Urocortin, a member of the corticotropin-releasing factor family, in normal and diseased heart

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Nishikimi, Toshio, Atsuro Miyata, Takeshi Horio, Fumiki Yoshiihara, Noritoshi Nagaya, Shuichi Takishita, Chikao Yutani, Hisayuki Matsuuo, Hiroaki Matsuoka, and Kenji Kangawa. Urocortin, a member of the corticotropin-releasing factor family, in normal and diseased heart. Am J Physiol Heart Circ Physiol 279: H3031–H3039, 2000.—In the present study we investigated the form of expression, action, second messenger, and the cellular location of urocortin, a member of the corticotropin-releasing factor (CRF) family, in the heart. Urocortin mRNA, as shown by quantitative RT-PCR analysis, is expressed in the cultured rat cardiac nonmyocytes (NMC) as well as myocytes (MC) in the heart, whereas CRF receptor type 2β (CRF-R2β), presumed urocortin receptor mRNA, is predominantly expressed in MC compared with NMC. Urocortin mRNA expression is higher in left ventricular (LV) hypertrophy than in normal LV, whereas CRF-R2β mRNA expression is markedly depressed in LV hypertrophy compared with normal LV. Urocortin more potently increased the cAMP levels in both MC and NMC than did CRF, and its effect was more potent in MC than in NMC. Urocortin significantly increased protein synthesis by [14C]Phe incorporations and atrial natriuretic peptide secretion in MC and collagen and increased DNA synthesis by [3H]prolin and [3H]Thy incorporations in NMC. An immunohistochemical study revealed that urocortin immunoreactivity was observed in MC in the normal human heart and that it was more intense in the MC of the human failing heart than in MC of the normal heart. These results, together with the recent evidence of urocortin for positive inotropic action, suggest that increased urocortin in the diseased heart may modulate the pathophysiology of cardiac hypertrophy or failing heart, at least in part, via cAMP signaling pathway.

heart failure; myocytes; hypertrophy; natriuretic peptides

corticotropin-releasing factor (CRF) is a 41-amino acid peptide produced in the hypothalamus as well as throughout the brain (31), where it plays an important role in the behavior and autonomic responses to stress (4). CRF belongs to a family of structurally related peptides that includes fish urotensin I (13), amphibian sauvinage (3), and a recently identified urotensin homolog discovered in mammals, urocortin (32). In addition to pituitary and central nervous system effects, peripheral effects of CRF have been observed involving the cardiovascular systems (12, 26, 27). The actions of these CRF-related peptides are mediated via binding to several recently characterized CRF receptors, which exhibit discrete and fairly exclusive distributions and are coupled with adenylyl cyclase. CRF receptor type 1 (CRF-R1) is expressed in high levels within the brain and pituitary (25), whereas CRF receptor type 2α (CRF-R2α) is confined to the central nervous system (14). A second splicing variant of the CRF receptor type 2β (CRF-R2β) is highly expressed in the heart as well as in other tissues, including the gastrointestinal tract, epididymis, and brain (24). Thus urocortin and CRF may bind to different receptor subtypes and stimulate adenylyl cyclase activity to different degrees. It has been reported that urocortin has ~10-fold higher affinity for CRF-R2β compared with CRF (32). In addition, Parkes et al. (23) reported that urocortin can produce potent and long-lasting actions to elevate cardiac contractility in conscious sheep, whereas CRF produced little effect on the cardiovascular system. These findings suggest that urocortin may participate in regulating cardiac function via CRF-R2β coupling cAMP signaling mechanism as an endogenous bioactive peptide in the heart. Indeed, a recent study reported the mRNA expression of urocortin in a rat cardiac cell line (21). However, little is known about the form of expression, other actions besides inotropic action, second messenger, and the cellular location of urocortin in the normal and diseased heart.

Therefore, the purpose of the present study is to investigate 1) whether urocortin and CRF-R2β are expressed in neonatal rat cultured cardiac myocytes (MC) or nonmyocytes (NMC) at mRNA levels, 2) whether urocortin and CRF-R2β mRNA expression is increased in left ventricular (LV) hypertrophy compared with that in normal LV in rats, 3) the cellular

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location of urocortin in the normal human heart, 4) and whether immunoreactivity of urocortin is increased in failing compared with normal human hearts. In addition, we examined the effects of urocortin, CRF, and α-helical CRF-(9–41) on cAMP levels in the rat cultured MC and NMC. With regard to the direct action of urocortin on MC and NMC, we studied the effect of urocortin on the [14C]phenylalanine (Phe) incorporation and atrial natriuretic peptide (ANP) release in cultured rat MC and on the [3H]prolin (Pro) and [3H]thymidine (Thy) incorporations in cultured rat NMC.

MATERIAL AND METHODS

Animals and materials. All procedures were in accordance with our institutional guidelines for animal research. Neonatal Wistar rats (day 1–2) were purchased from SLC (Shizuoka, Japan). Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) were purchased from Clear (Tokyo, Japan). The synthetic rat CRF, urocortin, and α-helical CRF-(9–41) were purchased from Peptide Institute (Osaka, Japan). The cAMP radioimmunoassay (RIA) kit was purchased from Yamasa Shoyu (Chiba, Japan). [14C]Phe was purchased from Amersham Life Science.

Cell culture. Enriched cultures of neonatal (day 1–2) cardiac MC and NMC were prepared from the hearts of Wistar rats by a method previously reported (11) with minor modifications (8, 18). In brief, apical halves of cardiac ventricles were recovered, and ventricular cardiac MC were dispersed in a balanced salt solution containing 0.06% collagenase II (Worthington Biochemical, Freehold, NJ) with agitation for 6 min at 37°C and then pipetted ~20 times. The differentiation of MC from NMC was performed by using the discontinuous Percoll gradient method. The purified MC were used for the experiments for the study of cAMP, [14C]Phe incorporation, ANP release, and RT-PCR. The NMC were allowed to grow to confluence and were then trypsinized and passed three times. Subconfluent NMC from the third passage, almost exclusively fibroblasts, were used in the experiments for the study of cAMP, [3H]Pro, and [3H]Thy incorporations and RT-PCR.

Quantification of mRNA using RT-PCR. Total RNA from MC and NMC was extracted by using the acid guanidinium thiocyanate-phenol-chloroform method, as previously reported (17), and RNA concentration was determined on the basis of absorbance at 260 nm. First-strand complementary DNA was synthesized from 5 μg of total RNA with murine transcriptase (Ready To Go, Pharmacia Biotech) using oligo(dT) primers (GIBCO-BRL). PCR was carried out with this cDNA mixture, 25 pmol of 5′- and 3′-primers, 0.2 mM 2′-deoxyribonucleoside 5′-triphosphates, and 0.5 units of Ampli Taq E polymerase (Perkin Elmer, Branchburg, NJ) in a reaction volume of 50 μl. Amplification was performed on a Perkin Elmer 4800 thermal cycler for 20–40 cycles at 1- to 2-cycle intervals on settings of 1 min of denaturation at 94°C, 1 min of annealing at 54°C (CRF-R1), 55°C (CRF-R2a and CRF-R2b), or 62°C (urocortin), and 1 min of extension at 72°C. The following sets of primers were used: urocortin, (forward) 5′-CGG CGA ATG TGG TCC AGG AT-3′, (reverse) 5′-CCG ATC ATC TGC CCA CCG AA; CRF-R1, (forward) 5′-GAC TGA ACC CTG TGT CCA-3′, (reverse) 5′-AGG TGG TCC ACG GAT GCA-3′; CRF-R2a, (forward) 5′-AAC TGC AGC CTG GCA CTG-3′, (reverse) 5′-ATC TGG TCC AAG GTC GTG-3′; and CRF-R2b, (forward) 5′-CTC TCT TCC CAG TGC ACA-3′, (reverse) 5′-ATC TGG TCC AAG GTC GTG-3′. PCR products were electrophoresed in a 1.5–2.0% agarose gel containing SYBR green (Takara, Tokyo, Japan), and the intensity of SYBR green luminescence was measured using a fluorescent image analyzer (FLA 2000; Fujifilm, Tokyo, Japan).

Reaction cycle-PCR product yield curves of each reaction mixture were plotted on semilogarithmic graphs as previously reported (10, 16). To estimate the initial amount of the template, we fitted regression equations of the form y = I × n^E, where y is the yield and n is the number of cycles, to the data in the linear portion of the curves. The coefficient I and the base E of the equations were expected to reflect the amount of the original template and the efficiency of amplification of each cycle, respectively. As an internal control, we measured glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels in the same manner with the use of specific primer: (forward) 5′-AAG GTC GGA GTC AAC GGA-3′ and (reverse) 5′-AGG GTG GAG GAG TGG GTG TCG-3′. Each product was subjected to DNA sequence analysis for confirmation (30). Quantification of mRNA of each molecule was obtained by dividing the amount of original template of each molecule by the amount of original template of GAPDH. The intra- and interassay coefficients of variation were 5% and 7%, respectively.

DOCA-salt SHR and WKY rats. DOCA-salt SHR is known to be a malignant hypertensive model with marked LV hypertrophy (28). After an acclimatization period of at least 7 days, 9-wk-old male SHR (n = 7) weighing from 200 to 240 g were treated with DOCA (Sigma Chemical, St. Louis, MO) and given 1% NaCl drinking water ad libitum. DOCA was administered once a week by subcutaneous injection (1 ml/kg of a suspension containing (per ml H2O) 50 mg of DOCA, 10.5 mg of methyl cellulose, 3 mg of carboxymethylcellulose, 1 mg of polysorbate 80, and 15 mg of NaCl) for 3 wk. At the end of the 3 wk of DOCA treatment, all rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg), and their body weights were measured. The measurements of mean arterial pressure (MAP) and heart rate (HR) were performed by using a previously described method (19). The heart was then arrested in diastole by an injection of 2 mmol of KCl through the carotid artery and was excised, and the LV was separated from the right ventricle (RV) and atrium and weighed. The LVs were frozen in liquid nitrogen and stored at ~80°C until RT-PCR analysis. The same procedure except for DOCA-salt treatment was performed in age-matched WKY rats (n = 7) as a control.

Quantification of mRNA of urocortin and CRF-R2b in the LV of DOCA-salt SHR and WKY rats was performed according to the methods described above.

Measurement of intracellular cAMP levels in MC and NMC. After each treatment of cardiac MC and NMC with various concentrations of urocortin (10−11–10−6 M) and CRF (10−11–10−6 M) with or without α-helical CRF-(9–41) (10−11–10−6 M) in the presence of 0.5 mM 3-isobutyl-1-methylxanthine, the medium was removed, and the cellular extract was obtained with the use of cold 70% ethanol, as previously reported (6, 18). The incubation time was 10 min, except for the time-course experiment. Each ethanol sample was evaporated in a vacuum until dry. The eluate was dissolved in RIA buffer. The cAMP level was measured using a RIA kit for cAMP.

Measurement of immunoreactive ANP levels. After cardiac MC were treated with various concentrations of urocortin (10−11–10−7 M) for 48 h, the culture medium was aspirated and stored at ~80°C. The medium (100 μl) was acidified with acetic acids, boiled to inactivate intrinsic proteases, and
lyophilized. The RIA for rat ANP was performed as previously reported (7).

Analysis of protein, DNA, and collagen syntheses. The effect of urocortin on protein, DNA, and collagen syntheses in cardiac MC and NMC from the incorporations of $[^{14}C]$Phe into cells was evaluated according to the method previously reported (29) with minor modifications (6, 7). After the preconditioning period, the cultured cells were replaced with fresh serum-free DMEM with various concentrations of urocortin ($10^{-11}$–$10^{-7}$ M). For protein synthesis in MC or collagen synthesis in NMC, either 0.2 µCi of $[^{14}C]$Phe or 0.5 µCi of $[^{3}H]$Pro was added, and the plates were then incubated for 24 h. For DNA synthesis in NMC, 0.5 µCi of $[^{3}H]$Thy was added 12 h after urocortin treatment, and cells were further incubated for 12 h. The cells were rinsed twice with cold phosphate-buffered saline (PBS) and incubated with 10% trichloroacetic acid at 4°C for 30 min. The precipitates were washed twice with cold 95% ethanol and solubilized in 1 M NaOH. The radioactivity of an aliquot was determined using a liquid scintillation counter.

Immunohistochemistry. For the immunohistochemical analysis, human heart tissues obtained from normal and failing LV were used. Normal LV tissues were obtained from an autopsied patient who had died of a cause other than cardiovascular disease and was without a history of cardiovascular disease ($n = 5$). Failing LV tissues were obtained from autopsied patients who had died because of heart failure from dilated cardiomyopathy ($n = 5$) or from a surgical sample of a Batista operation in patients with dilated cardiomyopathy ($n = 4$).

The immunohistochemical analysis was performed as previously reported (15) using a rabbit anti-urocortin-(3–40) antiserum (Y361; Yanaihara Institute, Shizuoka, Japan) diluted 1:3,000 in 0.1 M PBS containing 0.3% Triton-X. This polyclonal antibody was incubated with the sections for 5 days at 4°C (5). Nonimmune rabbit IgG was used as a control. The presence of immunoreactive urocortin was assessed with light microscopy by two trained observers without knowledge of the respective groups from which the tissue originated. The presence of urocortin immunoreactivity was evaluated to quantify the degree of staining of urocortin (0, no staining of urocortin; 1, minimal; 2, mild density; 3, moderate density; 4, maximal density). The mean values of urocortin stain scores in the failing hearts and normal hearts were calculated. To examine the immunohistochemical specificity of the reaction between antiserum and tissue, we performed absorption tests. The specificity was further confirmed by substitution of rabbit serum for primary antiserum.

Statistical analysis. All data are expressed as means ± SD. The multiple comparison was performed with a one-way ANOVA followed by Dunnett’s test. Student’s unpaired t-test was used to evaluate differences between the two groups. P values <0.05 were considered significant.

RESULTS

mRNA expression of urocortin, CRF-R1, CRF-R2α, and CRF-R2β in cardiac MC and NMC. Expression of urocortin, CRF-R1, CRF-R2α, and CRF-R2β mRNA in cardiac MC and NMC was analyzed using RT-PCR with (rat) urocortin-, CRF-R1-, CRF-R2α-, and CRF-R2β-specific primers, respectively. Specific bands of the predicted lengths (279 and 186 bp, respectively) were obtained with urocortin- and CRF-R2β-specific primers in cardiac MC and NMC. The PCR products of urocortin increased exponentially with each cycle until cycle 32 in both MC and NMC (Fig. 1A). There were no significant differences in mRNA levels of urocortin/GAPDH between MC and NMC (not significant, NS) (Fig. 1B). Similarly, the PCR products of CRF-R2β increased exponentially with each cycle until cycle 32 in MC (Fig. 1A). In contrast, PCR products of CRF-R2β in NMC were only observed in cycles 33 and 34 (Fig. 1A). The mRNA levels of CRF-R2β/GAPDH were obviously higher in MC than in NMC ($P < 0.0001$; Fig. 1B). No band was obtained with the CRF-R1 or CRF-R2α primers in cardiac MC and NMC (Fig. 1A).

mRNA expression of urocortin and CRF-R2β of LV in WKY rats and DOCA-salt SHR. Body weight (BW), LV weight (LVW), LVW/BW, MAP, and HR in WKY rats and DOCA-salt SHR are presented in Table 1. BW was higher in WKY rats than in DOCA-salt SHR ($P < 0.01$). LVW and LVW/BW were greater in DOCA-salt SHR than in WKY rats ($P < 0.01$). DOCA-salt SHR had a higher MAP than did WKY rats ($P < 0.01$); however, there were no differences in HR between the two groups.

The expression of urocortin and CRF-R2β mRNA in the LV of both DOCA-salt SHR and WKY was examined by quantitative RT-PCR analysis. Figure 2A shows a representative result of RT-PCR analysis in the LV of both groups. The expression of urocortin mRNA appears to be slightly higher in DOCA-salt SHR than in WKY rats, whereas the expression of CRF-R2β mRNA is remarkably depressed in DOCA-salt SHR compared with WKY rats. A quantitative analysis of these PCR products corrected for the level of GAPDH mRNA as an internal standard is shown in Fig. 2B. The mRNA levels of urocortin/GAPDH in the LV was greater in DOCA-salt SHR than in WKY rats ($P < 0.05$), whereas the mRNA levels of CRF-R2β/GAPDH was apparently lower in DOCA-salt SHR than in WKY rats ($P < 0.001$).

Effect of urocortin and CRF on cAMP levels and antagonistic effect of α-helical CRF-(9–41) on urocortin- and CRF-stimulated cAMP levels in MC and NMC. Urocortin increased the cAMP levels in the MC and NMC in a concentration-dependent manner, with an EC50 of 10−8 M in MC and 10−7 M in NMC (Fig. 3, A and C). In both the MC and NMC, CRF was less potent than urocortin (EC50 = 5 × 10−8 M in MC and 10−8 M in NMC) (Fig. 3, B and D). The maximum cAMP formations by urocortin and CRF were similar in both the MC and NMC. We also studied the time course of cAMP accumulation induced by urocortin in the cardiac MC and NMC. The cAMP level was significantly increased by urocortin at 2 min in MC ($P < 0.01$) and NMC ($P < 0.01$) and peaked at 10 min in MC and at 5 min in NMC. Thereafter, the cAMP levels gradually decreased (data not shown). The effects of α-helical CRF-(9–41) on the cAMP levels in the MC induced by urocortin and CRF are shown in Fig. 4, A and B. While α-helical CRF-(9–41) significantly inhibited the urocortin (10−10 M)-induced cAMP formation at concentrations >10−8 M ($P < 0.001$), α-helical CRF-(9–41) significantly inhibited the CRF (10−8 M)-induced cAMP formation at concentrations >10−8 M.
The effects of \( \alpha \)-helical CRF-(9–41) on the cAMP levels in the NMC induced by urocortin and CRF are shown in Fig. 4, C and D. \( \alpha \)-Helical CRF-(9–41) significantly attenuated the urocortin (5 \( \times \) \( 10^{-10} \) M)-induced cAMP levels at concentrations \( >10^{-8} \) M (\( P < 0.001 \)) and the CRF (10\( ^{-8} \) M)-induced cAMP levels at concentrations \( >10^{-8} \) M (\( P < 0.001 \)), respectively. Thus the receptors that have higher affinity for urocortin than for CRF or \( \alpha \)-helical CRF-(9–41) are expressed in the MC and NMC.

**Table 1.** Body weight, left ventricular weight, LVW/BW, mean arterial pressure, and heart rate in WKY and DOCA-salt SHR

<table>
<thead>
<tr>
<th>Variables</th>
<th>WKY</th>
<th>DOCA-Salt SHR</th>
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<tbody>
<tr>
<td>( n )</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>BW, g</td>
<td>311 ( \pm ) 2</td>
<td>285 ( \pm ) 14*</td>
</tr>
<tr>
<td>LVW, g</td>
<td>0.69 ( \pm ) 0.02</td>
<td>1.11 ( \pm ) 0.07*</td>
</tr>
<tr>
<td>LVW/BW, g/kg</td>
<td>2.21 ( \pm ) 0.07</td>
<td>3.91 ( \pm ) 0.35*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>123 ( \pm ) 15</td>
<td>194 ( \pm ) 23*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>389 ( \pm ) 36</td>
<td>376 ( \pm ) 30</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SD. BW, body weight; LVW, left ventricular weight; MAP, mean arterial pressure; HR, heart rate; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. *\( P < 0.01 \) vs. WKY.

Induced cAMP levels at concentrations \( >10^{-8} \) M (\( P < 0.001 \)) and the CRF (10\( ^{-8} \) M)-induced cAMP levels at concentrations \( >10^{-8} \) M (\( P < 0.001 \)), respectively. Thus the receptors that have higher affinity for urocortin than for CRF or \( \alpha \)-helical CRF-(9–41) are expressed in the MC and NMC.

**Effect of urocortin on ANP release and \([^{14}C]\)Phe incorporation in MC and on \([^{3}H]\)Pro and \([^{3}H]\)Thy incorporations in NMC.** As shown in Fig. 5A, urocortin significantly stimulated \([^{14}C]\)Phe incorporation at doses \( >10^{-10} \) M in cardiac MC (\( P < 0.05 \)). The effect of urocortin on ANP secretion in cultured cardiac MC is shown in Fig. 5B. Urocortin significantly increased ANP secretion at doses \( >10^{-10} \) M (\( P < 0.05 \)). These doses were comparable to the doses shown to increase cAMP levels in the MC. On the other hand, urocortin significantly increased \([^{3}H]\)Pro and \([^{3}H]\)Thy incorporations at doses \( >10^{-11} \) M in cardiac NMC (Fig. 5, C and
In contrast to the results in MC, these doses were markedly smaller doses shown to increase cAMP levels in NMC.

**Immunohistochemistry.** Hematoxylin-eosin staining in the hearts of patients with dilated cardiomyopathy revealed marked interstitial fibrosis and hypertrophy of the cardiac MC. A representative immunohistochemical staining for urocortin in the LV of failing and normal human hearts are illustrated in Fig. 6A. While weak immunostaining for urocortin was observed in the cardiac MC in the LV of the normal human heart (Fig. 6A), urocortin immunoreactivity was markedly more intense in cardiac MC in the LV of the failing heart (Fig. 6A). Consequently, the urocortin stain score
was significantly higher in the LV of failing hearts than in the LV of normal hearts (P < 0.05) (Fig. 6B). A positive urocortin immunostaining in fibrous tissue was not found in any group. Control slides with non-immune rabbit serum were negative for urocortin immunoreactivity (Fig. 6A). The sections treated with preabsorbed antiserum also showed no immunoreactivity for urocortin.

**DISCUSSION**

In addition to pituitary and central nervous system effects, peripheral effects of CRF involving the cardiovascular systems have been observed (12, 26, 27). CRF receptor subtypes CRF-R1, CRF-R2α, and CRF-R2β have been cloned, and while they show significant amino acid homology (~70%), they differ in their dis-
CRF-R1 is expressed predominantly in the brain and pituitary (14), and CRF-R2α is also expressed in the central nervous system (25), whereas the CRF-R2β is highly expressed in the heart as well as in other tissues, including the gastrointestinal tract, epididymis, and brain (24). In the present study CRF-R1 or CRF-R2α was not expressed in the cardiac MC or NMC, consistent with the view that CRF-R1 and CRF-R2α are receptors associated with the central nervous system. As for the peripheral actions of urocortin, a recent study reported that intravenous infusion of urocortin significantly increased cardiac output before changing peripheral vascular resistance (23), suggesting that urocortin may function as an endogenous bioactive peptide via the abundant CRF-R2β receptor in the heart. Indeed, Okosi et al. (21) recently reported the mRNA expression of urocortin in a rat cardiac cell line and in primary cultures of cardiac myocytes. However, there have been no reports of studies of the form of the expression of urocortin and the expression of its receptor in the MC and NMC. In the present study, we extended our investigation into the expression of urocortin and its receptor by the quantitative RT-PCR method in the MC and NMC and showed that urocortin was equally expressed in the MC and NMC, whereas CRF-R2β receptor was predominantly expressed in the MC compared with the NMC. These findings were supported by the cAMP study. Urocortin increased cellular cAMP levels more potently in the MC than in the NMC, and urocortin increased cellular cAMP levels in both the MC and NMC more potently than CRF, suggesting that the receptors with higher affinity for urocortin than CRF are expressed more in MC than in NMC. These observations, together with the recent evidence that tissue concentrations of urocortin in the rat heart are $10^{-9}$ M (20), which is enough to increase cAMP, ANP release, and protein synthesis in MC, suggest that urocortin produced in the MC and NMC may act mainly on MC, at least in part, via the cAMP signaling pathway in an autocrine and/or paracrine fashion.

Little is known about the action of urocortin on the heart. Recent studies reported that preincubation of urocortin in primary cardiac MC significantly reduced lactate dehydrogenase release into the medium during lethal hypoxia (21) and increased ANP and brain natriuretic peptide release into the medium (9), suggesting that urocortin has cardioprotective effects. With regard to the direct action of urocortin on MC and NMC, we investigated the effects of urocortin on protein, collagen, and DNA syntheses as well as ANP secretion in rat cultured MC and NMC. Recent studies have demonstrated that ANP is an inhibitory endogenous regulator of cardiac hypertrophy (7, 22). Urocortin...
CRF-R2 expression of urocortin, whereas mRNA expression of normal hearts. Furthermore, LV hypertrophy induced human hearts and that its immunoreactivity is more present in MC in the LV of normal pathway. In addition, we showed that urocortin immunoreactivity is markedly lower than concentrations that increase cAMP levels in NMC. These results suggest that urocortin may affect LV remodeling mediated by the actions for MC and NMC via possibly different signaling pathway. In addition, we showed that urocortin immunoreactivity is present in MC in the LV of normal human hearts and that its immunoreactivity is more intense in the LV of failing hearts than in the LV of normal hearts. Furthermore, LV hypertrophy induced by DOCA-salt treatment in SHR had higher mRNA expression of urocortin, whereas mRNA expression of CRF-R2β is markedly decreased compared with that in normal LV. Although the mechanism of increased mRNA expression or immunoreactivity of urocortin in LV hypertrophy or failing hearts remains unknown, the pathophysiological significance of increased urocortin in LV hypertrophy and failing hearts appears to be, in part, associated with not only the positive inotropic action but also the other actions such as hypertrophic effect.

Limitations. First, it is unclear whether increased urocortin expression is the cause or result of cardiac hypertrophy. The regulation of urocortin expression in the heart needs further study. Second, we could only show increased mRNA expression of urocortin and decreased mRNA expression of CRF-2β in LV hypertrophy induced by DOCA-salt SHR. Whether this phenomenon is common to the other cardiac hypertrophy or heart failure models needs further study. Finally, only immunohistochemical analysis was conducted in the human failing heart. A more detailed biochemical analysis is needed.

In conclusion, we obtained evidence showing that neonatal rat cardiac MC and NMC express mRNA of urocortin and the functional receptor of CRF-R2β, that MC and NMC respond to urocortin with a strong increase of intracellular accumulation of cAMP, and that the expression of CRF-R2β and the response of cAMP to urocortin is higher in MC than in NMC. Urocortin also increased protein synthesis and ANP secretion in rat cultured MC and collagen and DNA syntheses in NMC. LV hypertrophy in rat had higher mRNA expression of urocortin and lower mRNA expression of CRF-2β. Immunoreactivity of urocortin is more intense in MC of the human failing heart than in MC of the normal heart. Thus we propose that an intracardiac urocortin system is present and that it may modulate the pathophysiology of LV hypertrophy and the failing heart. The exact cellular mechanism of the effects of urocortin on the failing heart requires further study.

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