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Upregulation of corin gene expression in hypertrophic cardiomyocytes and failing myocardium

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Tran, Katherine L., Xiangru Lu, Ming Lei, Qingping Feng, and Qingyu Wu. Upregulation of corin gene expression in hypertrophic cardiomyocytes and failing myocardium. *Am J Physiol Heart Circ Physiol* 287: H1625–H1631, 2004. First published June 10, 2004; 10.1152/ajpheart.00298.2004.—High levels of plasma atrial natriuretic peptides (ANP) are associated with pathological conditions such as congestive heart failure (CHF). Recently, we have identified a cardiac serine protease, corin, that is the pro-ANP convertase. In this study, we examined the regulation of corin gene expression in cultured hypertrophic cardiomyocytes and in the left ventricular (LV) myocardium of a rat model of heart failure. Quantitative RT-PCR analysis showed that both corin and ANP mRNA levels were significantly increased in phenylephrine (PE)-stimulated rat neonatal cardiomyocytes in culture. The increase in corin mRNA correlated closely with the increase in cell size and ANP mRNA expression in the PE-treated cells ($r = 0.95$, $P < 0.01$; $r = 0.92$, $P < 0.01$, respectively). The PE-treated cardiomyocytes had an increased activity in converting recombinant human pro-ANP to biologically active ANP, as determined by a pro-ANP processing assay and a cell-based cGMP assay. In a rat model of heart failure induced by ligation of the left coronary artery, corin mRNA expression in the noninfarcted LV myocardium was significantly higher than that of control heart tissues from sham-operated animals, when examined by Northern blot analysis and RT-PCR at 8 wk. These results indicate that the *corin* gene is upregulated in hypertrophic cardiomyocytes and failing myocardium. Increased corin expression may contribute to elevation of ANP in the setting of cardiac hypertrophy and heart failure.

congestive heart failure; atrial natriuretic peptide; brain natriuretic peptide; serine protease

ATRIAL AND BRAIN NATRIURETIC PEPTIDES (ANP and BNP) are peptide hormones produced primarily by cardiomyocytes in the atrium and ventricle, respectively, and are important in maintaining sodium and body fluid homeostasis (7, 15, 19, 20, 30, 35). In response to volume or pressure overload, ANP and BNP are released from the heart. On binding to their receptor in target organs such as the kidney and peripheral blood vessels, these peptides stimulate the intrinsic guanylyl cyclase activity of the receptor, leading to accumulation of intracellular cGMP. The biological effects of ANP and BNP are to promote renal sodium and water excretion and decrease systemic vascular resistance, thereby reducing blood volume and pressure. Clinically, high plasma concentrations of ANP and BNP are found in patients with congestive heart failure (CHF) and the levels of these peptides often correlate with the extent of ventricular dysfunction and development of cardiac arrhythmias (1, 2, 11). BNP is currently used as a diagnostic and prognostic marker for CHF (21). More recently, recombinant human BNP has been approved as a short-term treatment for patients with decompensated CHF to improve hemodynamics and cardiac output (6).

In cardiomyocytes, both ANP and BNP are synthesized as prepropeptides. The signal peptide is removed to form pro-ANP and pro-BNP but a further proteolytic cleavage of the propeptide is required to convert the precursor to a biologically active peptide (16, 28, 33). This activation mechanism is critical in the regulation of the activity of the natriuretic peptides but the enzyme responsible for conversion had remained uncharacterized for many years. Previous studies (19) have established that the activation cleavage sites in human pro-ANP and pro-BNP are Leu94-Tyr95-Ala96-Pro97-Arg98 ↓ -Ser99 and Leu72-Arg73-Ala74-Pro75-Arg76 ↓ -Ser77, respectively, suggesting that a trypsin-like protease may be responsible for the activation cleavage. Recently, we isolated a cardiac serine protease, corin, that is a type II transmembrane protein with a unique mosaic domain structure (38, 39). With the use of in situ hybridization and Northern and Western blot analyses, Hooper et al. (13) and Yan et al. (39) showed that corin mRNA and protein were abundantly expressed in cardiomyocytes of both the atrium and ventricle. In functional studies (17, 40), recombinant corin converted pro-ANP to biologically active ANP in a highly sequence-specific manner. In cultured cardiomyocytes, overexpression of an active site mutant corin or transfection of small interfering RNA duplexes directed against the *corin* gene completely prevented the processing of pro-ANP (37). Recombinant corin also processed pro-BNP to BNP in cell-based assays (40). These results indicate that corin is the long-sought convertase for pro-ANP and pro-BNP.

In this study, we examined whether corin gene expression is increased in cultured hypertrophic neonatal cardiomyocytes in vitro and in failing myocardium after myocardial infarction in vivo. Our results showed that corin mRNA expression was significantly increased in hypertrophic cardiomyocytes induced by phenylephrine (PE) in culture and in the left ventricular (LV) myocardium from a rat model of heart failure. These results indicate that upregulation of corin expression is a hypertrophic response of cardiomyocytes, which may contribute to observed increases in ANP in pathological conditions such as heart failure.

METHODS

The protocol is in accord with guidelines published by the National Institutes of Health and all animals used in this study were treated humanely and in accordance with those guidelines.

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Primary culture of neonatal rat cardiomyocytes. Hearts were isolated from 1-day-old neonatal rats. The tissues, which contained both atria and ventricles, were minced and trypsinized (50 μg/ml) at 4°C overnight. The next day, the tissue samples were transferred to a 50-ml centrifuge tube and 1 ml of soybean trypsin inhibitor (2 mg/ml) was added. The tube was warmed at 37°C and 1,500 units of purified collagenase (Worthington Biochemical; Lakewood, NJ) were added. After 45 min at 37°C, the tissues were triturated using a 10-ml pipette and the cell suspension was filtered through a cell strainer (Worthington). Cells in the flow-through were collected and left undisturbed at room temperature for 20 min. The cells were then centrifuged at 100 *g* for 5 min and resuspended in DMEM-M199 supplemented with 10% FBS and 1% penicillin-streptomycin. The cells were preplated for 30 min to decrease contamination of nonmuscle cells and then plated at a density of 125,000 cells/cm^2 in culture flasks precoated with fibronectin (Becton-Dickinson; Franklin Lakes, NJ). The purity of the primary neonatal cardiomyocytes was >95%, as assessed by the beating phenotype of the cells.

Induction of hypertrophy in cardiomyocytes. To induce hypertrophy, cardiomyocytes were first incubated with serum-free medium (DMEM-M199) overnight and then treated with PE at various concentrations. Morphological changes of the PE-treated cells were monitored with a light microscope and F-actin staining. The size of the cells was measured semiautomatically by analyzing light microscopic photos using the SPOT Advanced program (version 3.1, Diagnostic Instruments). After 2 or 4 days, the cells were trypsinized and washed once with PBS. Total RNA was isolated using the RNeasy Maxi kit (Qiagen; Valencia, CA) according to the manufacturer's recommendation. Expression of corin and ANP mRNA was examined by real-time quantitative RT-PCR. To examine the role of adrenergic receptors in PE-induced changes in corin and ANP transcription, 500 ng/ml prazosin, 500 ng/ml yohimbine, or 500 ng/ml propranolol (Sigma; St. Louis, MO) (10) was added to the culture medium containing 100 nM PE. Cells were cultured for 4 days and total RNA was isolated for RT-PCR analysis of corin and ANP mRNA expression.

Real-time quantitative RT-PCR. To examine corin and ANP mRNA expression in cardiomyocytes, total RNA was isolated and analyzed by real-time quantitative RT-PCR. Labeled probes and primers were designed based on the rat corin (K. L. Tran and Q. Wu, unpublished observations) and ANP (27) cDNA sequences using PrimerExpress software (Applied Biosystems; Foster City, CA). The following oligonucleotide probes and primers were used in this study: corin [probe 5′(FAM)-AGT GAC CGT CCG ACA CCG TGT CC-(TAMRA)3′, sense primer 5′-GAA GAC TGT AAG GAC GGG AGT GA-3′, and antisense primer 5′-GTC AAG GCA ACC CCG ATC T-3′] and ANP [probe 5′(FAM)-CCC CGA CCC ACG CCA GCA T-(TAMRA)3′, sense primer 5′-CCG AGA CAG CAA ACA TCA GAT C-3′, and antisense primer 5′-TTG GTG ATG GAG AAG GAG CC-3′]. The control GAPDH primer and probe (*Taq*Man rodent GAPDH reagents) were purchased from Applied Biosystems. RT-PCR reactions were performed in a 50-μl mixture containing the following: 20 nM probe, 2 nM oligonucleotide primers, 12.8 units of murine leukemia virus RT, 5 units of RNase inhibitor, and 250 ng total RNA with one cycle at 48°C for 30 min, one cycle at 95°C for 10 min, and 40 cycles at 95°C for 15 s, and 60°C for 1 min. RT-PCR and the resulting relative increase in reporter fluorescent dye emission were monitored in real time with the use of a sequence detector (Prism 7700, Applied Biosystems).

Pro-ANP processing and Western blot analysis. Human embryonic kidney (HEK)-293 cells were grown in α-MEM containing 10% FBS in 6-well tissue culture plates. To express recombinant pro-ANP, the cells were transfected with a plasmid encoding human pro-ANP (pcDNAproANP) using a Lipofectamine 2000-based method (Invitrogen; Carlsbad, CA). Recombinant human pro-ANP contains a V5 tag at the COOH terminus that facilitated protein detection. Eighteen hours after the transfection, the conditioned medium containing recombinant pro-ANP was removed from HEK-293 cells, added to cultured rat neonatal cardiomyocytes that had been treated with PE, and incubated at 37°C for 2 h. The conditioned medium was then collected. Pro-ANP and its derivatives in the conditioned medium were analyzed by immunoprecipitation and Western blot analysis with the use of an anti-V5 antibody, as described previously (40).

cGMP-stimulating activity assay. Baby hamster kidney (BHK) cells were grown in essential MEM containing 10% FBS in 96-well plates. Conditioned medium (180 μl), which contained recombinant pro-ANP and was treated with rat neonatal cardiomyocytes, was added to the BHK cells and incubated at 37°C for 10 min. The cells were lysed with the use of a buffer (20 μl/well) containing 2% dodecyltrimethylammonium and 50 mM sodium acetate, pH 5.8. The intracellular cGMP concentration in the BHK cells was then determined using an enzyme immunoassay (EIA) kit (Amersham; Piscataway, NJ), as described previously (37). Each experimental condition was assayed in triplicate.

Rat model of heart failure. Myocardial infarction was induced in male Sprague-Dawley rats (200–300 g), as described previously (9, 29). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and artificially ventilated with a respirator. A left thoracotomy was performed to expose the left side of the heart. After the pericardium was opened, the left coronary artery was ligated with a suture between the pulmonary artery outflow tract and the left atrium. As controls, sham-operated rats underwent the same surgical procedure without coronary artery ligation. Eight weeks after the surgery, hemodynamic parameters were measured under pentobarbital anesthesia to confirm the development of heart failure. LV pressures were assessed by inserting a heparinized polyethylene-50 catheter (Becton-Dickinson; Sparks, MD) in the left ventricle via the right carotid artery. Measurements were made in the anesthetized state after an equilibration period (20–30 min) for stabilization after completion of cannulation. Mean arterial pressure was evaluated using the same catheter in the right carotid artery after removal from the left ventricle. All pressure and heart rates were measured with a Stratham pressure transducer (model P10EZ) connected to a bridge amplifier (model PCA-2, Sonometrics). Signals were fed into a PowerLab data-acquisition system (8SP). Hemodynamic parameters including mean arterial pressure, heart rate, LV end-diastolic pressure, and the first derivative of LV pressure were calculated with the use of a computer. At the end of the experiments, the animal was euthanized, and the heart weight, LV volume, LV wall thickness, and infarct size were measured as described previously (29). Infarct size was determined by endocardial circumferences of fibrotic and normal areas and expressed as a fraction of the total cross-sectional endocardial circumference of the LV (9). In separate experiments, rats from the treatment and control groups were euthanized at 1, 2, 4, and 8 wk, and heart tissues were collected for RT-PCR analysis of corin mRNA expression. All animals in this study were maintained and treated in an animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

RIA for ANP. Rat plasma samples were acidified with 10% trifluoroacetic acid (TFA) and loaded onto OASISa HLB cartridges (Waters; Milford, MA) that were prewashed with methanol and deionized water. The cartridges were washed with 0.1% TFA and eluted with 90% methanol in 0.1% TFA. ANP concentration in the plasma extracts was measured by a competition RIA. Briefly, plasma extracts were dried and reconstituted in a RIA buffer (Phoenix Pharmaceuticals; Mountain View, CA). A rabbit anti-ANP antibody and ^{125}I-labeled ANP, which was used as a tracer, were added and incubated at 4°C for 21 h. A goat anti-rabbit IgG antibody that was coupled to solid-phase beads was added to the mixture and incubated at room temperature for 90 min. The antibody-coupled beads were spun down and counted for radioactivity with the use of a gamma-counter (model 1470 Wizard, Wallac, Fisher Scientific; Unionville, Ontario, Canada). The values of bound relative to maximum binding percentages were

plotted. ANP concentrations in plasma samples were determined using an ANP standard.

Northern blot analysis. To determine corin mRNA expression, total RNA was isolated from frozen rat heart tissues using the RNeasy Maxi kit (Qiagen). To perform Northern blot analyses, total RNA was denatured in a loading buffer that contained 25% formaldehyde and separated on an agarose gel. RNA samples were transferred overnight onto a nylon membrane (NEN GeneScreen Hybridization Transfer Membrane) and UV cross linked. A partial rat cDNA probe was labeled with α-[^{32}P]dCTP (Amersham). Northern blots were hybridized with the labeled cDNA probe ($>2 \times 10^6$ counts·min^{-1}·ml^{-1}) overnight at 42°C in a buffer containing 50% formamide, 10% dextran sulfate, and 1% SDS. After being washed with 0.2× SSC and 0.2% SDS at 60°C, the blots were exposed to a phosphoimaging plate overnight. The blots were developed and specific hybridizing bands were quantified with a phosphoimager (Fujix BAS1000).

Statistical analysis. All data are given as means ± SD. Differences between two groups were compared by unpaired Student's *t*-test. For multigroup comparisons, ANOVA followed by Student-Newman-Kuels test was performed. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Corin and ANP mRNA expression in PE-treated cardiomyocytes. To induce hypertrophy in cardiomyocytes, primary rat neonatal cardiomyocytes were cultured in the presence of 100 nM PE, an α_1-adrenergic receptor agonist. Morphological changes in the PE-treated cells were monitored by light microscopy and F-actin staining. Four days after PE treatment, the cardiomyocytes displayed significant increases in size and enhanced expression of F-actin (data not shown). The PE-induced hypertrophic response was blocked by prazosin but not yohimbine or propranolol, confirming that the effect of PE was mediated by α_1-, but not α_2- or β-, adrenergic receptors.

To determine corin and ANP mRNA expression in the hypertrophic cardiomyocytes, total RNA was isolated from the PE-treated cardiomyocytes and analyzed by real-time quantitative RT-PCR. As shown in Fig. 1*A*, corin mRNA expression in the cells treated with 100 nM PE for 4 days was 3.5-fold higher than that in control cells. The PE-induced upregulation of corin mRNA expression was inhibited by prazosin but not yohimbine or propranolol. ANP mRNA expression was also upregulated in the PE-treated cardiomyocytes. As shown in Fig. 1*B*, ANP mRNA expression in the PE-treated cells was 3.8-fold higher than that in control cells. Similarly, prazosin, but not yohimbine or propranolol, inhibited the upregulation of ANP mRNA expression in the PE-treated cells. These results showed that both *corin* and *ANP* genes were upregulated in cultured cardiomyocytes in response to PE-induced hypertrophy and that these effects were mediated by the α_1-adrenergic receptor.

The upregulation of corin and ANP mRNA expression by PE in cultured cardiomyocytes was shown to be dose and time dependent. As shown in Fig. 2, *A* and *C*, increasing concentrations of PE stimulated corin and ANP mRNA expression in a dose-dependent manner. The effect of PE on corin and ANP mRNA expression in cultured cardiomyocytes was not detected at 2 days but became apparent after 4 days (Fig. 2, *B* and *D*). This time course is consistent with that of PE-induced hypertrophy in cardiomyocytes, which became evident 4 days after the treatment under our experimental conditions.

To examine correlations between the observed induction of corin mRNA expression and cell size or ANP mRNA expression, linear regression analysis was performed. The results showed that

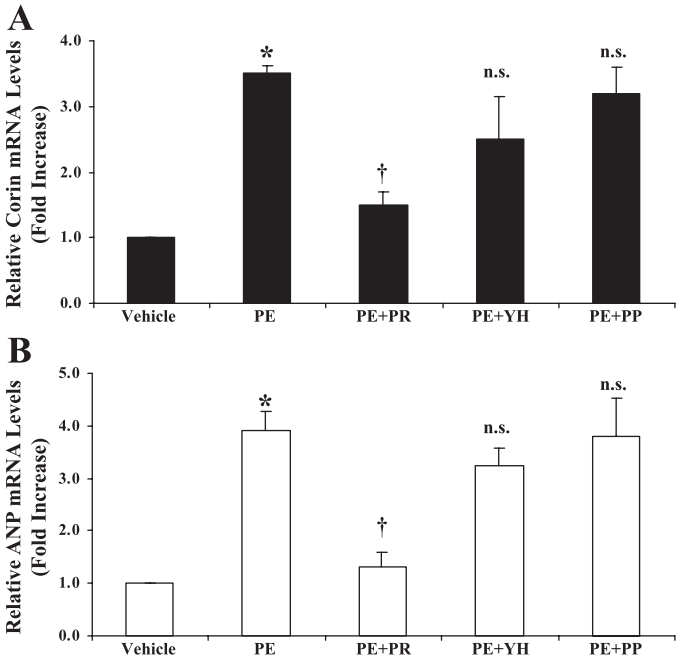

Fig. 1. Corin and atrial natriuretic peptide (ANP) mRNA expression in hypertrophic cardiomyocytes. Cardiomyocytes were stimulated with phenylephrine (PE; 100 nM) in the absence or presence of prazosin (PR; 500 ng/ml), yohimbine (YH; 500 ng/ml), and propranolol (PP; 500 ng/ml). Total RNA was isolated and corin and ANP mRNA was analyzed by RT-PCR. The relative corin (*A*) and ANP (*B*) mRNA levels were calculated by normalizing their values to the values obtained for GAPDH mRNA. The data are from four independent experiments and presented as means ± SD. ns, Not significant vs. PE. *$P < 0.01$ vs. vehicle control; †$P < 0.01$ vs. PE.

corin mRNA levels correlated closely with cell size ($r = 0.95$; $P < 0.01$) (Fig. 3*A*) and ANP mRNA levels ($r = 0.92$; $P < 0.01$) (Fig. 3*B*) in the PE-treated cardiomyocytes.

We also examined corin mRNA expression in hypertrophic cardiomyocytes induced by endothelin (100 nM) in culture. RT-PCR analysis showed that corin mRNA levels were 4.2-fold higher ($P < 0.01$) in the endothelin-treated cardiomyocytes than those in vehicle-treated control cells (data not shown), indicating that corin mRNA expression was enhanced in hypertrophic cardiomyocytes that were induced by different stimuli.

Processing of Pro-ANP by cultured cardiomyocytes. Because anti-human corin antibodies are not available, we were unable to directly measure corin protein expression in the PE-treated cardiomyocytes. Instead, we examined the activity of corin in these cells in a pro-ANP processing assay. Human recombinant pro-ANP was first expressed in 293 cells by transfection of an expression vector, pcDNAproANP. The conditioned medium containing recombinant pro-ANP was collected and incubated with cardiomyocytes treated with increasing concentrations of PE. Processing of pro-ANP was analyzed by immunoprecipitation and Western blot analysis. As shown in Fig. 4, *top*, production of recombinant ANP was significantly enhanced in the conditioned medium derived from PE-treated cardiomyocytes. Analysis of the Western blot with a densitometer showed that recombinant ANP level was ~2.8-fold higher in the conditioned medium from the cells treated with 500 nM PE than that from untreated control cardiomyocytes (Fig. 4, *bottom*). The effect of PE on pro-ANP processing

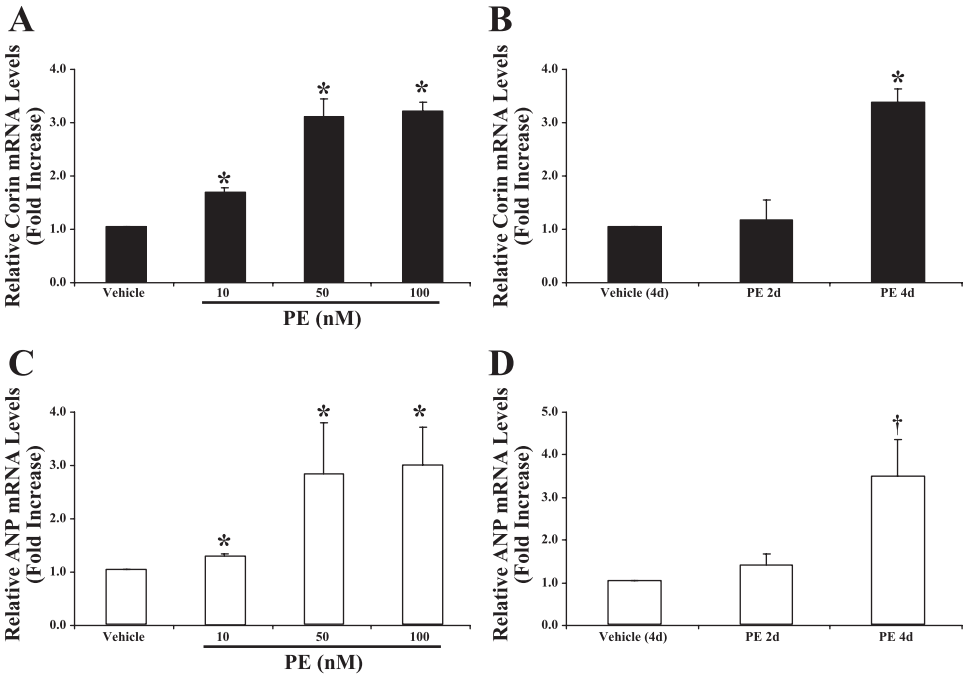

Fig. 2. Dose- and time-dependent induction of corin and ANP mRNA by PE. Cardiomyocytes were cultured for 4 days in the presence of increasing concentrations of PE (*A* and *C*) or in the presence of 100 nM PE for 2 or 4 days (*B* and *C*). Total RNA was isolated and corin and ANP mRNA was analyzed by RT-PCR. The relative corin (*A* and *B*) and ANP (*C* and *D*) mRNA levels were calculated by normalizing their values to the values obtained for GAPDH mRNA. The data are from three independent experiments and presented as means ± SD. *$P < 0.01$ vs. vehicle control; †$P < 0.05$ vs. vehicle control.

was completely blocked when the cells were cultured in the presence of the α_1-adrenergic receptor antagonist prazosin (1 μM). Although the Western blot-based studies are qualitative rather than quantitative, the data are consistent with the induction of corin mRNA by PE in hypertrophic cardiomyocytes and indicate that corin protein is present and functional on the surface of these cells.

Activity of cardiomyocyte-processed ANP. The biological function of ANP is mediated by binding to its receptor, stimulating the guanylyl cyclase activity of the receptor, and leading to generation of intracellular cGMP (20, 35). To determine whether the cardiomyocyte-processed recombinant ANP is biologically active and to better quantify the data, the

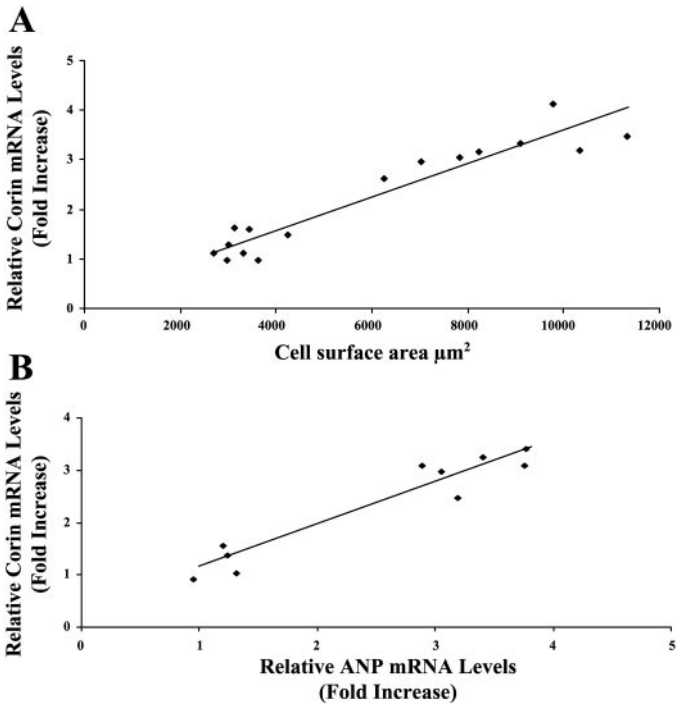

Fig. 3. Correlations between corin mRNA levels and cell size (*A*) or ANP mRNA levels (*B*). Linear regression analysis was performed with the use of VassarStats software to determine Spearman's rank correlation coefficients.

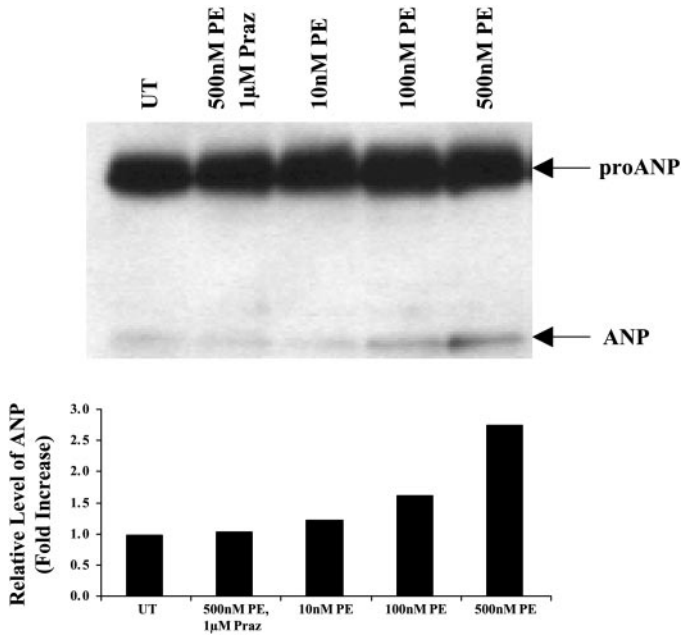

Fig. 4. Processing of recombinant pro-ANP in cardiomyocytes. Recombinant human pro-ANP was expressed in human embryonic kidney (HEK)-293 cells. The conditioned medium containing pro-ANP was collected and incubated with rat cardiomyocytes pretreated with increasing concentrations (10, 100, and 500 nM) of PE. As controls, untreated (UT) cardiomyocytes or cardiomyocytes treated with 500 nM of PE in the presence of 1 μM prazosin (Praz) were included. Recombinant pro-ANP and its derivatives in the conditioned medium were analyzed with Western blot analysis with the use of an anti-V5 antibody (*top*). To quantitate recombinant ANP, the Western blot was analyzed with a densitometer. Relative levels of ANP were calculated by normalizing their values to the ANP level obtained from the untreated cells (*bottom*).

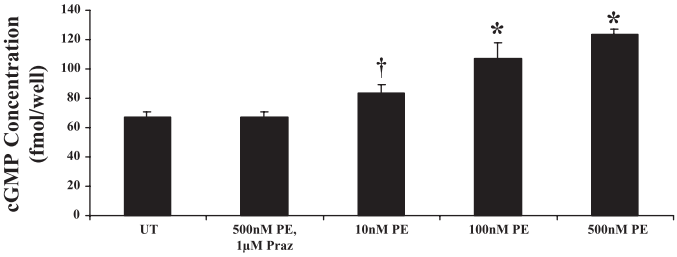

Fig. 5. cGMP-stimulating activity in the conditioned medium. The conditioned medium containing recombinant pro-ANP was incubated with cardiomyocytes pretreated with increasing concentrations of PE or control cells. The cGMP-stimulating activity in the conditioned medium was tested in a cell-based assay. Each experimental condition was assayed in triplicate. Data are presented as means ± SD from three independent experiments. *$P < 0.01$ vs. UT control group.

conditioned medium incubated with PE-treated cardiomyocytes was tested for cGMP-stimulating activity in a BHK cell-based assay. As shown in Fig. 5, cGMP-stimulating activities were significantly higher in the conditioned medium from cardiomyocytes treated with increasing concentrations of PE compared with that from untreated control cardiomyocytes. The results are consistent with the observed pro-ANP processing in PE-treated cardiomyocytes and indicate that recombinant ANP processed by these cells was biologically active.

Expression of corin mRNA in myocardium during heart failure. To examine corin mRNA expression in hypertrophic myocardium in vivo, a rat model of heart failure was performed by ligation of the left coronary artery. Eight weeks after the surgery, heart failure developed as indicated by significant elevation of LV end-diastolic pressure, decreased LV contractility, cardiac dilatation with increased LV volume, and hypertrophy of noninfarcted LV myocardium (Table 1).

By Northern blot analysis, corin mRNA expression in the noninfarcted LV myocardium from rats with heart failure was significantly increased compared with that in control samples from sham-operated animals (Fig. 6*A*). On average, corin mRNA expression was 3.3-fold higher in the rats with heart failure than that in control animals (Fig. 6*B*). Plasma ANP

Table 1. *Basal hemodynamic characteristics in heart failure and sham-operated rats*

	Sham ($n = 8$)	Heart Failure ($n = 8$)	P Value
Weight			
Body, g	538±22	531±17	NS
Heart, g	1.29±0.01	1.53±0.04	<0.01
Heart/body ratio, mg/g	2.43±0.1	2.87±0.04	<0.01
Left ventricle, g	0.96±0.02	1.06±0.03	<0.05
Right ventricle, g	0.25±0.01	0.32±0.01	<0.01
LV volume, μl	65±10	209±24	<0.01
LV wall thickness, mm	3.08±0.07	4.39±0.08	<0.01
MAP, mmHg	108±4	99±5	NS
HR, beats/min	356±11	362±17	NS
LVEDP, mmHg	6.0±0.6	12.7±2.3	<0.05
+dP/d*t*, mmHg/s	7,643±831	5,233±537	<0.05
−dP/d*t*, mmHg/s	6,661±569	4,639±544	<0.05
Infarct size, %	0	38.6±2.1	

Values are means ± SD; $n = 8$ hearts except for sham left ventricular (LV) wall thickness, where $n = 7$ hearts. NS, not significant; MAP, mean arterial pressure; HR, heart rate; LVEDP, LV end-diastolic pressure; dP/d*t*, first derivative of LV pressure. Significance indicates comparisons between the two groups by unpaired Student's *t*-test.

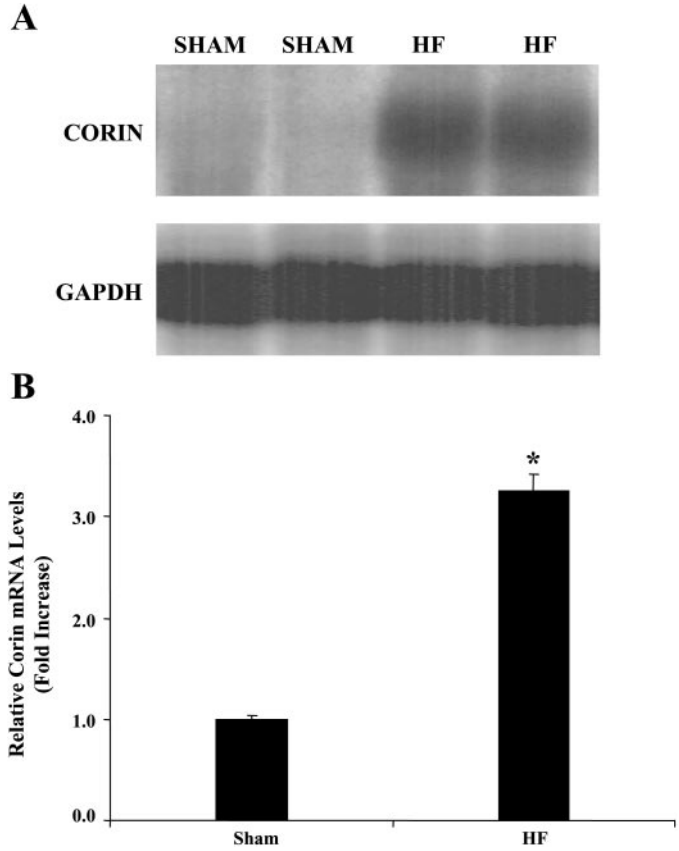

Fig. 6. Corin mRNA expression in a rat model of heart failure. Total RNA was isolated from heart tissues of rats with heart failure (HF) and sham-operated controls (Sham). Northern blot analysis was performed with the use of a partial rat corin cDNA. As a control, the same blot was reprobed with a rat GAPDH cDNA. *A*: representative blots from two rats with heart failure and two controls. The relative corin mRNA levels were quantitated by analyzing corin and GAPDH hybridizing bands using a densitometer. *B*: data from five rats in each group (means ± SD). *$P < 0.01$ vs. control.

concentrations in the experimental animals were measured with RIA. The results showed that plasma ANP concentrations were 4.8-fold higher in rats with heart failure that that in sham-operated controls (862.3 ± 243.9 vs. 180.2 ± 18.3 pg/ml; $n = 8$, $P < 0.01$).

We also examined the cardiac expression of corin mRNA during the course of heart failure in the rats. RT-PCR analysis of the noninfarcted LV myocardium showed that corin mRNA expression did not change significantly up to 4 wk after the surgery. By 8 wk, however, corin mRNA expression was threefold higher in the rats with heart failure than that in control animals (Fig. 7), confirming the finding by Northern blot analysis. Together, these results demonstrated that the *corin* gene was significantly upregulated in failing myocardium in the rat.

DISCUSSION

The ANP and BNP-mediated pathway plays an important physiological role in maintaining normal intravascular volume and blood pressure. Recently, we have identified a cardiac transmembrane serine protease, corin, that is the enzyme responsible for converting pro-ANP and pro-BNP to mature ANP and BNP (37, 39, 40). In this study, we examined the

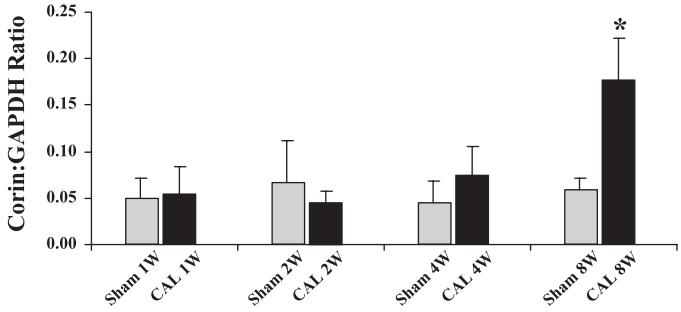

Fig. 7. Corin mRNA expression and plasma ANP levels in a rat model of HF at different times. Coronary artery ligation (CAL) or sham operation was performed in rats ($n = 5$ for each group). Total RNA was isolated from heart tissues of these rats at 1, 2, 4, and 8 wk after surgery. Corin mRNA expression was analyzed by quantitative RT-PCR. The relative corin mRNA levels were calculated by normalizing their values to the values obtained for GAPDH mRNA. *$P < 0.01$ vs. sham-operated control from the same time point.

regulation of corin gene expression in cultured cardiomyocytes during hypertrophic stimulation and in a rat model of heart failure, which is associated with myocardial hypertrophy in the noninfarcted LV myocardium. Our results showed that corin mRNA expression was significantly increased in hypertrophic cardiomyocytes induced by PE in culture. The upregulation of corin mRNA expression was also found in vivo in the noninfarcted myocardium of rats with heart failure after myocardial infarction.

High plasma concentrations of ANP and BNP are commonly associated with pathological conditions, such as hypertension, preeclampsia, myocardial infarction, and CHF (2, 5, 11, 26). Studies (20, 30, 35) have shown that in response to volume overload or hypertrophic stimuli, the production of these natriuretic peptides in cardiomyocytes are greatly enhanced. The increased expression of ANP and BNP has been attributed mainly to the enhanced synthesis and release of these natriuretic peptides in cardiomyocytes (8, 22). Our recent identification of corin as the pro-ANP convertase suggests that the activation of pro-ANP and pro-BNP could also be a critical step in the regulation of ANP and BNP production. In support of this hypothesis, we showed that overexpression of corin in cultured cardiomyocytes enhanced the processing of pro-ANP, leading to increased secretion of biologically active ANP (37). It was not known, however, whether the *corin* gene is regulated in cardiomyocytes under pathological conditions.

To test the hypothesis that the *corin* gene is upregulated in cardiomyocytes under hypertrophic conditions, we stimulated cultured rat neonatal cardiomyocytes by PE, an α_1-adrenergic receptor agonist. Hypertrophy of the cultured cells was confirmed by the increase in cell size and F-actin staining. RT-PCR analyses showed that corin mRNA levels were significantly increased in the hypertrophic cardiomyocytes. The induction of corin mRNA expression correlated directly with the size of the cardiomyocytes treated with different concentrations of PE for various time periods and with the induction of ANP mRNA expression in these cells. The PE-induced upregulation of corin mRNA expression was blocked by prazosin but not yohimbine or propranolol, confirming that the effects of PE are mediated by α_1-adrenergic receptor. In addition, similar upregulation of corin mRNA expression was also observed when cultured cardiomyocytes were stimulated by endothelin-1. It is known that activation of the α_1-adrenergic and endothelin receptors stimulates the $G\alpha_q$-mediated signaling pathway in cardiomyocytes and contributes to cardiac hypertrophy (14, 24, 31). Our results indicate that the upregulation of corin gene expression is a cellular response in hypertrophic cardiomyocytes that is likely mediated by the $G\alpha_q$ signaling pathway. Further experiments will be important to test whether corin gene expression is upregulated in cardiomyocytes stimulated by other agents, such as TGF-β or insulin-like growth factor, which activate receptor tyrosine kinases.

Cardiac hypertrophy is one of the compensatory mechanisms after myocardial infarction leading to heart failure. To confirm the induction of corin gene expression in vivo, we examined corin mRNA levels in a rat model of heart failure caused by ligation of left coronary artery. The failing myocardium in this model showed hypertrophy as indicated by increased heart weight, heart-to-body weight ratio, and LV wall thickness. Previous studies (41) reported that plasma ANP levels were highly elevated in this animal model of heart failure. By RIA, we also found that plasma ANP concentrations were significantly higher in rats with heart failure. Although the assay may also detect unprocessed pro-ANP molecules in plasma, the data are consistent with the overproduction of ANP in these animals. With the use of Northern blot and RT-PCR analyses, we showed that corin mRNA levels were significantly higher in the noninfarcted LV myocardium from rats with a late stage of heart failure. The results demonstrate that the corin gene is indeed upregulated in failing myocardium in vivo.

The role of corin in the pathophysiology of cardiac hypertrophy is not completely understood. Studies (12, 18, 23) have indicated that natriuretic peptide receptor signaling has local effects on cardiomyocytes that are independent of their systemic hemodynamic actions. Indeed, ANP has been shown to inhibit DNA synthesis and cell growth in cultured cardiomyocytes, fibroblasts, vascular smooth muscle, and endothelial cells (3, 4, 25) and to induce apoptosis in cultured neonatal rat cardiomyocytes (36). Studies (32) with knockout mice also demonstrate that BNP has a local antifibrotic effect in the heart. Recently, Tsuruda et al. (34) have shown that BNP promotes matrix metalloproteinase production in cultured cardiac fibroblasts. These data indicate that natriuretic peptides may also serve as a compensatory mechanism that acts locally to prevent cardiac hypertrophy and fibrosis. In this study, we have shown a close correlation, but not a causal relationship, between upregulation of corin expression and ANP levels. The possibility that other enzymes induced by PE in cardiomyocytes may contribute to the observed pro-ANP processing cannot be formally excluded. Furthermore, it is not known whether ANP itself regulates corin expression in cardiomyocytes. To address these issues, we tried RNA interference-based experiments in cultured cardiomyocytes but were unable to achieve sufficient levels of transfection to inhibit corin expression in the primary culture. Most recently, however, we created a corin knockout mouse and showed that pro-ANP processing was indeed abolished in these animals, confirming that corin is the physiological pro-ANP convertase (J. Chan and Q. Wu, unpublished observations). Together, our data support the notion that increased expression of corin contributes to elevation of ANP in the setting of cardiac hypertrophy and heart failure.

In summary, we have shown that the *corin* gene is highly upregulated in hypertrophic cardiomyocytes in vitro and failing myocardium in vivo. As the pro-ANP convertase, corin acts at

the top the ANP-mediated pathway. Our results suggest that the induction of corin expression is a cardiac response to hypertrophic stimuli and may play an important role in the pathophysiology of cardiac hypertrophy and heart failure.

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