Effects of cardiotrophin-1 on hemodynamics and endocrine function of the heart

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1Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, J apan 606-8507; 2Hypertension Research Laboratory, National Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka, J apan 565-8565; and 3Department of Anesthesiology, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, J apan 606-8507.

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Hamanaka, Ichiro, Yoshihiko Saito, Toshihiko Nishikimi, Tatsuo Magaribuchi, Shigeki Kamitani, Koichiro Kuwahara, Masashi Ishikawa, Yoshihiro Miyamoto, Masaki Harada, Emiko Ogawa, Noboru Kajiyama, Nobuki Takahashi, Takehiko Izumi, Gotaro Shirakami, Kenjiro Mori, Yoshito Inobe, Ichiro Kishimoto, Izuru Masuda, Kazuhiko Fukuda, and Kazuwa Nakao. Effects of cardiotrophin-1 on hemodynamics and endocrine function of the heart. Am J Physiol Heart Circ Physiol 279: H388–H396, 2000.—Cardiotrophin-1 (CT-1), a member of the interleukin-6 superfamily of cytokines, possesses hypertrophic actions and atrial natriuretic peptide (ANP)-producing activity in vitro. The goal of our study is to elucidate whether CT-1 affects the cardiovascular system in vivo. Intravenous injection of CT-1 (4–100 µg/kg) in conscious rats evoked significant declines in blood pressure and reflex increases in heart rate (HR) in a dose-dependent manner. CT-1 induced no significant change in cardiac output (from 260.7 ± 110.0 to 264.7 ± 26.6 ml·min⁻¹·kg⁻¹, P = not significant), which was compatible with the results from isolated perfused rat hearts; HR, change in pressure over time, left ventricular developed pressure, and perfusion pressure were unaffected. Northern blot and RT-PCR analyses revealed that CT-1 increased expression of inducible nitric oxide synthase (iNOS) in lung and aorta but not in heart or liver. Pretreatment with aminoguanidine, a specific iNOS inhibitor, inhibited both iNOS mRNA production and the depressor effect of CT-1. Interestingly, CT-1 increased ventricular expression of ANP and brain natriuretic peptide (BNP). The data demonstrate that CT-1 elicits its hypotensive effect via a nitric oxide-dependent mechanism and that CT-1 induces ANP and BNP mRNA expression in vivo.

Blood pressure; nitric oxide synthase; natriuretic peptide

GROWING EVIDENCE INDICATES that humoral factors such as angiotensin II (ANG II) (35), endothelin-1 (17, 27), and α-adrenergic receptor agonists (32) play obligatory roles in the pathogenesis and progression of ventricular remodeling and subsequent heart failure. These agents are all G protein-coupled receptor agonists that exert their cardiovascular actions, which include vasoconstriction, positive inotropy, and myocyte hypertrophy, by activating a mitogen-activated protein (MAP) kinase family pathway (26).

It has recently become apparent that another class of humoral factors, the cytokines, are also overexpressed during such human cardiac-related illnesses as congestive heart failure, ischemic heart disease, dilated cardiomyopathy, and septic cardiomyopathy (2, 16, 37). One well-characterized cytokine that functions significantly in the pathology of cardiac disease is tumor necrosis factor-α (TNF-α), which is in part synthesized in the heart itself. Bolus injection of TNF-α induces nitric oxide synthase (NOS) activation by nuclear factor-κB (NF-κB) (13, 15, 38) and nitric oxide (NO)-dependent decreases in arterial blood pressure and contractility.

Interleukin-6 (IL-6) is another multifunctional cytokine that mediates immune and inflammatory responses; plasma levels of IL-6 are reported to be elevated in cases of acute myocardial infarction, cardiac hypertrophy, and congestive heart failure (36, 40). IL-6 binding to its receptor and subsequent formation of the gp130 receptor complex activates the J anus kinase-signal transducer and activator of transcription (J AK-STAT) pathway in target cells. Although gp130 is abundant in the heart, little or no IL-6 or its receptor are expressed there. Accordingly, direct effects of IL-6 on the heart are not considered to be important. In fact, it was reported previously that injection of IL-6 in the dog did not alter the hemodynamic parameters (25).
Cardiotrophin-1 (CT-1) is a member of the IL-6 superfamily and was isolated from mouse embryoid body based on its ability to induce cardiac myocyte hypertrophy in vitro (22, 24). Substantial levels of CT-1 have been detected in the heart as well as in the kidney, lung, and aorta, and in skeletal muscle (7). In the heart, CT-1 mRNA is expressed both in myocytes and in surrounding nonmyocytes (12); it binds to the gp130/leukemia inhibitory factor (LIF) receptor complex in cardiac myocytes and activates the JAK-STAT and MAP kinase pathways (41). We previously showed that the expression of CT-1 mRNA is upregulated in the hypertrophied ventricles of genetically hypertensive rats (7). Moreover, using a specific anti-CT-1 antibody, we observed that CT-1 is released primarily from nonmyocytes and induces enlargement of myocyte size and atrial and brain natriuretic peptide (ANP and BNP, respectively) production in vitro (11, 30). The aim of the present study is to clarify whether CT-1 affects hemodynamics and endocrine function of the heart in vivo.

MATERIALS AND METHODS

Animals. Male Wistar rats (250–270 g, Shimizu Experiment, Tokyo) were housed in a light- and temperature-controlled room; they received rat chow and water ad libitum.

Reagents. Recombinant rat CT-1 was prepared using the glutathione S-transferase (GST) fusion system described in an earlier work (7). Briefly, the open reading frame of rat CT-1, which we previously cloned, was inserted into the E. coli site of the pGEM-4T3 expression vector (Amersham Pharmacia Biotech, Uppsala, Sweden). The GST fusion protein was expressed in bacteria, purified using a glutathione affinity column according to the manufacturer’s instruction (Amersham), and cleaved by thrombin. The purity of the CT-1 was verified by SDS-PAGE and quantified by colorimetric assay (Bio-Rad Laboratories, Hercules, CA).

The specific NOS inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), aminoguanidine (AG), and rat recombinant LIF were purchased from Sigma Chemical (St. Louis, MO).

Measurement of systemic arterial pressure. Systemic arterial blood pressure (BP) was measured in anesthetized and conscious animals as described in a previous work (21). Initially, rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital sodium (Abbott). A polyethylene-50 catheter (PE-50) filled with heparin and saline (200 IU/ml) was then implanted in the right internal carotid artery, and a second PE-50 catheter was implanted in the right external jugular vein. These catheters were passed subcutaneously to the back of the neck, where they were exteriorized and plugged with stainless steel pins. The incisions were then closed with sutures, and the animals were allowed to regain consciousness. Arterial BP was then measured using a pressure transducer (Fukuda Denshi, Tokyo) connected to the arterial cannula.

Administration of CT-1. CT-1 at concentrations of 4, 20, and 100 µg/kg body wt in 100 µl of phosphate-buffered saline (PBS) was injected via the intravenous catheter, and BP was monitored for 60 min. Control rats received an equal volume of PBS alone.

Measurement of cardiac output. Cardiac output was measured using the thermodilution method under pentobarbital sodium anesthesia. A thermodilution catheter connected to a computerized cardiac output monitor (Cardiotherm-500, Columbus Instruments) was advanced into the ascending aorta via the right carotid artery. To measure cardiac output, 0.1 ml of 0.9% saline at room temperature was injected as a bolus via the intravenous catheter (18). Measurements were made in triplicate shortly before and 15 min after CT-1 administration.

Isolated heart procedure. Rats were intraperitoneally injected with heparin (500 IU/kg) to block coagulation and then killed. The hearts were quickly removed and immersed in ice-cold perfusion fluid (modified Krebs-Henseleit solution). The aorta was cannulated superior to the aortic valve, and the isolated hearts were perfused at a constant rate of 6 ml/min at 37°C using the Langendorff technique without electrical pacing (31). Electrocardiograms and coronary perfusion pressure were monitored throughout the experiments using a San-Ei Biophysiograph 180 system (NEC San-Ei, Tokyo, Japan). Hearts were perfused for 60 min to allow stabilization; if arrhythmia was observed during the stabilization period, the heart was discarded. There was then a 30-min control period, after which hearts were perfused for 30 min with perfusion media alone (n = 10) or containing 10⁻⁹ (n = 9) or 10⁻⁸ (n = 5) mol/l of CT-1.

Isometric tension in aortic rings. Descending thoracic aorta was excised from rats euthanized by ether inhalation. The aorta was cleaned of adherent connective tissue and cut into transverse rings 4 mm in length. The rings were suspended between parallel hooks in a 10-ml tissue bath filled with Krebs-Henseleit solution at 37°C. A resting tension of 1.0 g was applied. Changes in isometric tension elicited by bath application of various drugs were recorded.

Intracerebroventricular injection of CT-1. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium. A stainless-steel intracerebroventricular cannula was placed 6.5 mm anterior to the lambda suture and 1.4 mm lateral to the midline at a depth of 4.5 mm, as described in a previous work (28). Using a microsyringe injector, we injected 5 µg of CT-1 in 10 µl of PBS.

RNA extraction. At selected times (shortly before injection and 15 min, 60 min, 2 h, 4 h, and 24 h after injection of CT-1), the lung, heart (left ventricle), liver, and aorta were removed from the rats and frozen at −80°C. Tissues were later homogenized in Trizol reagent (GIBCO BRL, Life Technologies, Grand Island, NY), and total RNA was isolated according to the manufacturer’s instructions.

Northern blot analysis. Northern blot analysis was performed as described in a previous work (19). Briefly, 20 µg of total RNA from each sample was separated on a 1.4% denaturing agarose gel and transferred onto nylon filters. The filters were incubated for 4 h at 42°C in prehybridization buffer consisting of 50% formamide, 5× Denhardt solution, 5× sodium-saline phosphate EDTA buffer, and 0.1% sodium dodecyl sulfate. The RNA on the filters was then hybridized for 24 h at 42°C to 32P-labeled probes at concentrations of 0.5 to 1 × 10⁶ cpm/ml.

RT-PCR analysis. First-strand cDNA synthesis was performed with 5 µg of total RNA isolated from the aorta using Superscript II (GIBCO BRL) according to the manufacturer’s instructions. The resulting cDNA was amplified by RT-PCR
using the following iNOS primers: sense, 5'-CCCTGCGAA GTTCTGGCAAAGCAGC-3', and antisense, 5'-GGCTGTCAGAGCCTC GTGGCTTTGG-3'. The protocol consisted of 35 cycles of incubation at 94°C for 45 s, 65°C for 45 s, and 72°C for 2 min followed by extension for 7 min at 72°C. The amplified iNOS product (693 bp) was analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light.

Statistical analysis. All values are presented as means ± SE. Comparisons between two groups were completed with use of unpaired Student's t-tests. ANOVA with subsequent Fisher exact test was used to determine significant differences among three or four groups. A value of P < 0.05 was considered significant.

RESULTS

Effect of intravenous infusion of CT-1 on hemodynamics in vivo in conscious rats. Intravenous injection of recombinant CT-1 (4 to 100 µg/kg) evoked dramatic decreases in mean BP in conscious rats (Fig. 1A). At each dose BP began to decline within 1 to 1.5 min after injection, and response was maximal within 10 min. BP subsequently returned almost to baseline levels over the course of 60 min. Heart rate (HR) showed reactive tachycardia in response to the decrease in BP (Fig. 1B).

For each parameter, the data used for the analysis were the area under the curve (AUC) or the area under the curve (AOC), calculated based on changes from baseline data. The trapezoidal method of calculating the area was used. As shown in Fig. 2A, CT-1-induced decline in mean BP was dose dependent at a dose of 4 to 100 µg/kg.

Cardiac output was measured by thermodilution method, which required the animals to be anesthetized. Baseline cardiac output was 260.7 ± 11.0 ml·min⁻¹·kg⁻¹, and it did not change after injection of 20 µg/kg CT-1 (264.7 ± 26.6 ml·min⁻¹·kg⁻¹; n = 5). As a control, we also measured BP and HR in rats with CT-1 injection. Mean BP significantly declined from 108.7 ± 0.67 to 71.7 ± 1.67 mmHg, although HR did not change (393.3 ± 45.5 vs. 397.7 ± 50.6 beats/min). Consequently, the calculated systemic vascular resistance (SVR) also decreased significantly (1.34 ± 0.06 vs. 0.88 ± 0.08 mmHg·min·ml⁻¹). The magnitudes of the
LIF is another IL-6 superfamily cytokine that binds to the gp130/LIF receptor heterodimer. Intravenous administration of 20 µg/kg LIF to anesthetized rats caused systemic hypotension that was virtually identical to the hypotension induced by the same dose of CT-1 (compare Fig. 3, A and C; see Table 1).

Isolated rat heart. To clarify the direct effects of CT-1, isolated rat hearts were perfused with and without $10^{-9}$ and $10^{-8}$ mol/l CT-1 for 30 min using the Langendorff technique. CT-1 had no effect on HR, maximum change in pressure over time (dP/dt), left ventricular developed pressure (LVDP), or perfusion pressure (Fig. 4). In contrast, $10^{-6}$ mol/l propranolol hydrochloride, a β-blocking agent, decreased HR, LVDP, and maximum dP/dt significantly (Fig. 4), indicating the validity of our Langendorff apparatus. Thus CT-1 had no direct chronotropic or inotropic effect on the heart.

Intracerebroventricular injection of CT-1. To rule out a central nervous system-mediated hypotensive effect, 20 µg/kg CT-1 was injected into the cerebral ventricles. There was no change in mean BP during the 30-min period following intracerebroventricular injection of CT-1 (data not shown). Via the same catheter, 10 µg/kg of pentobarbital sodium was administered 30 min after the CT-1 injection. BP decreased rapidly and transiently, suggesting that the intracerebroventricular catheter was correctly located, although 10 µg/kg of pentobarbital sodium never affected BP when it was administered peripherally.

Table 1. Changes in mean blood pressure after intravenous injection of agents in anesthetized rats

<table>
<thead>
<tr>
<th>Agent</th>
<th>n</th>
<th>Baseline Mean BP, mmHg</th>
<th>10 min Mean BP, mmHg</th>
<th>20 min Mean BP, mmHg</th>
<th>30 min Mean BP, mmHg</th>
<th>45 min Mean BP, mmHg</th>
</tr>
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<tbody>
<tr>
<td>CT-1 (20 µg/kg)</td>
<td>6</td>
<td>120.9±2.8</td>
<td>74.2±5.4</td>
<td>89.6±5.5</td>
<td>101.7±5.3</td>
<td>114.2±2.7</td>
</tr>
<tr>
<td>LIF (20 µg/kg)</td>
<td>4</td>
<td>122.2±2.8</td>
<td>65.8±5.6</td>
<td>79.1±8.3</td>
<td>91.0±11.9</td>
<td>110.0±3.3</td>
</tr>
<tr>
<td>CT-1 (20 µg/kg) after L-NAME (10 mg/kg)</td>
<td>4</td>
<td>135.8±0.9</td>
<td>136.1±1.1</td>
<td>136.2±3.1</td>
<td>135.1±0.9</td>
<td>137.0±1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals; CT-1, cardiotrophin-1; LIF, leukemia inhibitory factor; L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 vs. baseline.
Analysis of iNOS mRNA expression. Because eNOS was apparently not involved in the response to CT-1, alternative NOS activation was presumed. To test this hypothesis, expression of iNOS mRNA in CT-1-treated rats was analyzed by Northern blot in tissue samples from the lung, liver, heart, and spleen, and by RT-PCR in the aorta. Expression of iNOS mRNA was significantly increased in the lung (Fig. 5A) and aorta (Fig. 5B) 60 min after CT-1 administration, compared with that of rats injected with the same volume of PBS (Fig.)
no detectable increase in expression was present after 15 min. Interestingly, CT-1 had no effect on expression of iNOS mRNA in the heart (Fig. 5A).

Furthermore, to clarify the relation between the increment of iNOS expression and CT-1-induced hypotension, we examined the pretreatment effect of the iNOS-specific inhibitor AG on CT-1-induced hypotension. AG at a dose of 150 mg/kg was given subcutaneously 1 h before CT-1 treatment. We found that this dose given in this way showed no alteration in hemodynamics (data not shown) in Wistar rats as well as rabbits (33). As shown in Fig. 6A, AG pretreatment showed an 80% reduction in the AUC (P < 0.05) of mean BP compared with no pretreatment. In addition, the mechanism in iNOS inhibition by AG is partly due to the inhibition of iNOS production. We performed Northern blot analysis of iNOS mRNA in lung tissue of both AG-pretreated and nonpretreated rats. As shown in Fig. 6B, the induction of iNOS mRNA by CT-1 was almost completely suppressed.

Expression of mRNA for ANPs and BNPs. Our data show that the pharmacological dose of CT-1 has no effect on hemodynamic parameters in the heart. However, because CT-1 stimulates ANP and BNP synthesis and secretion in cultured ventricular myocytes (6), we investigated whether or not CT-1 affected ventricular expression of ANP and BNP mRNA in vivo. Figure 7 shows that expression of BNP mRNA was significantly augmented 1 h after injection of CT-1, reached a maximum 2 h after injection, and returned to the baseline levels within 24 h. Interestingly, ANP mRNA was not changed significantly until 24 h after the injection, and thereafter it was increased.

**DISCUSSION**

The present study demonstrates that intravenous administration of CT-1 causes dose-dependent systemic hypotension in both conscious and anesthetized rats via a NO-dependent mechanism. Cardiac output was unaffected by CT-1; moreover, results from experiments carried out in isolated hearts showed that there was no direct suppressive effect of CT-1 on cardiac function. Rather, the hypotensive effect resulted from a significant decline in SVR caused by NO-evoked vasodilation. We also showed for the first time that LIF elicits a hypotensive response that is virtually identical to that of CT-1. Because CT-1 and LIF share the LIF/gp130-receptor heterodimer complex, and both activate the JAK-STAT pathway, it is likely that the hypotension is

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**Fig. 6.** A: iNOS-specific inhibitor, amino-guanidine (AG), at a dose of 150 mg/kg was given subcutaneously 1 h before 20 µg/kg of CT-1 treatment. BP was monitored for 1 h after CT-1 injection. Bar graph shows AUC of mean BP with (+) and without (−) AG pretreatment. +P < 0.01. B: Northern blot analysis of iNOS mRNA in lung tissue of AG-pretreated and nonpretreated rats. Induction of iNOS mRNA by CT-1 was almost completely suppressed by AG pretreatment.

**Fig. 7.** Expression of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) mRNA in hearts from rats treated with CT-1. Rats were killed 15 min, 1 h, 2 h, 4 h, or 24 h after CT-1 injection. A 15-µg sample of total RNA from each rat was transferred to membranes for Northern blot analysis. Representative blots of ANP (A) and BNP (B) at indicated times are shown. Bar graph depicts relative quantities of message (ANP/GAPDH, BNP/GAPDH) which is normalized to 0-min (baseline) value and was arbitrarily assigned a value of 10. Expression of ANP mRNA significantly increased 24 h after CT-1 injection, whereas expression of BNP mRNA significantly increased and peaked 2 h after injection. Data are means ± SE from six samples. *P < 0.05 vs. 0 min.
a common effect of agonists sharing the LIF/gp130-receptor heterodimer complex signaling pathway.

The present study indicates that CT-1 did not directly affect cardiac function itself in vivo and in vitro. In isolated perfused hearts, CT-1 had no inotropic or chronotropic effects. In conscious rats, HR increased after CT-1 injection, almost certainly due to baroreceptor reflex to the decrease in BP. Cardiac output was not changed in vivo. These findings are consistent with the recent data from Jim et al. (9) in which it is mentioned that CT-1 does not induce a change in either calculated stroke volume or ventricular contractility. The doses we used in the isolated perfused hearts are considered valid, because CT-1 induces myocyte hypertrophy and ANP and BNP production in cultured media in a dose-dependent manner at a range from $10^{-11}$ to $10^{-8}$ mol/l. Moreover, $10^{-9}$ mol/l of CT-1 induces phosphorylation of signal transducers and activators of transcription-3 (STAT-3) in myocytes (11). The doses we used for in vivo studies are also considered valid, because plasma CT-1 concentrations are probably $10^{-8}$ mol/l just after injection of 20 µg/kg of CT-1 intravenously, when the plasma volume is taken into account.

It was also possible that the intravenously administered peptide directly acted on the central nervous system; this is the case for leptin (1, 4), a newly discovered satiety factor whose receptor is also a member of the gp130 family (20). However, no significant changes in systemic hemodynamics were observed when CT-1 was intracerebroventricularly injected, indicating that the site of action of intravenously administered CT-1 is the cardiovascular system in the periphery. Our finding that CT-1-induced hypotension was completely blocked by l-NAME is indicative of the role played by NOS. As is well known, three different types of NOS are recognized: eNOS, iNOS, and neuronal NOS. Many growth factors and cytokines cause hypotension by activating eNOS or iNOS. Platelet-derived growth factor BB causes an endothelium-dependent, NO-mediated relaxation of rat aorta (3), and insulin-like growth factor 1 induces NO production in endothelial cells by activating eNOS (39). In this context, first we assessed the endothelium dependence of CT-1-induced hypotension in aortic ring preparations and found none. Furthermore, we detected no nitrite production in cultures of bovine aortic endothelial cells after CT-1 stimulation by the Greise method (data not shown). Thus it appears unlikely that eNOS-mediated vasorelaxation was involved in the response to CT-1. To clarify the next possibility, we assessed iNOS expression because it is well established that inflammatory cytokines such as interleukin-1β (22) and TNF-α (15) cause vasorelaxation by inducing iNOS. Indeed, we observed augmented expression of iNOS mRNA in the aorta and lung 60 min after CT-1 administration, suggesting the possible participation of iNOS in the hypotensive effect of CT-1. Furthermore, our data using the iNOS-specific inhibitor AG strengthened this conclusion. However, there was a discrepancy in the time courses between CT-1-induced hypotension and expression of iNOS mRNA: systemic hypotension developed within several minutes, whereas increases in iNOS were not detectable for 60 min after the CT-1 injection. Considering that even in the case of lipopolysaccharide (LPS)-induced hypotension (which is recognized to be mediated by iNOS induction), the mRNA level of iNOS upregulation was just detectable more than 1 h after LPS injection (14), and this discrepancy could be accounted for. Furthermore although TNF-α increases iNOS mRNA expression in many organs, including the heart, and thereby induces ventricular dysfunction (10, 29), iNOS mRNA was selectively upregulated by CT-1 in the aorta and lung but not in the heart or liver. The absence of an effect on perfusion pressure, LVDP, and maximum dP/dt by CT-1 on the isolated heart is consistent with the finding that the expression of iNOS mRNA was not upregulated in the heart. Consequently, although it is evident that CT-1-induced hypotension is NO mediated, further studies will be necessary to clarify the precise site of action and mechanism for CT-1-induced vasorelaxation.

Of particular interest to us was the finding that CT-1 affects endocrine function, or ANP and BNP production, although it does not affect the cardiac pumping function, as mentioned above. As previously reported, CT-1 stimulates ANP and BNP secretion and increases cell size in cultured ventricular myocytes (6, 11, 24). Until now, most humoral stimulators for ANP and BNP secretion, such as G protein-coupled receptor agonists, increased loading conditions in vivo when they were intravenously injected (8). In this point, CT-1 seems to be another class of stimulator for ANP and BNP and probably has different roles than G protein-coupled receptor agonists in the cardiovascular system. It is not clear what the precise mechanism is for the upregulation of ANP and BNP production by CT-1 in vivo, nor whether it includes the direct or indirect action of CT-1. However, considering that CT-1 stimulates ANP and BNP secretion in vitro and that CT-1 does not affect cardiac pumping function for at least 1 h after injection in vivo, it may be possible that CT-1 stimulates ANP and BNP production in vivo independent of hemodynamic changes. The significance of increased expression of ANP and BNP genes by CT-1 is not clear at present. Although ANP and BNP have hypotensive properties in vivo, it is unlikely that ANP and BNP play an obligatory role in the decrease in blood pressure by CT-1 infusion, because the hypotensive effect of ANP and BNP is not blocked by l-NAME.

Recently Talwar et al. (34) demonstrated that plasma CT-1 concentration is upregulated in patients with heart failure. Considering this finding and our present data, CT-1 may play a part in reducing afterload in certain pathological conditions. To understand the precise role of endogenous CT-1 or the long-term effects of CT-1, the development of mice lacking or overexpressing the CT-1 gene is needed in the near future.

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