that U-II may impair diastolic function via increasing collagen deposition and induction of myocyte hypertrophy. These effects were ameliorated by ROCK inhibition. These data therefore suggest an important contribution of U-II to myocardial diastolic dysfunction, and the ROCK signaling pathway mediating these effects.

U-II has previously been shown to have effects on the humoral system by stimulating the release of adrenocorticotropin hormone and epinephrine and causing potent chronotropic and inotropic actions (28). Future studies that examine the role of the humoral system in response to U-II may help further our understanding of the role of U-II in cardiovascular disease.
In summary, we have shown that chronic infusion of U-II exerts direct effects on the heart in mediating diastolic dysfunction. Our in vitro studies also demonstrate that U-II increased collagen deposition by NCF and induced NCM hyperfunction. Our in vitro studies also demonstrate that U-II in exerts direct effects on the heart in mediating diastolic dysfunction.

**ACKNOWLEDGMENTS**

We thank GlaicoSmithKline (King of Prussia, PA) for kindly providing the selective ROCK inhibitor GSK-576371 and rat urotensin-II. We thank Walter Thomas (University of Queensland, Australia) for kindly providing UT adenosine for the study.

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