C-Type Natriuretic Peptide Increases Myocardial Contractility and Sinus Rate Mediated by Guanylyl Cyclase-Linked Natriuretic Peptide Receptors in Isolated, Blood-Perfused Dog Heart Preparations

MASAMICHI HIROSE, YASUYUKI FURUKAWA, FUMIO KUROGOUCHI, KOICHI NAKAJIMA, YUSUKE MIYASHITA and SHIGETOSHI CHIBA

Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390-8621, Japan

Accepted for publication March 20, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

There are no available data on the direct effect of C-type natriuretic peptide (CNP) and brain natriuretic peptide (BNP) on the myocardial contractility in mammalian hearts. Thus we studied the inotropic and chronotropic effects of CNP-22 and BNP-32 compared with those of atrial natriuretic peptide (ANP)-28 using the isolated, blood-perfused canine right atrial or left ventricular preparations. CNP increased the atrial contractile force in a dose-dependent manner with a small increase in sinus rate in isolated atria, whereas neither ANP nor BNP changed atrial force and rate. CNP but not BNP also increased the ventricular contractile force in isolated ventricles. Pretreatment with a high dose (3 nmol) of CNP attenuated the positive inotropic response to CNP at a low dose (1 nmol) but not to norepinephrine. A guanylyl cyclase-linked natriuretic peptide receptor antagonist, HS-142–1, inhibited the increases in atrial contractile force and sinus rate in response to CNP, but it did not affect the positive cardiac responses to norepinephrine. Propranolol did not block the positive cardiac responses to CNP. 3-Isobutyl-1-methylxanthine in rates of 0.6 to 1.3 μmol/min attenuated the CNP-induced positive inotropic responses, when it potentiated the positive inotropic response to norepinephrine. On the other hand, parasympathetic nerve stimulation attenuated the positive cardiac responses to CNP and norepinephrine. These results demonstrate that CNP increases myocardial contractile force with a small increase in sinus rate mediated by guanylyl cyclase-linked natriuretic peptide receptors, probably type B receptors in the dog heart, and suggest that the positive inotropic response to CNP is influenced by the cyclic adenosine 3′,5′-monophosphate-dependent signal transduction.

The natriuretic peptide family is made up of three distinct peptides: ANP, BNP and CNP. These peptides are thought to play an important role in cardiovascular homeostasis (Brown et al., 1993). ANP is a 28-amino-acid peptide that is secreted from atria and acts as a cardiac hormone with a variety of biological actions including natriuresis, diuresis, vasorelaxation and inhibition of renin and aldosterone secretion (Nakao et al., 1992). BNP is a 32-amino-acid peptide that shares structural and biological similarity to ANP and is a novel cardiac hormone which is synthesized in and secreted from the ventricle and atria (Mukoyama et al., 1991; Ogawa et al., 1991). CNP is a newly identified 22-amino-acid peptide that demonstrates structural similarity to the cardiac hormones ANP and BNP (Komatsu et al., 1991). This peptide is distributed widely within the vascular endothelium (Espanier, 1994) and may play a role in the regulation of vascular tone (Clavell et al., 1993). Additionally, CNP mRNA has been detected in the rat heart (Vollmar et al., 1993), and the existence of CNP has been demonstrated in the human ventricle (Wei et al., 1993).

Both ANP and BNP function biologically via NPR-A that is highly expressed in endothelial cells (Koller et al., 1991). CNP functions via NPR-B that is highly expressed in vascular smooth muscles. All three peptides are cleared and degraded intracellularly by NPR-C (Koller et al., 1991). mRNA transcripts for these three receptors were all detectable in the rat and human heart (Nunez et al., 1992). Therefore, all three natriuretic peptides may have a potential role in cardiac function modulation.

Recently, Beaulieu et al. (1996) observed that CNP but not
ANP increased sinus rate in anesthetized and isolated dog hearts and that its positive chronotropic effect was not blocked by prazosin, atropine, indomethacin, losartan, cimetidine or mepyramine. However, no report is available on the effects of CNP and BNP on myocardial contractility and whether the positive chronotropic response to CNP is mediated by natriuretic peptide receptors. Therefore, in this study, we investigated the direct effects of CNP and BNP as well as ANP on the atrial and ventricular contractility and SA nodal pacemaker activity in the isolated, blood-perfused right atrial and left ventricular preparations of the dogs. We observed the positive inotropic and chronotropic responses to CNP. Thus, to determine whether the positive cardiac responses to CNP are mediated by guanylyl cyclase-linked natriuretic receptors, we examined the effects of HS-142–1 on the positive cardiac responses to CNP in the isolated right atrial preparations. HS-142–1 is an inhibitor of the guanylyl cyclase-linked natriuretic peptide receptors, i.e., NPR-A and NPR-B (Matsuda and Morishita, 1993). We also studied the effects of a phosphodiesterase inhibitor, IBMX, and intracardiac parasympathetic nerve stimulation on the positive cardiac responses to CNP to investigate whether the positive cardiac responses to CNP interacts with the cAMP-dependent signal transduction.

**Materials and Methods**

The animal experiments were approved by the Shinshu University School of Medicine Animal Studies Committee.

**Isolated, blood-perfused dog heart preparations.** Isolated right atra and left ventricles were obtained from 22 dogs (weighing 9–15 kg) anesthetized with sodium pentobarbital (35 mg/kg i.v.). Each preparation was perfused with heparanized arterial blood from a second, support dog. The details of these preparations have been described earlier (Chiba et al., 1975; Chiba, 1976).

The 22 support dogs, weighing 11 to 35 kg, were anesthetized with pentobarbital sodium (35 mg/kg i.v.) and ventilated artificially through a cuffed tracheal tube with room air by use of a Harvard respirator (Harvard Apparatus Co., Inc., South Natick, MA, model 607). Sodium heparin (500 USP U/kg i.v.) was administered to each dog at the beginning of the perfusion of the isolated atrial or ventricular preparation, and 200 USP U/kg were given subsequently at 1-h intervals.

After sodium heparin (200 USP U/kg i.v.) was administered, the right atrium or left ventricle was excised and immersed in cold Ringer's solution of the following composition (millimolar): NaCl, 154.0; KCl, 5.6; CaCl₂, 2.2 and NaHCO₃, 3.6. The wet weight of the isolated right atrial and left ventricular preparations varied from 5 to 14 g and from 13 to 24 g, respectively. The sinus node artery of the isolated right atrium or the anterior descending branch of the left coronary artery of the isolated left ventricle was cannulated, and each preparation was perfused with heparanized blood from the carotid artery of the anesthetized support dog by the aid of a peristaltic pump (Harvard Apparatus, model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mm Hg. The venous effluent from the preparation was led to a collecting funnel and returned to the support dog through an external jugular vein.

The preparation was anchored to a stainless steel bar and placed in a cup-shaped glass container kept at 37°C. The upper part of the cardiac preparation was connected to a force-displacement transducer (Nihon Kohden, Tokyo, Japan, AP-620C) by a silk thread. The cardiac tissue usually was stretched to a resting tension of 2 g. Isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden, RTA-1200). A pair of bipolar silver electrodes was brought into contact with the epicardial surface of the isolated preparation to record the atrial electrogram or to drive the left ventricle electrically. The left ventricular preparation was stimulated electrically by a voltage (4 V) greater than threshold with a pulse width of 1 msec and at a frequency of 2 Hz by an electrical stimulator (Nihon Kohden, SEN 7103). The atrial rate was derived from the electrogram with a cardiotachometer (Nihon Kohden, AT-600G).

Another pair of electrodes was placed posteriorly on the fatty tissue of the caval margin of the atrium and was used to stimulate intracardiac parasympathetic nerve fibers (Furukawa et al., 1980). The femoral arterial blood pressure, heart rate derived from lead II of the electrocardiogram of the support dog and the rate of blood flow to the preparation were monitored simultaneously.

**Experimental protocols.** We carried out three series of experiments after 30 min stabilization. In the first series, to examine the direct effects of ANP, BNP and CNP on the SA nodal pacemaker activity, atrial contractility and ventricular contractility, we studied the changes in sinus rate and atrial contractive force in response to ANP (1–30 nmol, n = 3), BNP (1–30 nmol, n = 3), CNP (0.1–3 nmol, n = 6) and norepinephrine (0.1, 0.3 or 1 nmol, n = 6) in the isolated, blood-perfused right atrium and the changes in the ventricular contractile force in response to BNP (1–30 nmol, n = 3), CNP (0.1–10 nmol, n = 6) and norepinephrine (0.1, 0.3 or 1 nmol, n = 6) in the isolated, blood-perfused left ventricle. Additionally, to investigate whether the cardiac responses to CNP cause tachyphylaxis, we studied the effects of CNP at 3 nmol (n = 5) on the positive cardiac responses to CNP at 1 nmol and norepinephrine at 0.1, 0.3 or 1 nmol in isolated right atrial preparations. The responses to CNP were obtained 2 and 30 min after the high dose of CNP.

In the second series, to determine whether the cardiac responses to CNP are mediated by natriuretic peptide receptors, we studied the effects of a natriuretic peptide receptor antagonist, HS-142–1 (2 mg, n = 5) on the positive cardiac responses to CNP (1 nmol) and norepinephrine (0.1, 0.3 or 1 nmol) in the isolated right atrium. The responses to CNP were obtained 2 and 30 min after HS-142–1 treatment. Additionally, to study whether the responses to CNP are mediated by adrenergic mechanism, we examined the effects of propranolol (30 nmol, n = 5) on the positive cardiac responses to CNP (1 nmol) and norepinephrine (0.1 nmol) in the isolated right atrium. The responses to CNP were observed 2 min after propranolol.

In the third series, to determine whether the cardiac responses to CNP are influenced by phosphodiesterase inhibition, we examined the effects of IBMX, a nonspecific phosphodiesterase inhibitor, on the positive cardiac responses to CNP (1 nmol, n = 5) and norepinephrine (0.1, 0.3 or 1 nmol, n = 5) in the atrial preparations. IBMX in low (0.06–0.15 μmol/min) or high rates (0.6–1.3 μmol/min) was infused into the sinus node artery. The responses to CNP were observed 2 min after IBMX infusion started. To examine whether acetylcholine released at stimulation of vagal nerves attenuates the positive cardiac responses to CNP, we also studied the effects of intracardiac parasympathetic nerve stimulation on the positive cardiac responses to CNP (1 nmol) and norepinephrine (0.1 or 0.3 nmol) in five isolated perfused right atria.

Enough recovery time (usually 30 min after injection of CNP) was allowed to prevent the effects of the former injection of CNP from affecting the next injection of CNP through the experiment.

**Drugs.** Drugs were mixed fresh for each experiment. Atrial natriuretic peptide-28 (human) (ANP-28, Peptide Institute Inc., Osaka, Japan), brain natriuretic peptide-32 (human) (BNP-32, Peptide Institute Inc.) and C-type natriuretic peptide-22 (human) (CNP-22, Peptide Institute Inc.) were dissolved in distilled water, kept frozen at −20°C as stock solutions and diluted immediately before use. A nonpeptide natriuretic peptide receptor antagonist, HS-142–1, which was a generous gift of Y. Matsuda at Tokyo Research Laboratory, Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan, was dissolved in distilled water before use. Norepinephrine hydrochloride (Sankyo, Tokyo, Japan), propranolol hydrochloride (Sigma, St Louis, MO) and IBMX (Aldrich, Milwaukee, WI) were dissolved and diluted in 0.9%
Drugs were injected or infused into the sinus node artery or the anterior descending branch of the left coronary artery through a rubber tube by a microsyringe. The amount of drug solution injected was 0.01 to 0.03 ml during a 4-sec period.

**Statistical analysis.** All data were presented as percent changes from the respective control and expressed as mean ± S.E. An analysis of variance with Bonferroni’s test was used for the statistical analysis of multiple comparisons of data. Student’s *t*-test for unpaired data was used for comparison between the two groups. *P* < .05 was considered statistically significant.

**Results**

**Effects of ANP, BNP and CNP on the SA nodal pacemaker activity and myocardial contractility.** When CNP (0.3–3 nmol) was injected into the sinus node artery of an isolated, blood-perfused right atrium, CNP increased the sinus rate and atrial contractile force dose dependently (fig. 1A). Figure 2 summarizes data of the effects of ANP, BNP, CNP and norepinephrine on the isolated right atrial preparation. CNP (0.1–3 nmol) increased the sinus rate (*P* < .05) and atrial contractile force (*P* < .01). On the other hand, ANP and BNP did not affect the sinus rate and atrial contractility significantly even when the doses of peptides were increased to 30 nmol. The threshold dose for the positive inotropic effect of CNP was 0.1 nmol. The percentage increases in atrial force in response to CNP were greater than those in sinus rate. The chronotropic and inotropic responses to norepinephrine were characterized for comparison with the responses to peptides.

When CNP (1–10 nmol) was injected into the anterior descending branch of the left coronary artery of an isolated, blood-perfused left ventricle, it increased the ventricular contractile force dose dependently (fig. 1B). Figure 3 summarizes data of the effects of BNP, CNP and norepinephrine on the isolated left ventricular preparation. CNP (0.1–10 nmol) increased the ventricular contractile force in a dose-dependent manner (*P* < .001), whereas BNP did not affect the ventricular contractile force. The threshold dose for the positive inotropic effect of CNP was 0.3 nmol.

To investigate whether the positive inotropic and chronotropic responses to CNP cause tachyphylaxis, we studied the effects of CNP at 3 nmol on the positive cardiac responses to CNP at 1 nmol and norepinephrine at 0.1 to 1 nmol in isolated right atria (fig. 4). Pretreatment with a high dose of 3 nmol of CNP attenuated (*P* < .001) the positive inotropic response to CNP at a low dose of 1 nmol but not to norepinephrine. Thirty minutes after treatment with a high dose of CNP, the positive inotropic response to CNP almost recovered to the control level (fig. 4). Three nanomoles of CNP tended to depress the positive chronotropic response to 1
nmol of CNP (table 1, part I), although the positive chronotropic response to CNP at 1 nmol was small.

**Effects of HS-142-1 and propranolol on the positive cardiac responses to CNP.** To investigate whether the positive inotropic and chronotropic responses to CNP were mediated by guanylyl cyclase-linked natriuretic peptide receptors, we studied the effects of HS-142–1 (2 mg) on the positive inotropic and chronotropic responses to CNP (1 nmol) and norepinephrine (0.1–1 nmol) in five isolated right atria. HS-142–1 blocked the positive inotropic response to CNP (P \(<\) .001) but not to norepinephrine (fig. 5). When CNP was rechallenged 30 min after HS-142–1 treatment, it caused increases in atrial contractile force similar to those before HS-142–1. HS-142–1 abolished the small positive chronotropic response to CNP (table 1, part II). HS-142–1 did not affect the basal sinus rate and atrial contractility in the isolated atria.

We tested whether the positive cardiac responses to CNP were mediated by beta adrenoceptors in isolated right atria. When propranolol (30 nmol) completely suppressed the positive inotropic responses to norepinephrine (0.1 nmol) (0.8 ± 0.1 g vs. 0 g, P \(<\) .001), it did not attenuate the positive inotropic responses to CNP (1 nmol) (0.8 ± 0.2 g vs. 0.8 ± 0.2 g, P = NS) in five isolated right atrial preparations. Propranolol also did not attenuate the positive chronotropic response to CNP (table 1, part VI).

**Effects of IBMX and intracardiac parasympathetic stimulation on positive cardiac responses to CNP.** We investigated whether the positive inotropic and chronotropic responses to CNP were mediated by guanylyl cyclase-linked natriuretic peptide receptors, we studied the effects of HS-142–1 (2 mg) on the positive inotropic and chronotropic responses to CNP (1 nmol) and norepinephrine (0.1–1 nmol) in five isolated, blood-perfused right atria. Vertical bars show S.E. Mean increases in atrial force in response to CNP were 0.9 ± 0.07 g in the control experiment. The basal atrial force in five right atria was 2.1 ± 0.4 g, not significant vs. control; P \(<\) .001 vs. control. NE, norepinephrine.

**TABLE 1**

Effects of each blocker on the positive chronotropic responses to CNP and norepinephrine in isolated, blood-perfused dog atria

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control</th>
<th>After drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Change in sinus rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>After drug treatment</td>
</tr>
<tr>
<td></td>
<td>beats/min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>After drug treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. A high dose of CNP (3 nmol, n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP (1 nmol)</td>
<td>2 ± 0.7</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>NE (0.1–1 nmol)</td>
<td>39 ± 18.5</td>
<td>39 ± 18.6</td>
</tr>
<tr>
<td>II. HS-142-1 (2 mg, n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP (1 nmol)</td>
<td>3 ± 1.3</td>
<td>0*</td>
</tr>
<tr>
<td>NE (0.1–1 nmol)</td>
<td>40 ± 7.2</td>
<td>38 ± 6.8</td>
</tr>
<tr>
<td>III. Low rates of IBMX (0.06–0.13 μmol/min, n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP (1 nmol)</td>
<td>2 ± 0.9</td>
<td>6 ± 2.5</td>
</tr>
<tr>
<td>NE (0.1–1 nmol)</td>
<td>35 ± 9.6</td>
<td>43 ± 14.6</td>
</tr>
<tr>
<td>IV. High rates of IBMX (0.6–1.3 μmol/min, n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP (1 nmol)</td>
<td>5 ± 2.2</td>
<td>9 ± 7.7</td>
</tr>
<tr>
<td>NE (0.1–1 nmol)</td>
<td>19 ± 6.1</td>
<td>16 ± 6.2</td>
</tr>
<tr>
<td>V. SAPS (10–30 Hz, n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP (1 nmol)</td>
<td>3 ± 1.3</td>
<td>0*</td>
</tr>
<tr>
<td>NE (0.1–0.3 nmol)</td>
<td>38 ± 8.3</td>
<td>18 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(59 ± 15.6%)*</td>
</tr>
<tr>
<td>VI. Propranolol (30 nmol, n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP (1 nmol)</td>
<td>3 ± 1.3</td>
<td>4 ± 1.1</td>
</tr>
<tr>
<td>NE (0.1–0.3 nmol)</td>
<td>28 ± 9.3</td>
<td>0*</td>
</tr>
</tbody>
</table>

*NE, norepinephrine; SAPS, intracardiac parasympathetic nerve stimulation; HS-142-1, natriuretic peptide receptor antagonist. Control sinus rate before a high dose of CNP treatment in five dogs was 99 ± 5.9 (mean ± S.E.) beats/min and it was not significantly different from the sinus rates of other experimental groups.

* P < .05 vs. control. Parentheses show percentage changes.
responses to CNP interact with the cAMP-dependent signal transduction. Low infusion rates (0.06–0.13 μmol/min) of IBMX increased the sinus rate by 22 ± 6.2 beats/min (17 ± 4.9%) and atrial contractile force by 0.8 ± 0.1 g (28 ± 5.2%) from the control levels in five dog atria. High infusion rates (0.6–1.3 μmol/min) of IBMX also increased the sinus rate by 65 ± 10.1 beats/min (55 ± 7.6%) and atrial contractile force by 4.6 ± 0.9 g (135 ± 16.2%) in another five atria. Two minutes later, the sinus rate and contractile force reached the steady-state levels that were maintained during IBMX infusion. IBMX at low rates did not affect the positive inotropic response to CNP, whereas it potentiated (P < .001) the positive inotropic response to norepinephrine (fig. 6A). However, IBMX at high rates attenuated (P < .001) the positive inotropic response to CNP, but it still potentiated the positive inotropic response to norepinephrine (fig. 6B). Neither low rates nor high rates of IBMX significantly affect the positive chronotropic responses to CNP and norepinephrine significantly (table 1, parts III and IV).

When intracardiac parasympathetic nerves were stimulated at a frequency of 10 or 30 Hz with a pulse duration of less than 0.03 msec and a voltage of 10 V, parasympathetic nerve stimulation decreased the sinus rate by 19.03 msec and a voltage of 10 V, parasympathetic nerve stimulation decreased the sinus rate by 19 ± 2.3 beats/min (20 ± 2.6%) and atrial contractile force by 0.6 ± 0.1 g (48 ± 5.6%) in five dog atria. Two minutes later, the sinus rate and contractile force reached the steady-state levels which were maintained during stimulation. Parasympathetic stimulation significantly reduced the positive cardiac responses to CNP and norepinephrine (fig. 7 and table 1, part V).

**Discussion**

**Cardiac effects of natriuretic peptides.** We demonstrated in the present study that CNP directly and dose-dependently increased atrial and ventricular contractile forces in isolated, blood-perfused dog heart preparations (figs. 1, 2 and 3). Our results also showed that the positive inotropic response to CNP was much greater than the positive chronotropic response to CNP, whereas norepinephrine increased the sinus rate and myocardial contractile force to a similar degree (fig. 2). Other cardiotonic peptides such as vasoactive intestinal peptide, pituitary adenylyl cyclase-activating polypeptide and glucagon increased sinus rate more than atrial contractile force in comparison with those effects of norepinephrine in isolated perfused right atrial preparations of the dog (Furukawa et al., 1986; Karasawa et al., 1990; Yonezawa et al., 1996). It is suggested, therefore, that CNP and its analogs may be very useful cardiotonic agents. On the other hand, BNP did not affect the atrial and ventricular contractility as well as sinus rate in the present study (figs. 2B and 3), although BNP is a cardiac hormone that is synthesized in and secreted from the ventricle (Mukoyama et al., 1991; Ogawa et al., 1991). In addition, ANP that is secreted from atria had no effect on the atrial contractility and sinus rate (fig. 2A). Therefore, ANP and BNP may not have a potential role as a paracrine hormone in cardiac contractility and sinus rate in the dog heart.

Clavell et al. (1993) have shown that CNP circulates in low picomolar concentrations in canine plasma. The isolated atrial preparation used in the present study was perfused with arterial blood from a support dog. However, when HS-142–1, a natriuretic peptide receptor blocker, was injected into the sinus node artery of the isolated atrial preparation, it did not affect the basal sinus rate and atrial contractility in this preparation. Therefore, endogenous CNP may not have a potential role on cardiac function in physiological states.

**Mechanisms for the CNP-induced positive inotropic and chronotropic responses.** In the present study, we
demonstrated that a high dose of CNP attenuated the increases in atrial contractility and sinus rate in response to a low dose of CNP but not norepinephrine (fig. 4). HS-142–1 blocked the positive cardiac responses to CNP (fig. 5 and table 1, part II). HS-142–1 is a specific natriuretic peptide receptor antagonist. The affinity cross-linking study demonstrated that HS-142–1 specifically abolished the labeling of the 135 kdalton band which was derived from the labeling of the guanylyl cyclase-linked natriuretic peptide receptors (Matsuda and Morishita, 1993). However, HS-142–1 had no effect on the labeling of the 60 kdalton band which was derived from the guanylyl cyclase-free receptors, i.e., NPR-C (Matsuda and Morishita, 1993). HS-142–1 inhibited cGMP production stimulated by ANP, BNP and CNP with almost equal potency in PC12 cells (Matsuda and Morishita, 1993). From the present results, therefore, we suggest that CNP increases the myocardial contractile force and sinus rate mediated by the guanylyl cyclase-linked natriuretic peptide receptors but not by the guanylyl cyclase-free receptors in the dog heart.

Recently, Koller et al. (1991) described ligand specificity of the two different subtypes of guanylyl cyclase-linked natriuretic peptide receptors, i.e., NPR-A and NPR-B. NPR-A has high affinity for ANP and BNP, whereas NPR-B binds only CNP with high affinity. Dose-response curves for stimulation of guanylyl cyclase of NPR-A and NPR-B demonstrated that both ANP and BNP could stimulate NPR-A effectively, and that BNP was approximately 10-fold less potent than ANP (Koller and Goeddel, 1992). In contrast, CNP did not increase intracellular cGMP significantly in cells expressing NPR-A. In NPR-B-expressing cells, only CNP could stimulate cGMP production effectively (Koller and Goeddel, 1992). In the present study, we demonstrated that CNP caused the positive inotropic and chronotropic effects, whereas ANP and BNP had no effects on the cardiac contractility and sinus rate (figs. 1, 2 and 3). Therefore, it is likely that the positive inotropic and chronotropic effects of CNP are mediated by NPR-B in the dog heart. On the other hand, propranolol did not affect the positive inotropic and chronotropic responses to CNP, which indicates that the cardiac responses to CNP are not mediated through beta adrenoceptors in the dog heart.

Most of the biological activities of the natriuretic peptides are thought to be mediated by intracellular accumulation of cGMP through the activation of particulate guanylyl cyclase (Inagami, 1989; Song et al., 1988). NPR-A and NPR-B are members of the family of receptor guanylyl cyclases. Our present results demonstrated that the positive inotropic response to CNP was mediated by NPR-B, which suggests that the increase in the production of cGMP induced by CNP may cause the positive inotropic response to CNP, although we did not determine changes in tissue cGMP and cAMP concentrations. Several cGMP effectors, namely, cGs-PDE (type II PDE), cGi-PDE (type III PDE) and G-kinase have been reported in vertebrate heart cells (Lohmann et al., 1991). Physiologically relevant targets of cGMP action in mammalian hearts include cGi-PDE and G-kinase (Lohmann et al., 1991). cGi-PDE is a dominant isozyme regulating intracellular cAMP in the heart of many species (Rapundalo et al., 1989; Beavo and Reifsnyder, 1990; Bohm et al., 1991). Ono and Trautwein (1991) reported that cGMP further increased L-type calcium current activated by isoproterenol, forskolin or intracellular dialysis with cAMP in guinea-pig ventricular cells. They suggested that inhibition of cGMP-sensitive PDE induced by cGMP increased tissue cAMP and that cGMP, as an intrinsic inhibitor for cGi-PDE, had a regulatory function under physiological conditions. cGMP also has been reported to increase both L-type calcium current and myocardial contractility in single beat cells of guinea-pig ventricular myocytes (Shirayama and Pappano, 1986). On the other hand, Kumar et al. (1997) suggested that cGMP increased the basal L-type calcium current mediated by G-kinase-dependent phosphorylation of calcium channels in newborn rabbit ventricular cells. Our present results demonstrated that the high infusion rates of IBMX (0.6–1.3 μmol/min) decreased the positive inotropic response to CNP. When 0.6 to 1.3 μmol/min of IBMX attenuated the positive inotropic response to CNP, intra-arterial concentrations of IBMX were approximately 60 to 130 μM. This concentration is about 5- to 10-fold greater than the IBMX concentrations that inhibit type I to IV PDEs in guinea-pig isolated ventricular myocytes (Bethke et al., 1992). Therefore, it is conceivable that cGMP increased by CNP could not act on cGi-PDE further after infusion of IBMX at high rates, because cGi-PDE already had been inhibited by high rates of IBMX in the isolated atrium. In addition, we demonstrated that when we investigated the effect of intracardiac parasympathetic nerve stimulation on the positive cardiac responses to CNP and norepinephrine, parasympathetic nerve stimulation attenuated the positive cardiac responses to these substances (fig. 7). Furukawa et al. (1989) reported that IBMX and norepinephrine-induced positive cardiac responses were depressed by parasympathetic nerve stimulation in isolated dog atria. Lindemann and Watanabe (1985) reported that acetylcholine attenuated the increase in cAMP, phospholamban phosphorylation and calcium uptake produced by IBMX in guinea-pig ventricles, and suggested that cholinomimetics inhibited cAMP-dependent effects at a site or sites in the cAMP cascade distal the catalytic unit of adenylyl cyclase. Therefore, the cAMP-dependent signal transduction may be involved in the positive cardiac responses to CNP.

However, sodium nitroprusside markedly elevated atrial cGMP levels but produced little or no functional change (Diamond et al., 1977; Taniguchi et al., 1979; Endoh and Yamashita, 1981). Sodium nitroprusside had no effect on cardiac contractility in isolated, blood-perfused dog heart preparations (Chiba et al., 1982). In addition, there is no report of the positive inotropic effect of ANP in the heart, although several reports demonstrate that ANP activates a receptor containing particulate guanylyl cyclase activity and increases cGMP in isolated rabbit and rat cardiac myocytes (Cramb et al., 1987; Neyes and Vetter, 1989). Therefore, further studies and measurements of tissue cGMP and cAMP levels are needed to demonstrate the intracellular mechanisms for the positive inotropic response to CNP in the dog heart, because it is not clear if all of the CNP-induced action can be explained by a cGMP-mediated mechanism.

Acknowledgments

We thank Dr. Y. Matsuda and Dr. S. Nakanishi (Tokyo Research Laboratories, Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan) for kindly providing HS-142–1.
References


Send reprint requests to: Y. Furukawa, M.D., Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390-8621, Japan.

Hirose et al.