Journal of the American College of Cardiology Vol. 45, No. 4, 2005

Published by Elsevier Inc.
ISSN 0735-1097/05/$30.00
doi:10.1016/j.jacc.2004.10.067

C-Type Natriuretic Peptide, a Novel Antifibrotic and Antihypertrophic Agent, Prevents Cardiac Remodeling After Myocardial Infarction

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OBJECTIVES We assessed the hypothesis that in vivo administration of C-type natriuretic peptide (CNP) might attenuate cardiac remodeling after myocardial infarction (MI) through its antifibrotic and antihypertrophic action.

BACKGROUND Recently, we have shown that CNP has more potent antifibrotic and antihypertrophic effects than atrial natriuretic peptide (ANP) in cultured cardiac fibroblasts and cardiomyocytes.

METHODS Experimental MI was induced by coronary ligation in male Sprague-Dawley rats; CNP at 0.1 μg/kg/min (n = 34) or vehicle (n = 35) was intravenously infused by osmotic mini-pump starting four days after MI. Sham-operated rats (n = 34) served as controls. After two weeks of infusion, the effects of CNP on cardiac remodeling were evaluated by echocardiograpic, hemodynamic, histopathologic, and gene analysis.

RESULTS C-type natriuretic peptide markedly attenuated the left ventricular (LV) enlargement caused by MI (LV end-diastolic dimension, sham: 6.7 ± 0.1 mm; MI+vehicle; 8.3 ± 0.1 mm; MI+CNP: 7.7 ± 0.1 mm, $p < 0.01$) without affecting arterial pressure. Moreover, there was a substantial decrease in LV end-diastolic pressure, and increases in dP/dt_{max}, dP/dt_{min}, and cardiac output in CNP-treated MI rats compared with vehicle-treated MI rats. Importantly, CNP infusion markedly attenuated an increase in morphometrical collagen volume fraction in the noninfarct region (sham: 3.1 ± 0.2%; MI+vehicle: 5.7 ± 0.5%; MI+CNP: 3.9 ± 0.3%, $p < 0.01$). In addition, CNP significantly reduced an increase in cross-sectional area of the cardiomyocytes. These effects of CNP were accompanied by suppression of MI-induced increases in collagen I, collagen III, ANP, and β-myosin heavy chain messenger ribonucleic acid levels in the noninfarct region.

CONCLUSIONS These data suggest that CNP may be useful as a novel antiremodeling agent. (J Am Coll Cardiol 2005;45:608–16) © 2005 by the American College of Cardiology Foundation

The mammalian natriuretic peptide system consists of three structurally homologous peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (1). The actions of the natriuretic peptides are modulated through membrane-bound receptors, two of which are guanylyl cyclase (GC)-coupled receptors (GC-A and GC-B). These receptors are linked to the cyclic guanosine monophosphate (cGMP)-dependent signaling cascade and mediate the biological actions of the peptides (2). Atrial natriuretic peptide and BNP are mainly released from the heart to act as circulating hormones, which bind to their specific receptor, GC-A, in the vascular tissue, kidney, and adrenal gland and induce vasodilation, natriuresis, and diuresis (3). C-type natriuretic peptide, which was originally isolated from porcine brain extracts (4), not only acts in the central nervous system, but also plays a role in the local regulation such as the suppression of neointimal formation after vascular injury (5) through its specific receptor, GC-B. Recently, we have shown that CNP was synthesized in cultured cardiac fibroblasts and that CNP inhibited both deoxyribonucleic acid (DNA) and collagen synthesis of cardiac fibroblasts more potently than ANP and BNP (6). C-type natriuretic peptide also has more potent antihypertrophic effects than ANP in cultured cardiomyocytes (7). These findings might be due to the relative abundance of GC-B over GC-A in cardiac fibroblasts and in cardiomyocytes (6,7). In addition, in a recent clinical study, CNP was produced by the hearts of patients with chronic heart failure, and its level in the coronary sinus correlated with mean pulmonary wedge pressure (8). These basic and clinical results suggest that CNP might represent an important local mediator in the heart.

Left ventricular (LV) remodeling after myocardial infarction (MI) is a major cause of subsequent heart failure and death (9). Postinfarction remodeling has been divided into an early phase (within 72 h), which involves expansion of the infarct zone, and a late phase (after 72 h), which is associated with time-dependent LV dilation, mural hypertrophy, and cardiac fibrosis (10). Given the inhibitory effects of CNP on cardiac fibrosis and hypertrophy in vitro, CNP might act against the progression of cardiac late remodeling after MI. Furthermore, because intravenously administered CNP has been demonstrated to have much less potent

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Manuscript received May 28, 2004; revised manuscript received October 22, 2004, accepted October 25, 2004.

Abbreviations and Acronyms

ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
CNP = C-type natriuretic peptide
cGMP = cyclic guanosine monophosphate
GC = guanylyl cyclase
LV = left ventricle/ventricular
MHC = myosin heavy chain
MI = myocardial infarction
PKG = cyclic guanosine monophosphate-dependent protein kinase
RV = right ventricle/ventricular
TGF = transforming growth factor

vasorelaxant and natriuretic activities than ANP (4,11), CNP is not expected to perturb systemic hemodynamics after massive MI while ANP or BNP is. However, there has been no in vivo evidence to directly prove these beneficial effects of CNP after MI. Therefore, in the present study, we have assessed the hypothesis that in vivo administration of CNP might attenuate cardiac late remodeling after MI. In addition, to elucidate the mechanism involved in the antifibrotic action of CNP, we investigated the action of cGMP/cGMP-dependent protein kinase (PKG) pathway on collagen synthesis by cardiac fibroblasts in vitro, and to clarify whether CNP is an important local mediator in the heart, we investigated the degree and source of endogenous CNP production in the infarcted heart.

METHODS

Model of MI. All experimental procedures were performed according to the guidelines for animal experimentation of National Cardiovascular Center. Male Sprague-Dawley rats (Nihon SLC, Hamamatsu, Japan) weighing 180 to 220 g were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally). After left thoracotomy, the left coronary artery was ligated 2 to 3 mm from its origin using a 6-0 Prolene suture. The chest was closed, and the rats were allowed to recover. Sham-operated rats underwent the identical surgical procedure as described above without the actual coronary artery ligation.

Administration of CNP. Four days after coronary ligation, the rats with MI were randomly divided into two groups: one to be infused with synthetic CNP (MI+CNP, n = 36) and the other with vehicle (MI+vehicle, n = 42). The CNP group was then fitted with subcutaneous osmotic minipumps (model 2ML2, Alza Corp., Palo Alto, California) filled with synthetic CNP dissolved in a 5% glucose solution and set to release 0.1 μg/kg/min of the peptide for two weeks. The dose of CNP was chosen because our preliminary study revealed that CNP at this dose has no effects on arterial blood pressure and heart rate in rats. Glucose solution was infused in a similar manner in the control group. The pumps were connected to the left jugular vein by a polyethylene catheter. The synthetic CNP was kindly provided by Daiichi Suntory Pharma (Tokyo, Japan).

Noninvasive blood pressure and pulse rate. Systolic blood pressure and pulse rate were measured before MI and one day, one week, and two weeks after MI by the tail-cuff method without use of anesthesia (Softron, Tokyo, Japan).

Echocardiographic and hemodynamic studies. Echocardiographic studies were performed using an echocardiographic system equipped with a 15-MHz phased-array transducer (SONOS 5500, Hewlett Packard, Andover, Massachusetts) under anesthesia with sodium pentobarbital (30 mg/kg, intraperitoneally) 4 and 18 days after the experimental MI or sham operation as described previously (12). Rats with >20% fractional shortening or an early filling wave (E) velocity to atrial filling wave (A) velocity ratio of <3 in the echocardiographic study performed four days after MI were excluded from the study.

Eighteen days after the coronary ligation or sham operation, hemodynamic studies were performed under anesthesia as previously described (12). After completion of hemodynamic measurements, the hearts were arrested by the injection of 30 mM potassium chloride through the carotid artery, excised, and weighed.

Histological examination. After fixation, three cross-sections through the ventricles were obtained and embedded (n = 17 to 19 in each group). Paraffin sections (2 μm) were stained with Masson's trichrome for measurement of infarct size, hematoxylin and eosin for measurement of myocyte size, and Sirius red F3BA for determination of collagen volume fraction. The infarct size was expressed as previously described (13). For the measurement of cardiomyocyte cross-sectional area and diameter in the noninfarcted LV, a total of 30 myocytes sectioned transversely for area and longitudinally for diameter at the level of the nucleus were randomly chosen from each section at ×400 magnification, and traced. To measure collagen volume fraction, 16 fields in the border and remote myocardium of the noninfarcted LV and right ventricle (RV) walls per section were scanned at a magnification of ×200. The interstitial collagen volume fraction was measured while omitting fibrosis of the perivascular, epi-, and endocardial areas from the study. The collagen volume fraction was obtained by calculating the mean ratio of connective tissue to the total tissue area of all the measurements of the section. The collagen-positive areas from all sections were determined by a single investigator who was unaware of the experimental groups.

Northern blot analysis. Total ribonucleic acid (10 μg/lane) was extracted from the RV, noninfarcted LV, and infarcted LV (n = 10 in each group). Hybridization was carried out with cDNA probes for rat α-1 (type I) collagen, rat α-1 (type III) collagen, rat fibronectin, rat transforming growth factor (TGF)-β-1, rat ANP, and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH). We also used synthetic oligonucleotide probes for the α- and β-myosin heavy chain (MHC) messenger ribonucleic

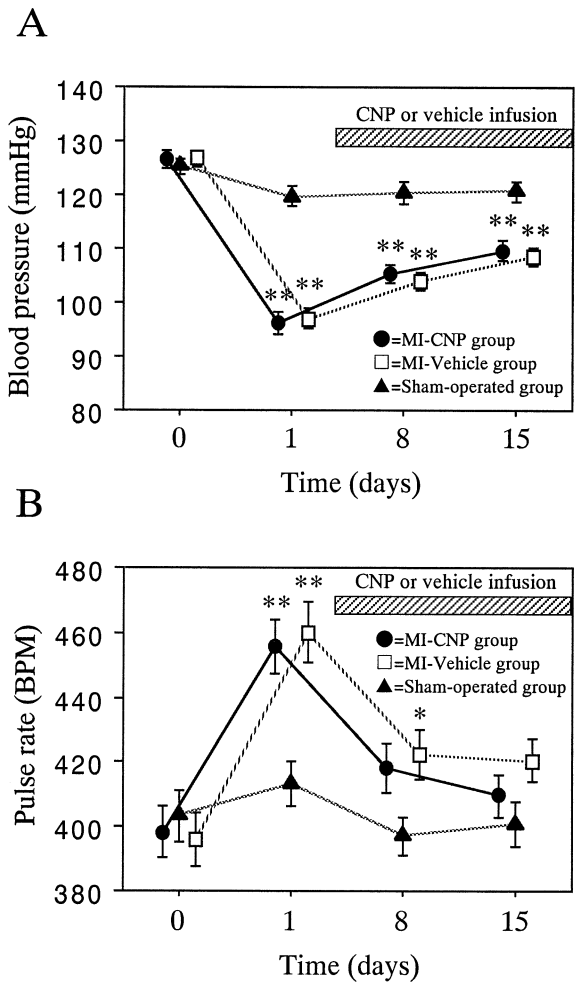

Figure 1. Time course of systolic blood pressure **(A)** and pulse rate **(B)** in sham-operated rats **(closed triangles)** and in rats with myocardial infarction (MI) before and during infusion of 0.1 μg/kg/min C-type natriuretic peptide (CNP) **(closed circles)** or vehicle (5% glucose solution) **(open squares)**. Values are mean ± SEM. A p value for systolic blood pressure by two-way analysis of variance: group <0.001; time course <0.001; group/time course interaction <0.001, and a p value for pulse rate by two-way analysis of variance: group <0.05; time course <0.001; group/time course interaction <0.01. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with the sham-operated group at same stage by Bonferroni multiple-comparison *t* test. BPM = beats/min.

acids (mRNA). The band intensity was estimated by a radioimage analyzer (BAS-5000, Fuji Film, Tokyo, Japan).

Collagen synthesis in vitro. Neonatal cardiac fibroblasts were prepared as described previously (14). The effects of CNP and a cGMP analog on collagen synthesis in cardiac fibroblasts were evaluated on subconfluent cultures by the incorporation of [^{3}H]proline into cells as previously described (6). In brief, after the preconditioning period, CNP with or without Rp-8-pCPT-cGMP (Calbiochem, San Diego, California), or 8-Bromo cGMP (Sigma, St. Louis, Missouri) was added, and 0.5 μCi of [^{3}H]proline was also added. After the cells were incubated for 24 h, the radioactivity of aliquots of the trichloroacetic acid-insoluble material was determined using a liquid scintillation counter.

Table 1. Echocardiographic Parameters

	Sham	MI+Vehicle	MI+CNP
4th day (before treatment)			
AWT diastole, mm	1.2 ± 0.01	1.0 ± 0.01*	1.0 ± 0.01*
PWT diastole, mm	1.3 ± 0.01	1.3 ± 0.01	1.3 ± 0.01
LVDd, mm	6.4 ± 0.1	7.0 ± 0.1*	7.0 ± 0.1*
FS, %	34 ± 1	16 ± 0.3*	15 ± 0.3*
E velocity, cm/s	89 ± 3	102 ± 2*	103 ± 3*
A velocity, cm/s	49 ± 2	18 ± 1*	19 ± 1*
E/A	1.9 ± 0.1	5.8 ± 0.2*	5.6 ± 0.1*
18th day (after treatment)			
AWT diastole, mm	1.2 ± 0.01	0.9 ± 0.02*	0.9 ± 0.01*
PWT diastole, mm	1.3 ± 0.01	1.5 ± 0.02*	1.4 ± 0.02*†
LVDd, mm	6.7 ± 0.1	8.3 ± 0.1*	7.7 ± 0.1*†
FS, %	35 ± 1	16 ± 0.4*	18 ± 0.4*†
E velocity, cm/s	88 ± 2	112 ± 3*	102 ± 3*‡
A velocity, cm/s	51 ± 2	19 ± 1*	26 ± 1*†
E/A	1.8 ± 0.05	6.2 ± 0.2*	4.2 ± 0.2*†

Values are mean ± SEM. *p < 0.01 compared with sham-operated group; †p < 0.01, ‡p < 0.05 compared with MI+vehicle group by analysis of variance and Bonferroni multiple-comparison t test.

A = atrial filling wave; AWT = anterior wall thickness; CNP = C-type natriuretic peptide; E = early filling wave; FS = fractional shortening; LVDd = left ventricular end-diastolic dimensions; MI = myocardial infarction; PWT = posterior wall thickness.

Quantitative reverse transcription-polymerase chain reaction. Endogenous mRNA expressions of ventricular CNP were evaluated in rats killed on day 3, 7, and 18 after MI (without CNP treatment) and on day 3 after sham operation (n = 6 in each group) with quantitative reverse transcription-polymerase chain reaction using a LightCycler system (Roche Applied Science, Penzberg, Germany) according to the manufacturer's instruction.

Immunohistochemical analysis. Immunohistochemical studies were performed to localize endogenous CNP in LV myocardium after MI. The section on day 7 after MI (in rats without CNP treatment) was stained with goat anti-CNP antibody (Santa Cruz Biotechnology, Santa Cruz, California) followed by Alexa-Fluor donkey anti-goat IgG antibody (Molecular Probes, Eugene, Oregon) and stained with rabbit fibronectin antibody (Sigma, St. Louis, Missouri) followed by tetrarhodamine isothiocyanate-conjugated goat anti-rabbit IgG antibody (DakoCytomation, Glostrup, Denmark).

Statistical analysis. All values are expressed as mean ± SEM. Differences among the groups were evaluated by one-way analysis of variance and two-way analysis of variance for repeated measurements, as appropriate. When a statistical difference was detected by analysis of variance, the Bonferroni method of adjusting for multiple pairwise comparisons was used. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The effect of CNP on survival rate and infarct size. Among the MI rats, two of the CNP-infused rats and seven of the vehicle-infused rats died during the two-week infusion period. The survival rate of the MI+CNP group (94%) was higher than that of the MI+vehicle group (83%), but this

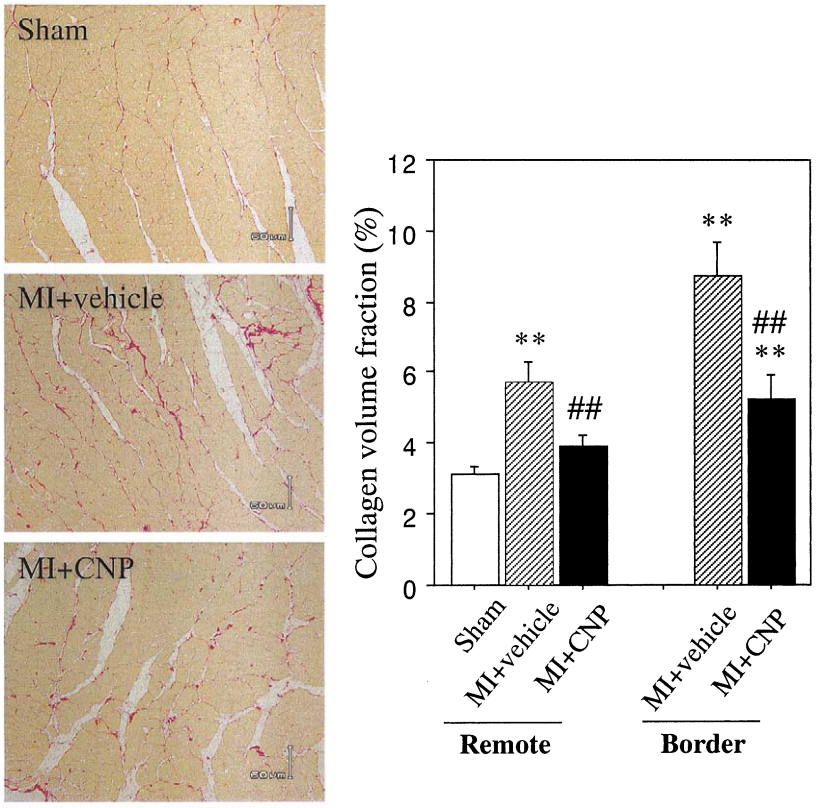

Figure 2. The effect of C-type natriuretic peptide (CNP) infusion on collagen volume fraction in the remote and border noninfarcted left ventricular area after myocardial infarction (MI). Representative photomicrographs of collagen volume stained with Sirius red in the remote noninfarcted LV (×200 magnification) **(left)** and quantitative morphometric analysis **(right)**. Values are mean ± SEM. **$p < 0.01$ compared with the sham-operated group; ##$p < 0.01$ compared with the MI+vehicle group by analysis of variance and Bonferroni multiple-comparison *t* test.

difference was not statistically significant by Kaplan-Meier survival analysis ($p = 0.13$). No rats died in the sham group. Therefore, the total numbers for final analysis were 34 rats in the MI+CNP group, 35 in the MI+vehicle group, and 34 in the sham group. There was no difference in infarct size between the MI+CNP and MI+vehicle groups (45 ± 1% and 46 ± 1%, respectively).

Serial change of noninvasive blood pressure and pulse rate. A significant reduction in the systolic blood pressure was observed in MI+CNP or MI+vehicle rats compared with the sham-operation rats during two weeks after the operation. As shown in Figure 1A, the systolic blood pressure was not perturbed by CNP infusion at any time points. The pulse rate in MI groups significantly increased at day 1 compared with sham animals and decreased gradually. The pulse rate was not significantly affected by CNP treatment at any time points (Fig. 1B).

The effect of CNP on echocardiographic and hemodynamic parameters. Table 1 shows echocardiographic assessments of cardiac geometry and function for the three groups of rats at the 4th and 18th days after MI. At the 4th day (before CNP infusion), when compared with sham, LV enlargement, decreased fractional shortening, and increased ratio of E to A velocities were seen in similar degree in both MI groups. At the 18th day (after two weeks of CNP infusion), hypertrophy of the posterior wall and the LV cavity enlargement caused by MI were significantly attenuated by CNP infusion, although thinning of the anterior wall was not changed; CNP also ameliorated the decrease of fractional shortening. Furthermore, CNP significantly improved LV diastolic filling pattern, resulting in a marked reduction in the ratio of E to A velocities (Table 1).

Table 2 also shows hemodynamic assessments for the three groups of rats at the 18th day after MI. No significant difference was noted in heart rate among the three groups. Mean arterial pressure and LV systolic pressure were lower

Table 2. Hemodynamic Parameters

	Sham	MI+Vehicle	MI+CNP
HR, beats/min	412 ± 5	421 ± 6	410 ± 5
MAP, mm Hg	120 ± 2	99 ± 2*	103 ± 2*
LVSP, mm Hg	139 ± 2	116 ± 2*	118 ± 2*
LVEDP, mm Hg	7 ± 0.4	18 ± 1*	13 ± 1*†
LV dP/dt_{max}, mm Hg/s	7,970 ± 156	5,019 ± 155*	5,743 ± 155*†
LV dP/dt_{min}, mm Hg/s	−6,216 ± 158	−3,791 ± 151*	−4,644 ± 147*†
CO, ml/min	98 ± 2	73 ± 2*	81 ± 2*†

Values are mean ± SEM. *$p < 0.01$ compared with sham-operated group; †$p < 0.01$ compared with MI+vehicle group by analysis of variance and Bonferroni multiple-comparison *t* test.

CNP = C-type natriuretic peptide; CO = cardiac output; HR = heart rate; LV dP/dt max or min = peak rate of left ventricular rise or fall; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; MI = myocardial infarction.

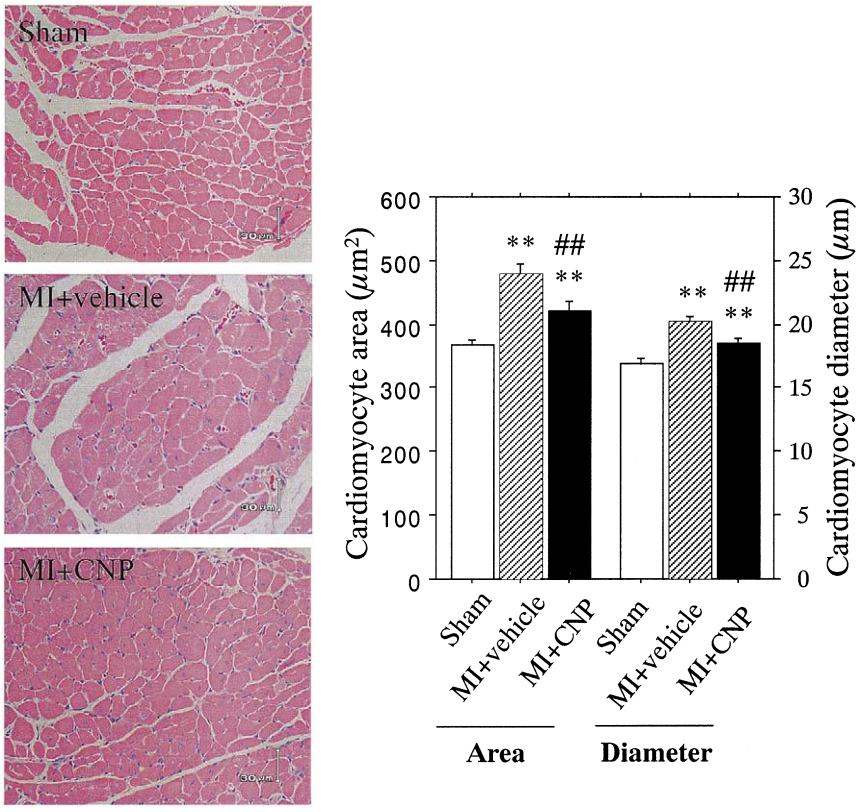

Figure 3. The effect of C-type natriuretic peptide (CNP) infusion on cardiac hypertrophy in the noninfarcted left ventricle. Representative photomicrographs of cardiomyocyte size stained with hematoxylin and eosin (×400 magnification) **(left)** and quantitative morphometric analysis of cardiomyocyte area and diameter **(right)**. Values are mean ± SEM. **$p < 0.01$ compared with the sham-operated group; ##$p < 0.01$ compared with the myocardial infarction (MI) + vehicle group by analysis of variance and Bonferroni multiple-comparison *t* test.

in the MI+vehicle and MI+CNP groups than in sham, but there were no differences in these parameters between the two MI groups. Left ventricular end-diastolic pressure was higher, and the peak rate of contraction (dP/dt_{max}), the peak rate of relaxation (dP/dt_{min}), and the cardiac output were lower in MI+vehicle than in sham. As shown in Table 2, the MI-induced systolic and diastolic LV dysfunction was markedly improved by CNP.

The effect of CNP on cardiac collagen volume and hypertrophy. To clarify the mechanism of improved cardiac performance caused by CNP, we examined the effects of CNP treatment on collagen volume and mural hypertrophy in the noninfarcted region; CNP significantly ($p < 0.01$) attenuated an increase in morphometrical collagen volume fraction in the remote LV (Fig. 2) and RV (sham: 3.3 ± 0.3%; MI+vehicle: 5.5 ± 0.5%; MI+CNP: 4.2 ± 0.3%). Furthermore, CNP reduced an increase in collagen volume fraction more effectively in the border region of MI, in which fibrosis was more prominent compared with the remote zone (Fig. 2).

The cross-sectional area and diameter of myocytes in the noninfarcted LV significantly increased in MI+vehicle compared with sham, and hypertrophy of the myocytes was significantly ($p < 0.01$) inhibited by CNP infusion (Fig. 3). In agreement with the above results, the heart-weight-to-body-weight ratio, which was increased in the two MI groups compared with sham, was significantly ($p < 0.01$) lowered by CNP treatment (sham: 3.29 ± 0.03 g/kg; MI+vehicle: 3.96 ± 0.09 g/kg; MI+CNP: 3.69 ± 0.06 g/kg).

The effect of CNP on gene expression. To confirm the effects of CNP on cardiac remodeling, we examined the expression of several mRNAs associated with fibrosis and hypertrophy in the noninfarcted LV and RV after MI (Figs. 4A, representative autoradiograms, and 4B, quantitative analysis, n = 10 in each group). As shown in Figure 4, the increased mRNA expression of collagen type I and collagen type III after MI was significantly suppressed by treatment with CNP. The increased fibronectin mRNA expression tended to be decreased by CNP, but it was not significant. At the 18th day, mRNA expression of TGF-β-1, which is well known to be a fibrotic cytokine and to be upregulated in the acute phase of MI (15), was not different between MI rats with or without CNP infusion; CNP treatment resulted in suppression of the ANP mRNA level, which is a useful marker of cardiac fetal phenotype modulation after MI, and the β-/α-MHC ratio, which is a qualitative marker of cardiac hypertrophy, in the noninfarcted LV (Fig. 4). In the infarcted LV, mRNA levels of collagen type I, collagen type III, fibronectin, TGF-β-1, ANP, and β-/α-MHC were all increased in the MI+vehicle and MI+CNP groups compared with sham, but there was no difference in these

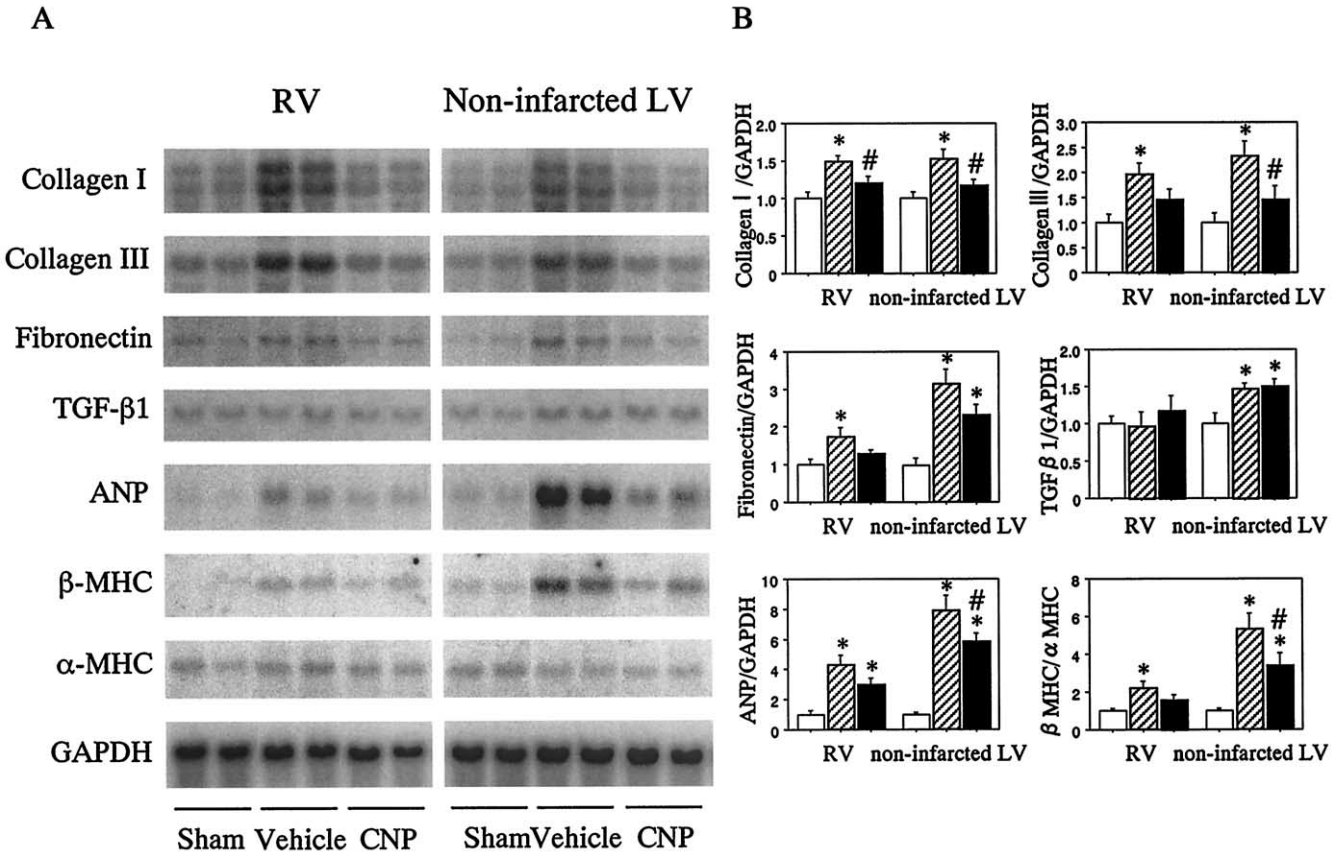

Figure 4. The effect of C-type natriuretic peptide (CNP) on messenger ribonucleic acid (mRNA) expression associated with cardiac remodeling after myocardial infarction (MI). **(A)** Typical autoradiograms of Northern blot analysis of mRNA levels in right ventricle (RV) and noninfarcted left ventricle (LV) for collagen type I, collagen type III, fibronectin, transforming growth factor-(TGF)-β-1, atrial natriuretic peptide (ANP), β- and α-myosin heavy chain (MHC), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) at the 18th day after MI. **(B)** Quantitative analyses of the abundance of each gene in the RV and noninfarcted LV at the 18th day after MI (n = 10 in each group). In individual samples, each mRNA value was corrected for the GAPDH mRNA value. Levels in sham-operated rats were arbitrarily assigned a value of 1.0. Values are mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with the sham-operated group; $\#p < 0.05$ compared with the MI+vehicle group by analysis of variance and Bonferroni multiple-comparison *t* test. **Open bars** = sham; **striped bars** = MI+vehicle; **solid bars** = MI+CNP.

parameters in the infarcted LV between the two MI groups (data not shown).

Cellular mechanism of the antifibrotic action of CNP. C-type natriuretic peptide and 8-Bromo cGMP, an analog of cGMP, decreased the incorporation of [^{3}H]proline into cardiac fibroblasts in a dose-dependent manner (Figs. 5A and 5B). The decrease of [^{3}H]proline incorporation by CNP was completely blocked by Rp-8-pCPT-cGMP, a cell-permeable inhibitor of PKG type I and type II, at a concentration of 10^{-5} mol/l (Fig. 5C).

Endogenous CNP expression after MI. C-type natriuretic peptide mRNA expression increased on day 3 by about four-fold in the infarcted LV and two-fold in the noninfarcted LV compared with sham rats, and gradually decreased from day 7 to day 18 (Fig. 6A). C-type natriuretic peptide mRNA expression in RV slightly increased on day 7 only (1.5-fold) (Fig. 6A). In addition, immunohistochemical study revealed that CNP was stained mainly in fibrotic area of the infarct and border region on day 7 after MI (Fig. 6B). These results suggest that endogenous local CNP might play an important role in the infarcted heart.

DISCUSSION

In this study, we have demonstrated that in vivo administration of CNP improved cardiac function and protected against cardiac remodeling after MI in rats. The beneficial effects of CNP treatment in the heart after MI included attenuation of cardiac fibrosis, hypertrophy, and LV enlargement.

In addition, continuous treatment with CNP had no effects on mean arterial pressure and LV systolic pressure at the time of sacrifice 18 days after MI. The serial change in noninvasive blood pressure during the recovery period after MI was also similar in rats with and without CNP treatment. These findings are consistent with previous studies, which showed that CNP infusion had little vasodepressor or natriuretic activities in rats and healthy humans (4,11).

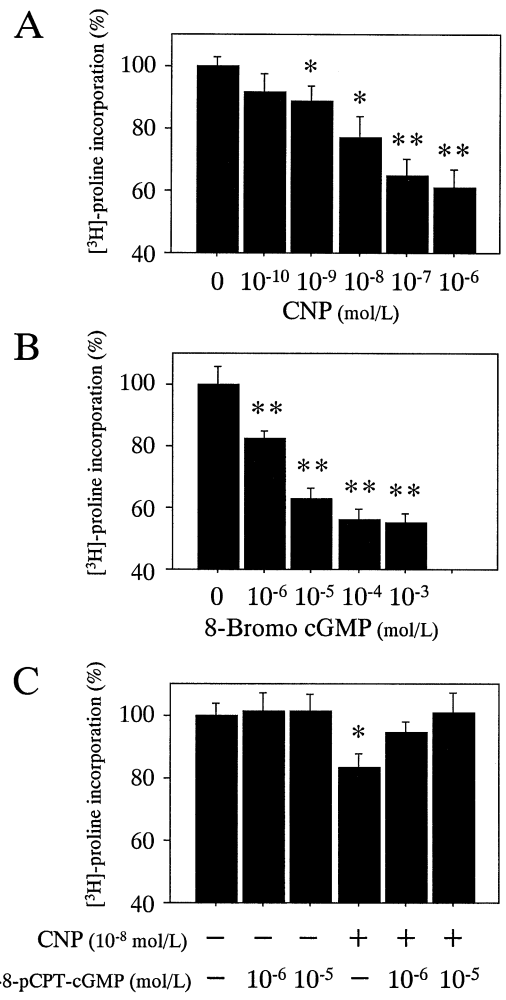

Figure 5. The effect of C-type natriuretic peptide (CNP) on collagen synthesis via the cyclic guanosine monophosphate (cGMP)/cyclic guanosine monophosphate-dependent protein kinase (PKG) pathway in cultured neonatal rat cardiac fibroblasts. **(A and B)** The effect of CNP and 8-Bromo cGMP on [^{3}H]proline incorporation in cardiac fibroblasts. **(C)** [^{3}H]proline incorporation in the presence or absence of 10^{-8} mol/l CNP with or without Rp-8-pCPT-cGMP, PKG inhibitor. Values are mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with control by analysis of variance and Bonferroni multiple-comparison *t* test.

Similarly, the heart rate was not significantly affected by CNP infusion throughout the study period.

The effect of CNP on cardiac performance. Chronic administration of CNP improved cardiac performance in MI rats, as indicated by increases in LV fractional shortening, cardiac output, and LV $dP/dt_{max/min}$, and by decreases in E/A ratio and LV end-diastolic pressure, which were accompanied by improvement of LV enlargement. Because the effect of CNP on pre- or after-load, heart rate, and infarct size was very little, a mechanism other than hemodynamic improvement or reduction in infarct size is probably the cause of the beneficial effects of CNP on cardiac performance.

The beneficial effect of CNP on cardiac remodeling through its antifibrotic action. One possibility is that CNP directly inhibits myocardial fibrosis because we have previously demonstrated that CNP directly inhibited both DNA and collagen synthesis by cardiac fibroblasts in vitro (6). In the present study, we have, therefore, examined the in vivo effect of CNP on fibrosis and found that CNP significantly attenuated an increase in morphometrical collagen volume fraction in the noninfarcted LV and RV. In addition, the effect of CNP was more prominent in the border region of MI, in which fibrosis was more increased, than that in the remote region. Because our preliminary study showed that a number of fibroblasts shift toward a myofibroblastic phenotype indicated by α-smooth muscle actin in the border region of MI, CNP might have more potent inhibitory effect on myofibroblast-like cells than on fibroblasts. Furthermore, the mRNA levels of collagen type I and III in the noninfarcted LV and RV were suppressed by treatment with CNP. These results provide in vivo evidence that CNP is a potent "fibrosis-inhibitory agent" after MI. The amount of myocardial collagen deposition in the infarcted and noninfarcted regions during healing after MI was reported to influence and to be integral to the process of ventricular remodeling (16). In addition, it has been shown that excessive accumulation of myocardial collagen might result in rigidity of the myocardium and severely impaired relaxation (17). Therefore, improved LV $dP/dt_{max/min}$ by CNP after MI might reflect improved myocardial rigidity in the noninfarcted region caused by the reduction in cardiac fibrosis.

Given the in vivo antifibrotic action of CNP, we further explored the cellular mechanisms of this action in vitro. Consistent with our previous study (6), CNP or 8-Bromo cGMP, an analog of cGMP, potently inhibited collagen synthesis of cultured cardiac fibroblasts. In the present study, the inhibitory effect of CNP was completely blocked by Rp-8-pCPT-cGMP, an inhibitor of PKG (Fig. 5), indicating that CNP inhibited collagen synthesis by activating cGMP/PKG pathway.

Some experimental data suggest that antifibrotic agents could potentially enhance the remodeling of the extracellular collagen matrix in the infarct zone during very early stage of healing after MI (18). However, in the present study, the death rate of the MI+CNP group (6%) was lower than that of the MI+vehicle group (17%) during the two-week infusion period, and two dead rats with CNP treatment showed no findings of LV rupture. The late start of CNP infusion at the fourth day of MI might reduce the potential adverse effects of antifibrosis such as wall thinning of infarct zone. Further studies are needed to determine the best timing of CNP treatment after MI.

The beneficial effect of CNP on cardiac remodeling through its antihypertrophic action. Another possible mechanism of cardioprotection by CNP might be attenuated myocardial hypertrophy after MI. As shown in the Results section, CNP effectively reduced the MI-induced myocardial hypertrophy in vivo. The findings are in agreement with previous in vitro studies, which showed that natriuretic peptides including CNP prevented hypertrophy

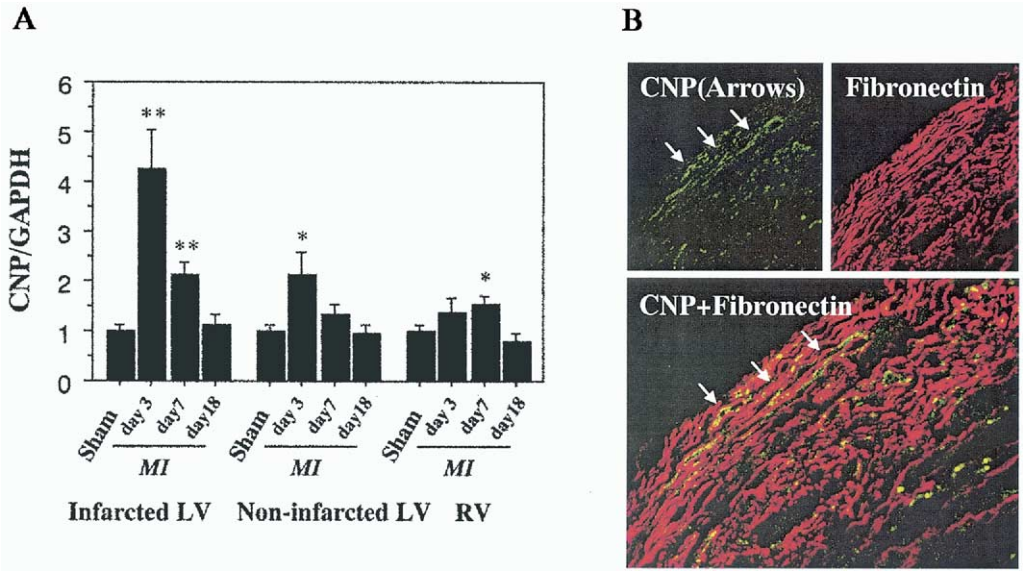

Figure 6. Endogenous C-type natriuretic peptide (CNP) expressions after myocardial infarction (MI). **(A)** Endogenous CNP mRNA abundance in the infarcted left ventricle (LV), noninfarcted LV, and right ventricle (RV) at different times after MI and on day 3 after sham operation. Values are mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with sham by analysis of variance and Bonferroni's multiple-comparison *t* test. **(B)** Immunofluorescent microscopic CNP expression. Upper photomicrographs show the section on day 7 after MI stained by the specific antibody against CNP-53 **(left, green)** and stained by antifibronectin antibody **(right, red)**. Lower photomicrograph shows the merged image (×600 magnification). GAPDH = glyceraldehydes-3-phosphate dehydrogenase.

of cultured cardiomyocytes (7,19). Although the precise mechanism by which CNP inhibits cardiac hypertrophy remains unknown, our previous study (7) suggested that CNP inhibits hypertrophy of cardiac myocytes directly by activating cGMP-dependent mechanism and indirectly by reducing endothelin-1 secretion from nonmyocytes.

On the other hand, because hypertrophy after MI is an adaptive response that offsets increased load, attenuates progressive dilation, and stabilizes contractile function (20), decreased cardiac hypertrophy shown in the present study might be caused by the indirect effect of CNP via decreased LV systolic wall stress. Further studies are needed to determine the contribution ratio of direct and indirect effects of CNP on cardiac hypertrophy.

Comparison with other antiremodeling therapies after MI. A number of therapeutic approaches to limiting ventricular remodeling in MI have been reported. These agents include angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor blockers, β-adrenergic blockers, aldosterone antagonists, and matrix metalloproteinase inhibitors. Although a number of these other agents have been given orally and, in this regard, they have an advantage over CNP, CNP treatment has some advantages concerning short treatment period and fewer side effects. Actually, in previous studies, it took more than four weeks for other agents to attain similar reduction of collagen volume fraction as two weeks treatment of CNP. Furthermore, these synthetic agents might cause harmful effects such as severe hypotension by vasodilators (21) or musculoskeletal toxicity by matrix metalloproteinase inhibitors (22). Because CNP does not affect blood pressure, it can be used in hemodynamically unstable patients as often seen in acute MI.

Study limitations. Because the effects of CNP were evaluated after two weeks of therapy in the present study, its long-term effects on the cardiac remodeling after MI remain unclear. For future clinical application, further study is necessary to examine if the antiremodeling effects of CNP persist for the long-term follow-up period.

Summary. Our study has demonstrated that continuous administration of CNP improved LV dysfunction and attenuated the development of cardiac remodeling after MI. Because CNP has much weaker vasorelaxant and natriuretic activities, but has much more potent antifibrotic and antihypertrophic actions than ANP or BNP, these beneficial effects of CNP might be associated with direct effects on the failing heart. In conclusion, CNP is potentially useful as a new antiremodeling agent through its novel mechanism of action.

Acknowledgments

The authors are grateful to Dr. Yujiro Hayashi, Daiichi Suntory Pharma, for the kind gift of CNP. We also thank Ms. Chika Fukuhara for her excellent technical assistance.

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REFERENCES

1. Rosenzweig A, Seidman CE. Atrial natriuretic factor and related peptide hormones. Annu Rev Biochem 1991;60:229–55.
2. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med 1998;339:321–8.

3. Nakao K, Ogawa Y, Suga S, et al. Molecular biology and biochemistry of the natriuretic peptide system. I: Natriuretic peptides. J Hypertens 1992;10:907–12.
4. Sudoh T, Minamino N, Kangawa K, Matsuo H. C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. Biochem Biophys Res Commun 1990;168:863–70.
5. Furuya M, Miyazaki T, Honbou N, et al. C-type natriuretic peptide inhibits intimal thickening after vascular injury. Ann NY Acad Sci 1995;748:517–23.
6. Horio T, Tokudome T, Maki T, et al. Gene expression, secretion, and autocrine action of C-type natriuretic peptide in cultured adult rat cardiac fibroblasts. Endocrinology 2003;144:2279–84.
7. Tokudome T, Horio T, Soeki T, et al. Inhibitory effect of C-type natriuretic peptide (CNP) on cultured cardiac myocyte hypertrophy: interference between CNP and endothelin-1 signaling pathways. Endocrinology 2004;145:2131–40.
8. Kalra PR, Clague JR, Bolger AP, et al. Myocardial production of C-type natriuretic peptide in chronic heart failure. Circulation 2003; 107:571–3.
9. Hammermeister KE, DeRouen TA, Dodge HT. Variables predictive of survival in patients with coronary artery disease: selection by univariate and multivariate analysis from the clinical, electrocardiographic, exercise, arteriographic, and quantitative angiographic evaluations. Circulation 1979;59:421–30.
10. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation 2000;101: 2981–8.
11. Igaki T, Itoh H, Suga SI, et al. Effects of intravenously administered C-type natriuretic peptide in humans: comparison with atrial natriuretic peptide. Hypertens Res 1998;21:7–13.
12. Nagaya N, Uematsu M, Kojima M, et al. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation 2001;104:1430–5.
13. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. Am J Pathol 1978;90:57–70.
14. Horio T, Nishikimi T, Yoshihara F, et al. Production and secretion of adrenomedullin in cultured rat cardiac myocytes and nonmyocytes: stimulation by interleukin-1 beta and tumor necrosis factor-alpha. Endocrinology 1998;139:4576–80.
15. Thompson NL, Bazoberry F, Speir EH, et al. Transforming growth factor beta-1 in acute myocardial infarction in rats. Growth Factors 1988;1:91–9.
16. Jugdutt BI, Joljart MJ, Khan MI. Rate of collagen deposition during healing and ventricular remodeling after myocardial infarction in rat and dog models. Circulation 1996;94:94–101.
17. Doering CW, Jalil JE, Janicki JS, et al. Collagen network remodelling and diastolic stiffness of the rat left ventricle with pressure overload hypertrophy. Cardiovasc Res 1988;22:686–95.
18. Jugdutt BI. Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? Circulation 2003;108: 1395–403.
19. Rosenkranz AC, Woods RL, Dusting GJ, Ritchie RH. Antihypertrophic actions of the natriuretic peptides in adult rat cardiomyocytes: importance of cyclic GMP. Cardiovasc Res 2003;57:515–22.
20. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990;81:1161–72.
21. Jugdutt BI. Myocardial salvage by intravenous nitroglycerin in conscious dogs: loss of beneficial effect with marked nitroglycerin-induced hypotension. Circulation 1983;68:673–84.
22. Drummond AH, Beckett P, Brown PD, et al. Preclinical and clinical studies of MMP inhibitors in cancer. Ann NY Acad Sci 1999;878: 228–35.