Cellular distribution of GPR14 and the positive inotropic role of urotensin II in the myocardium in adult rat

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Submitted 26 May 2004; accepted in final form 13 July 2004

Gong, Hui, Yan-Xia Wang, Yi-Zhun Zhu, Wen-Wei Wang, Ming-Jie Wang, Tai Yao, and Yi-Chun Zhu. Cellular distribution of GPR14 and the positive inotropic role of urotensin II in the myocardium in adult rat. J Appl Physiol 97: 2228–2235, 2004. First published July 23; doi:10.1152/japplphysiol.00540.2004.—Urotensin II is a cyclic neuropeptide recently shown to play a role via its receptor GPR14 in regulating vascular tone in the mammalian cardiovascular system. The existence of GPR14 in rat heart has been validated by ligand binding assay and RT-PCR. In the present study, we investigated the cellular distribution of GPR14 protein in rat heart by using immunohistochemistry and confocal microscopic immunofluorescence double staining with antipeptide polyclonal antibodies against GPR14 and cell type markers for myocytes and endothelial cells. The direct effect of urotensin II on left ventricular contractility was further evaluated in isolated left ventricular papillary muscles of the rat. Paraffin-embedded heart sections, positive immunohistochemical staining was observed in the left ventricle but not in the right ventricle and atria. Immunofluorescence double staining revealed the cardiac myocyte as the only cell type expressing GPR14 protein in frozen heart sections as well as in isolated cardiac myocytes. There was no visible signal for GPR14 in intramyocardial coronary arteries and capillaries. The existence of GPR14 protein in rat heart was further validated by immunoprecipitation and Western blot analysis. Urotensin II has recently been shown to be upregulated in infarcted rat heart (28) and in congestive heart failure in humans (11), suggesting a possible role of urotensin II in cardiac remodeling. Agonists (28) and in congestive heart failure in humans (11), suggesting a possible role of urotensin II in cardiac remodeling. Agonists (8, 17) and antagonists (2, 12, 24) for GPR14 have been assumed as tools for pharmacological research and potential drug leads for the treatment of cardiovascular diseases. These reports give rise to the hypothesis that rat myocardium contains GPR14 receptor, which may be responsible for the biological effects of urotensin II in this tissue. It is possible that GPR14 receptors may also occur in coronary vessels in the myocardium. Systemic administration of human urotensin II to anesthetized monkeys resulted in a complex dose-dependent hemodynamic response including a decrease in cardiac contractility and stroke volume with a concomitant increase in total peripheral resistance, leading eventually to fatal cardiovascular collapse (1). Intravenous bolus injection of human urotensin II into the rat produced a dose-dependent decrease in mean arterial pressure, left ventricular systolic pressure, and cardiac contractility (18). In contrast, urotensin II elicited a concentration-dependent increase in myocardial contractile force of isolated rat left atrial trabeculae (25) and an increase in coronary resistance in isolated perfused rat heart (16). Despite the accumulating evidence for the role of urotensin II in the regulation of peripheral vascular tone, little has been known with regard to its putative direct effect on cardiac contractility. It is not clear yet whether the inotropic effect of systemic administration of urotensin II is resulted from its direct action on the heart (particularly the left ventricle) or secondary to the changes in the total peripheral resistance. On the other hand, urotensin II has been shown to affect the process of cell growth. It induces a hypertrophic response (32, 28) and an activation of the extracellular signal-regulated protein kinase 1/2 (32) in cultured cardiac myocytes. To identify the receptor for urotensin II, Ames et al. (1) cloned a human G-protein-coupled receptor similar to the rat GPR14, which selectively bind with urotensin II. Urotensin II has been proved by many authors to be the endogenous ligand for GPR14 (19, 22, 23). It has been demonstrated that GPR14 mRNA is widely expressed in human cardiovascular tissues, such as the cardiac myocytes, vascular smooth muscle cells, and endothelial cells (1), as well as in the spinal cord (7) and endocrine tissues (5). Accumulating lines of evidence now indicate that the cardiovascular effects of urotensin II are mediated by its receptor GPR14 (1, 19, 22, 28). Urotensin II has recently been shown to be upregulated in infarcted rat heart (28) and in congestive heart failure in humans (11), suggesting a possible role of urotensin II in cardiac remodeling. Agonists (8, 17) and antagonists (2, 12, 24) for GPR14 have been assumed as tools for pharmacological research and potential drug leads for the treatment of cardiovascular diseases. These reports give rise to the hypothesis that rat myocardium contains GPR14 receptor, which may be responsible for the biological effects of urotensin II in this tissue. It is possible that GPR14 receptors may also occur in coronary vessels in the myocardium.

UROTENSIN II, A CYCLIC NEUROPEPTIDE initially isolated from the urophysis of teleost fish (3), has recently been cloned in several mammalian species including human. Gibson et al. (15) first reported a role of urotensin II in regulating cardiovascular function in the rat. More recently, urotensin II was identified in both the vascular and cardiac tissues and shown to effectively constrict the isolated arteries from rats (14), rabbits (10), dogs (10), pigs (10), nonhuman primates (1), and humans (20). In several recent reports, urotensin II has been recognized as the most potent vasoconstrictor with 8- to 110-fold potent than endothelin 1 (9). On the other hand, however, the reported depressor and regionally selective vasodilator effects (4, 13, 27) of urotensin II endow its vasoactive effects with complexity.

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dium and may mediate the vasoactive effects of urotensin II in regulating coronary blood flow. Therefore, it is important to make clear the cellular localization of urotensin II receptor GPR14 in the heart, which has not been investigated in detail.

The present study was aimed to clarify the cellular distribution of GPR14 protein in normal rat heart using immunohistochemistry and confocal microscopic immunofluorescence double-staining techniques. Experiments were also performed to identify the gene expression of GPR14 in the heart by using immunoprecipitation, Western blot analysis, and RT-PCR. The direct effect of urotensin II on left ventricular contractility was further observed in isolated rat left ventricular papillary muscle preparations.

METHODS

Male Sprague-Dawley (SD) rats weighing 200–250 g were obtained from the Department of Experimental Animals, Chinese Academy of Sciences. The investigation was approved by the Shanghai Committee for Animal Experiments and conforms with the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996).

Immunohistochemistry and immunofluorescence double staining. After chloral hydrate overdose, the rat heart was immediately excised, cut into 2-mm-thick circular pieces, and immersed in 10% neutral buffered formalin. Eight rats were used in the morphological studies. After shearing, five or more consecutive sections were obtained from both sides of each 2-mm-thick circular heart piece. For immunohistochemical staining, the specimens were hydrated, embedded in paraffin, and then cut into 6-μm-thick sections and deparaffinized with a graded series of xylene and ethanol solutions. The sections were incubated with 0.1% trypsin for 10 min at 37°C. After being washed in PBS, the sections were incubated in PBS containing 0.3% hydrogen peroxide and then incubated with 10% normal goat serum for 30 min at 37°C. Afterward, the sections were incubated in polyclonal rabbit anti-rat GPR14 antibodies (1:100), which were raised against a synthesized peptide corresponding to the sequence of 322nd–339th amino acids of rat GPR14 protein (Alpha Diagnostic International, San Antonio, TX), for 1 h at 37°C followed by an overnight incubation at 4°C. The sections were washed in PBS and incubated with biotinylated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at 37°C. After a wash in PBS, the sections were incubated with the avidin-biotin-peroxidase complex (Santa Cruz Biotechnology) for 1 h at 37°C. After a final wash in PBS, the sections were treated with diaminobenzidine as the chromogen and counterstained with hematoxylin. Replacement of the primary antibody with the nonimmune rabbit IgG served as the negative control.

For immunofluorescence double staining, the snap-frozen sections were cut into 6-μm-thick sections and incubated with 10% normal goat serum for 30 min at 37°C. Then the sections were incubated with polyclonal rabbit anti-rat GPR14 antibodies (1:100; Alpha Diagnostic International) and polyclonal goat anti-troponin-C antibodies (1:50; Santa Cruz Biotechnology) or MRC OX43, a mouse anti-rat endothelial cell IgG1 (1:100; Serotec, Oxford, UK) for 1 h at 37°C, washed,
and incubated with donkey anti-rabbit IgG conjugated with rhodamine (1:100) and mouse anti-goat IgG conjugated with FITC (1:100) or goat anti-mouse IgG conjugated with FITC (1:100) (Santa Cruz Biotechnology), respectively, at 37°C for 1 h.

Isolated cardiac myocytes were fixed in 4% formaldehyde, permeabilized with 0.2% Triton-X, and double stained for GPR14 and troponin-C by the same methods described above for heart section staining.

All immunofluorescent images were visualized with confocal microscopy as described (26). A Leica confocal microscope (Leica TCS SP2, Wetzlar, Germany) was used with argon-krypton laser to excite the dyes at 488 and 568 nm. The emission wavelengths were set at 530 nm for FITC and 590 nm for rhodamine. Sections were viewed with a ×200 or ×400 objective. The images were collected at 512 × 512 pixels.

Isolation of cardiac myocytes. Cardiac myocytes were obtained from hearts of three SD rats (200–250 g) by a enzymatic dissociation method that was similar to that described by Wittenberg et al. (29) with some modifications. On a Langendorff apparatus, the heart was perfused with a HEPES-buffered solution containing (in mmol/l) 117 NaCl, 5.7 KCl, 4.4 NaHCO3, 1.5 NaH2PO4, 1.7 MgCl2, 20 HEPES, 11 glucose, 10 creatine, and 20 taurine, and the pH was adjusted to 7.4 with NaOH. The heart was then perfused with fresh buffer mixed with 0.3 mg/ml collagenase type II (Sigma, St. Louis, MO) and 20 μmol/l CaCl2 for 5–6 min. Both the left and right ventricles were then cut off and chopped separately into small pieces. After 10 min of shaking, the cells were then washed in turn with 500 μmol CaCl2 and 1 mmol CaCl2. After a mild centrifugation, rod-shaped cells with clear striations were obtained and used for immunostaining.

Immunoprecipitation and Western blot analysis. Protein was extracted from the hearts of three male SD rats (200–250 g) according to the methods of Moore et al. (21). The lysates were incubated overnight with 5 μl of rabbit polyclonal anti-GPR14 antibodies (1:100; Alpha Diagnostic International) and 100 μl of protein-A-agarose in a rocker to acquire the immunocomplexes. The protein samples were separated in 12% SDS-PAGE, transferred to polyvinylidene difluoride membranes (Gelman-Pall, Ann Arbor, MI), and incubated with either rabbit polyclonal anti-GPR14 antibodies (1:500) (Alpha Diagnostic International) or the nonimmune rabbit IgG overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated anti-rabbit antibodies (1:1,000) (Calbiochem-Novabiochem, Darmstadt, Germany) for 1 h at room temperature.

RNA isolation and RT-PCR amplification. The total RNA was extracted from the right and left ventricle of three SD rats and reverse transcribed into first-strand complementary DNA. PCR was carried out using the following primers: sense 5’-CTGTGACTGAGCTGCTGGTGAC-3’, antisense 5’-GGTGCTATGAGGAGGATGC-3’ for GPR14 cDNA, which would theoretically yield a PCR product of 272 bp; and sense 5’-CATGTCTCAGTGTTCCAGT-3’, antisense 5’-GGCTAACAGGTTGTTGTC-3’ for GAPDH, which would theoretically yield a PCR product of 349 bp. PCR was run 25 cycles for GAPDH and GPR14 cDNA in a Perkin-Elmer 9600 thermal cycler (Applied Biosystem, Foster City, CA). Three-step PCR of denaturing, annealing, and extension reactions proceeded at 94°C for 1 min, at 64°C (GAPDH) or at 55°C (GPR14) for 1 min, and at 72°C for 1 min.

Isolated papillary muscle preparation. The anterior and posterior papillary muscles of the left ventricle were dissected free from eight

Fig. 2. Immunofluorescence double staining with anti-GPR14 antibodies and anti-troponin-C antibodies shows positive signals for GPR14 protein on the cardiac myocytes imaged with a confocal microscope. Left ventricular sections are double stained for troponin-C (A, green signal, arrow) and GPR14 (B, red signal, arrowhead). C is a superimposition of A and B showing the colocalization of GPR14 protein (yellow signal, arrowhead) and the cardiac myocyte (bar = 40 μm). D–F are from an isolated rat left ventricular myocytes double stained for troponin-C (D, green signal, arrow) and GPR14 (E, red signal, arrowhead). F is a superimposition of D and E showing that GPR14 protein is preferentially localized on the cell membrane of the cardiac myocyte (F, yellow signal, arrowhead; bar = 20 μm).
Fig. 3. Photomicrographs show detection and localization of the GPR14 protein and the endothelial cells (green signal) with immunofluorescence double staining in rat heart imaged with a confocal microscope. Left ventricular sections are doubly stained with the endothelial cell type marker, MRC OX43 (Aa, Ba, and Ca, green signal, arrow) and anti-GPR14 antibodies (Ab, Bb, and Cb, red signal, arrowhead). Ac, Bc, and Cc are the superimpositions of Aa and Ab, Ba and Bb, and Ca and Cb, respectively, showing that the cellular localization of GPR14 protein does not colocalize with the endothelial cells. Ad, Bd, and Cd are the phase-contrast images of panels Aa–Ac, Ba–Bc, and Ca–Cc, respectively. Aa–Ad: a double-stained left ventricular section showing GPR14 protein and the capillary endothelial cells. Ba–Bd: a double-stained left ventricular section showing GPR14 protein, the capillary endothelial cells, and a cross sectional intramyocardial coronary artery. Ca–Cd: a feature view of a cross-sectional intramyocardial coronary artery. As can be taken from Bc–Bd and Cc–Cd, the GPR14 protein (red signal, arrowhead) is located neither on the endothelial cells (green signal, arrow) nor on the vascular smooth muscle layer (open arrowhead) of the intramyocardial coronary artery. (Aa–Ad, Ba–Bd, bar = 40 μm; Ca–Cd, bar = 20 μm)
Results

Immunohistochemical staining for GPR14 in the rat heart. Immunohistochemical staining was carried out to localize GPR14 protein in different portions of the heart, such as the atria, ventricles, and aortic valves. In the paraffin-embedded heart sections, GPR14 protein was identified by anti-GPR14 primary antibodies and visualized by using the avidin-biotin-peroxidase complex method. The immunoreactive signals for GPR14 protein were only localized in the left ventricle. In the left ventricle, the positive signals were observed in the myocardium (Fig. 1B, arrow). No positive signal was observed in the aortic valves (Fig. 1F). There was no visible staining for GPR14 in the left atrium (Fig. 1D), right atrium (Fig. 1E), or right ventricle (Fig. 1C). In all the rats examined for GPR14 protein, positive signals were observed only in the left ventricle and not in other parts of the heart. In the myocardium where significant immunohistochemical signals for GPR14 were identified, the cardiac myocyte appeared to account for the positive signals for GPR14. The cellular distribution pattern of GPR14 protein in the left ventricle was further visualized with immunofluorescent double staining.

Cellular localization of GPR14 protein in the cardiac myocytes. Left ventricular sections were double stained for GPR14 protein and the cell type markers for myocytes or vascular endothelial cells and imaged with confocal microscopy. Colocalization of GPR14 protein and troponin-C, a cardiomyocyte-specific element, was identified by immunofluorescence double staining. All the GPR14 signals were overlaid with the cells immunostained for troponin-C, suggesting that GPR14 protein was mostly expressed in the cardiac myocytes (Fig. 2, A–C; green, arrow, troponin-C; red, arrowhead, GPR14). To further verify the existence of GPR14 protein in the cardiac myocytes, isolated rod-shaped rat left ventricular myocytes were subjected to immunofluorescence double staining using GPR14 antibodies and the cell type marker for the myocyte. Observation on a single cardiac myocyte confirmed that the positive staining for GPR14 was localized on the cell membrane and also in the cytoplasm, although the subcellular distribution of GPR14 could not be further determined with this method (Fig. 2, D–F; green, arrow, troponin-C; red, arrowhead, GPR14). In the left ventricular sections double stained for GPR14 protein and the vascular endothelial cell type marker OX43, both the capillary endothelial cells and the endothelial cells in the intramyocardial coronary arteries were visualized, but they did not colocalize with the signals for GPR14 protein (Fig. 3; green, arrow, endothelial cell type marker; red, arrowhead, GPR14; open arrowhead, vascular smooth muscle layer). The cross-sectional wall area of the intramyocardial coronary artery could be made out by the circular endothelium in the immunofluorescence image (Fig. 3, Ba, Bc, Ca, and Cc; endothelial cell type marker; red, arrowhead, GPR14; open arrowhead, vascular smooth muscle layer) and in the light microscopic phase-contrast image (Fig. 3, Bd and Cd; open arrowhead, vascular smooth muscle layer). Evidently, these areas were not recognized by GPR14 antibodies. We have looked for the receptor in sections from proximal, distal, epicardial, and endocardial regions of the left ventricle and confirmed that the GPR14 receptor is not present in the coronary vessels. In all the rats examined with double staining for GPR14 and cell type markers for cardiac or endothelial cells, we were only able to detect the localization of GPR14 in cardiac myocyte. Minimal variability between samples from the same animal or between animals was observed. Therefore, observations with the immunofluorescence double-staining technique confirmed that GPR14 protein was only localized in the cardiac myocytes but
not in the endothelial cells nor in the vascular smooth muscle cells in rat heart.

Detection of cardiac GPR14 expression by immunoprecipitation and Western blot analysis and RT-PCR. To further validate the existence of GPR14 protein in the heart and to test the specificity of the antibodies for GPR14 protein used in the immunohistochemical studies, the homogenates of the left and right ventricle were immunoprecipitated by GPR14 antibodies and consequently detected by Western blot analysis. The proteins immunoprecipitated by GPR14 antibodies were subjected to Western blot analysis using rabbit anti-GPR14 antibodies. As can be seen in Fig. 4A, a band at ~43 kDa corresponding to the molecular weight of GPR14 protein was identified in addition to a 53-kDa band of rabbit IgG in the left ventricle (Fig. 4A, lane 2). In the right ventricle, the band for GPR 14 was not detected (lane 1). Lane 3 and lane 4 are negative controls for lane 1 and lane 2, respectively, in which the proteins immunoprecipitated by GPR14 antibodies were immunoblotted with nonimmune rabbit IgG instead of the GPR14 antibodies, showing only a 53-kDa rabbit IgG band.

The mRNA for GPR14 was also detected in the left ventricle by RT-PCR, whereas the corresponding band for the right ventricle was faint. In gel electrophoresis, the PCR products yielded from GPR14 mRNA were displayed at ~272 bp (Fig. 4B). Sequence analysis confirmed that the sequence of the 272-bp band corresponds to position 58 bp-330 bp of the GPR14 full-length cDNA of rat in GenBank.

Effect of urotensin II on myocardial contractility of the isolated rat papillary muscles. In isolated rat papillary muscle strips, cumulative concentrations of urotensin II (10^{-8}, 10^{-7}, and 10^{-6} mol/l) induced an increase in isometric contractile forces represented as active tension of the percentage baseline contraction (Fig. 5). Other isometric contractile parameters such as dT/dt, electromechanical delay, time to peak tension, and time to 50% relaxation were not altered by urotensin II (Table 1). The data represent the average values of eight independent experiments.

DISCUSSION

In the present study, we provide clear evidence for the existence of GPR14 protein in the cardiac myocytes, but not the endothelial and vascular smooth muscle cells. The distribution of urotensin II receptor GPR14 protein on the cardiac working cells suggests possible direct inotropic and/or chronotropic effect of urotensin II on the heart. Actually, in isolated rat papillary muscle preparations, we demonstrated the direct positive inotropic effect of urotensin II.

In the present study, cardiac expression of the GPR14 protein was further validated by Western blot analysis that revealed a single band of ~43 kDa matching well with the theoretical molecular weight of 42.7 kDa for GPR14 protein (1). Moreover, the specificity of the primary antibodies used to recognize GPR14 protein in heart sections for morphological examinations in the present study was confirmed by the single immunoreactive band displayed in Western blot analysis. The gene expression for GPR14 at the transcription level in rat heart was also examined with RT-PCR. In line with our present observations, Ames and coworkers (1) identified urotensin II binding sites in rat heart with the radioligand binding methods, which, however, falls short of clarifying the type of the cells in which the urotensin II receptors are located. We used immunofluorescence double-staining method and were able to clarify the type of the cells in the myocardial tissues that expressed GPR14 protein. Our findings are in line with Liu’s in situ hybridization study, which revealed the expression site of GPR14 mRNA to be in the cardiac myocytes (19).

On the other hand, several in vivo studies implied that urotensin II exhibited inotropic effect under physiological conditions, e.g., systemic administration of urotensin II induced a decrease in left ventricular contractility in anesthetized monkeys (1, 31) and rats (18). However, the possibility that the inotropic effect was secondary to urotensin II-induced alterations in total peripheral resistance and blood pressure (1, 31, 18) cannot be excluded. Russell et al. (25) reported a stimulant effect of urotensin II in isolated human right atrial trabeculae. However, the data for a direct inotropic effect of urotensin II on the left ventricle were scarce. In the present study, we demonstrated a urotensin II-induced increase in contractile force of isolated rat left ventricular papillary muscles, suggesting that the urotensin II receptor plays a role in the physiology of cardiac function. Again, this observation further confirms the existence of GPR 14 protein in the left ventricle of normal rat heart.

Table 1. Effects of urotensin II on the isometric contraction parameters of the isolated rat papillary muscles

<table>
<thead>
<tr>
<th>Concentrations of Urotensin II, M</th>
<th>-dT/dt, %</th>
<th>dT/dt, %</th>
<th>EMD, ms</th>
<th>TTP, ms</th>
<th>RT1/2, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102±1</td>
<td>105±2</td>
<td>19±1</td>
<td>157±5</td>
<td>93±4</td>
</tr>
<tr>
<td>Urotensin II</td>
<td>107±2</td>
<td>105±2</td>
<td>18±1</td>
<td>152±5</td>
<td>87±7</td>
</tr>
<tr>
<td>10^{-7}</td>
<td>103±2</td>
<td>109±2</td>
<td>20±1</td>
<td>152±4</td>
<td>92±3</td>
</tr>
<tr>
<td>10^{-6}</td>
<td>106±5</td>
<td>116±2</td>
<td>19±1</td>
<td>149±4</td>
<td>83±6</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8. dT/dt, maximal rate of rise of tension, expressed as the percentage of baseline contraction; -dT/dt, maximal rate of decrease of tension, expressed as the percentage of baseline contraction; EMD, electromechanical delay; TTP, time to peak tension; RT1/2, time to 50% relaxation.
Recently, Tzanidis et al. (28) reported an upregulation of GPR 14 in the cardiac myocytes as well as in the endothelial cells and fibroblasts in rat heart after coronary artery ligation, though GPR 14 protein was not identified in any cell type in sham-operated rat. Their study suggests an implication for the urotensin II receptor to be linked with the process of cardiac remodeling after myocardial infarction. This hypothesis is validated by the direct actions of urotensin II on cardiac cell growth in vitro, i.e., the urotensin II-induced increase in collagen mRNA and protein in cardiac fibroblast, and the urotensin II-dependent activation of hypertrophic signaling in neonatal cardiomyocytes expressing recombinant GPR14 receptor (28). This study indicates that urotensin II may play a role in the pathology of heart, resulting in deleterious effects such as cardiac hypertrophy and cardiac fibrosis. In contrary to Tzanidis et al., who have not detected GPR14 receptor in sham-operated rat heart, we localized GPR14 receptor in the left ventricle of normal rat heart with RT-PCR, immunohistochemical staining, immunoprecipitation, and Western blot analysis, and a urotensin II-induced increase in contractile force in isolated left ventricular papillary muscles. The idea that GPR14 receptor is present in normal rat myocardium is also supported by the identification of GPR14 mRNA in normal rat heart (19).

Emerging evidence has been composing a picture of the actions of urotensin II on the heart: inotropic and chronotropic effects (1, 18, 25, 31), growth-stimulating effects on cardiac myocytes (32, 28, 11, 30), stimulation of the release of certain peptides such as atrial natriuretic peptide and brain-derived natriuretic peptide (32), regulation of intracellular signaling elements such as extracellular signal-regulated protein kinase 1/2 (32), and stimulation of collagen synthesis in cardiac fibroblasts (28). However, the cardiac actions of urotensin II remain largely unknown, e.g., the role of urotensin II in ventricular hypertrophy, the signal-transduction pathways after GPR14 activation, and the putative interactions between the ligand-receptor system of urotensin II and that of other cytokines. In this context, therefore, observations of the cellular localization of urotensin II receptor GPR14 protein and a direct urotensin II-induced positive inotropic effect in rat heart may shed some light on the understanding of the cardiac actions and underlying mechanisms of urotensin II. The absence of GPR14 protein in intramyocardial coronary arteries and capillaries observed in the present study is in close agreement with Liu et al. (19). In contrast, mRNA of GPR14 was shown to be expressed in the endothelial and smooth muscle cells of the coronary artery as well as in cardiac myocyte in human heart (1, 11). Moreover, coronary vasodilator and vasoconstrictor actions of urotensin II has been reported in the rat (4, 16), suggesting the existence of urotensin II receptor in the coronary vessels. We speculate that this controversy may imply the existence of unidentified receptor(s) in rat coronary endothelial and vascular smooth muscle through which urotensin II mediates its vasodilator and constrictor actions. Therefore, the possible existence and functions of urotensin II receptors in the endothelial and vascular smooth muscle cells remain to be further elucidated.

In conclusion, the present study provides the first evidence that the urotensin II receptor GPR14 protein is localized in the cardiac myocytes but not in the endothelial cells or the vascular smooth muscle cells in normal adult rat heart. The cardiac GPR14 protein mediates a positive inotropic effect of urotensin II, suggesting its role in the regulation of the pumping capacity of the heart.

ACKNOWLEDGMENTS

The authors thank Professor Jin-Sheng Zhang for helpful consultation and Ying-Jiong Ding, Ying Chen, and Li Zhou for expert technical assistance.

GRANTS

This work was supported by a grant from the National Natural Science Foundation of China (30270548).

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