Effect of endogenous natriuretic peptide system on ventricular and coronary function in failing heart

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Yamamoto, Kazuhiro, John C. Burnett, Jr., and Margaret M. Redfield. Effect of endogenous natriuretic peptide system on ventricular and coronary function in failing heart. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2406–H2414, 1997.—Ventricular concentrations of atrial, brain (BNP) and C-type natriuretic peptide are enhanced in congestive heart failure (CHF). Natriuretic peptide receptors are present on ventricular myocytes and stimulate guanosine 3′,5′-cyclic monophosphate (cGMP) production. cGMP has been demonstrated to affect myocyte function in vitro. Thus we hypothesized that the intracardiac natriuretic peptide system may modulate myocardial and coronary function in CHF. To test this hypothesis, the effects of an intracoronary infusion of the natriuretic peptide receptor antagonist HS-142–1 on ventricular and coronary function were examined in anesthetized dogs with chronic CHF. To determine whether receptor stimulation had contrasting effects to those of receptor blockade, intracoronary BNP was infused in anesthetized normal and CHF dogs. Low-dose HS-142–1 delayed and slowed left ventricular (LV) relaxation and decreased coronary blood flow without changes in LV pressures. Higher doses further impaired LV relaxation without further decreases in coronary blood flow. In normal and CHF dogs, exogenous BNP produced the opposite effect with a quicker onset and faster rate of LV relaxation without effects on LV pressures or coronary blood flow. The endogenous natriuretic peptide system has an autocrine-paracrine role to modulate LV and coronary vascular function in CHF.

ventricular function; coronary circulation; guanosine 3′,5′-cyclic monophosphate; heart failure

IN THE NORMAL HEART, brain natriuretic peptide (BNP) is of atrial and ventricular origin (20), whereas atrial natriuretic peptide (ANP) is produced primarily by the atrial myocyte (13) and C-type natriuretic peptide (CNP) is produced in the endothelial cells (34). In the presence of congestive heart failure (CHF) (37), there are increased concentrations of these peptides in the left ventricular (LV) myocardium. BNP and ANP activate the particulate guanylate cyclase-linked natriuretic peptide A receptor (NPR-A) and CNP binds to the particulate guanylate cyclase-linked B receptor (NPR-B) to produce the natriuretic peptide second messenger guanosine 3′,5′-cyclic monophosphate (cGMP) (14). As circulating hormones, the actions of the natriuretic peptides on systemic loading conditions and renal and humoral function are well defined (3, 7, 33). However, the messenger RNAs for NPR-A and NPR-B are present in the ventricle (15, 23), and isolated myocytes produce cGMP when exposed to BNP or ANP (15, 22), suggesting that the ventricular myocyte may be a target for, as well as a source of, the natriuretic peptides. Although the effects of cGMP on LV function are controversial (18), in vitro studies in normal isolated myocytes or papillary muscles have reported favorable effects of cGMP on myocardial relaxation (18, 28, 29), and BNP has been demonstrated to improve exercise hemodynamics in patients with diastolic heart failure, suggesting that these in vitro effects may be clinically relevant (6).

Because the natriuretic peptides and their receptors are present in the LV (24) in the presence of CHF and because the natriuretic peptide second messenger has been demonstrated in vitro to have effects on myocyte function, we hypothesized that in addition to their humoral actions, the natriuretic peptides may have an autocrine-paracrine role to modulate ventricular function in CHF. Furthermore, because administration of exogenous natriuretic peptides has been demonstrated to produce coronary vasodilatation (5, 29, 38), we postulated that endogenous activation of the natriuretic peptide receptors modulate coronary vascular tone in CHF.

Thus this study was designed to determine the functional role of the endogenous natriuretic peptide system in the modulation of ventricular and coronary vascular function in the failing canine heart. We utilized an intracoronary infusion of the natriuretic peptide receptor antagonist HS-142–1 (19) at concentrations that were designed to provide blockade of local intracardiac and then systemic natriuretic peptide receptors in a model of chronic CHF produced by rapid ventricular pacing. We then confirmed that the effects of HS-142–1 were mediated by antagonism of the natriuretic peptides by infusing one of the natriuretic peptides (BNP) into the coronary arteries of normal and CHF dogs to determine whether selective natriuretic peptide receptor stimulation produced contrasting responses to those of receptor antagonism.

METHODS

Three protocols were performed. First, a graded intracoronary infusion of HS-142–1, which blocks NPR-A and NPR-B and has been shown to antagonize effects of ANP, BNP, and CNP (19, 42), was performed in dogs with CHF. The doses of HS-142–1 were chosen to provide first a selective intracardiac blockade of the natriuretic peptide receptors and then blockade of systemic natriuretic peptide receptors (study 1). Next, the effects of natriuretic peptide receptor stimulation with a graded intracoronary infusion of exogenous BNP on ventricular function were examined in the normal dog. This infusion was conducted in the absence and presence of the β-adrenergic receptor antagonist esmolol hydrochloride to control for the effects of potential activation or inhibition of the sympathetic nervous system on ventricular function (7, 10) (study 2). Finally, the effects of further natriuretic peptide receptor stimulation with exogenous BNP were examined in...
dogs with CHF and endogenous activation of the natriuretic peptide system (study 3). These three protocols were conducted in 26 mongrel dogs (weighing 17–27 kg, mean 22 kg). This study conformed to the guiding principles of the American Physiological Society and was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic and Mayo Foundation.

Model of tachycardia-induced cardiomyopathy. In the CHF dogs programmable pacemakers (model 8329 or 5985, Medtronic) were implanted under pentobarbital sodium anesthesia (30 mg/kg) using an epidural lead as previously reported (26, 30). After a 2-wk recovery period, rapid ventricular pacing was commenced at 180 beats/min for 10 days, followed by 200 beats/min for 7 days, 210 beats/min for 7 days, and 225 beats/min for 7 days (39).

Preparation for acute experiments. On the day before the acute experiment, echocardiography was performed in sinus rhythm in the conscious state to measure LV cavity size and ejection fraction (26, 30, 39). The night before the acute experiments, the dogs were fasted but were allowed access to water. On the day of the acute experiment, dogs were sedated with Pentothal Sodium (10–20 mg/kg) before induction of general anesthesia [intravenous infusion of fentanyl citrate (0.25 mg/kg) and midazolam hydrochloride (0.75 mg/kg) over 15–30 min] and were intubated for artificial ventilation. The dogs were a Harvard respirator (Harvard apparatus) using room air supplemented with 100% oxygen. After the induction of general anesthesia, intravenous infusion of anesthetics (0.175 mg·kg⁻¹·h⁻¹ fentanyl citrate, 0.59 mg·kg⁻¹·h⁻¹ midazolam hydrochloride, 0.06 mg·kg⁻¹·h⁻¹ pancuronium bromide) was continued until the end of the experiment. In the CHF dogs the pacemaker was deprogrammed just before the sedation, and it was not restarted. The right femoral artery was cannulated for monitoring arterial pressure and withdrawal of blood. A 7-Fr high-fidelity manometer-tipped catheter (Millar Instrument) was introduced retrograde across the aortic valve into the LV through the left femoral artery for the measurement of LV pressure. The manometer was calibrated relative to atmospheric pressure before introduction into the LV. A 7-Fr flow-directed thermomil lumination pulmonary artery catheter (American Edwards Laboratories) was advanced into the pulmonary artery through the right jugular vein for the measurement of right atrial, pulmonary arterial, and pulmonary capillary wedge pressures and cardiac output. The heart was exposed through a left thoracotomy, and the pericardium was widely incised. The proximal portions of the left anterior descending and the left circumflex coronary arteries were dissected from the surrounding tissue, and right angle 27-gauge needles connected with polyethylene tubing were inserted into the left anterior descending and the left circumflex coronary arteries for drug administration. A calibrated electromagnetic flow probe (Carolina Medical Electronics) was positioned on the left circumflex coronary artery to measure coronary blood flow. A temporary pacing lead was connected to the right or left atrial appendage, and atrial pacing was started at a rate of 10–20 beats/min over the spontaneous heart rate to keep heart rate constant throughout the experimental protocol. Aortic, right atrial, pulmonary arterial, and pulmonary capillary wedge pressures were measured with a fluid-filled pressure transducer (Honeywell). Before pressure recording the manometric LV pressure was aligned with the pressure measured by its fluid-filled lumen connected to a fluid-filled pressure transducer. Cardiac output was obtained in triplicate by the thermodilution method. Electrocardiographic tracing, manometric LV pressure, and the first derivative of the LV pressure (dP/dt) were recorded at a paper speed of 100 mm/s on a strip recorder (Gould Electronics). The electrocardiogram was monitored carefully during placement of the flow probe and coronary needles as well as during the infusions for evidence of S-T segment change.

Study 1. Effects of natriuretic peptide receptor antagonism with intracoronary HS-142–1 on LV and coronary function in CHF. This protocol included five CHF dogs. After surgical preparation and the commencement of atrial pacing, the dogs were allowed to equilibrate for 30 min before the collection of baseline data. Hemodynamic recordings and blood withdrawal for humoral analysis were performed. After the data collection under atrial pacing, atrial pacing was stopped for ≤1 min, and intrinsic heart rate was also measured. After the collection of the control data, three graded doses of HS–142–1 were infused into the left anterior descending and the left circumflex coronary arteries at a rate of 5 µg·kg⁻¹·min⁻¹ (2.5 µg·kg⁻¹·min⁻¹ in each coronary artery) for 30 min after a bolus injection of 125 µg/kg (62.5 µg/kg in each coronary artery), at a rate of 10 µg·kg⁻¹·min⁻¹ (5 µg·kg⁻¹·min⁻¹ in each coronary artery) for 30 min after a bolus injection of 250 µg/kg (125 µg/kg in each coronary artery), and then at a rate of 20 µg·kg⁻¹·min⁻¹ (10 µg·kg⁻¹·min⁻¹ in each coronary artery) for 30 min after a bolus injection of 500 µg/kg (250 µg/kg in each coronary artery). A concentration of HS–142–1 in the left circumflex coronary artery at each of the three doses calculated with a continuously infused dose and a coronary blood flow at steady state was 2.4 ± 1.7, 4.8 ± 3.4, and 10.7 ± 8.0 µg/ml, respectively. At the end of each infusion the same data collected during the control period were collected.

Study 2. Effects of natriuretic peptide receptor stimulation with exogenous intracoronary BNP in normal dogs in absence and presence of β-adrenergic receptor antagonist. Six normal dogs were prepared as described, and baseline measurements were made. Then human BNP (hBNP, Phoenix Pharmaceuticals) was infused into the left anterior descending and the left circumflex coronary arteries at 12.5 ng·kg⁻¹·min⁻¹ (6.25 ng·kg⁻¹·min⁻¹ in each coronary artery) for 30 min and at 25 ng·kg⁻¹·min⁻¹ (12.5 ng·kg⁻¹·min⁻¹ in each coronary artery) for 30 min. Data were collected at the end of each infusion. In six additional normal dogs, the β-adrenergic receptor antagonist esmolol hydrochloride was administered intravenously after the atrial pacing was started, utilizing a bolus dosage of 500 µg/kg followed by a continuous infusion of 150 µg·kg⁻¹·min⁻¹. The control data were collected after 30 min of the infusion of esmolol hydrochloride, and the same protocol as previously described was conducted under the continuous infusion of esmolol hydrochloride and the atrial pacing.

The concentration of infused BNP in the left circumflex coronary artery at each of the two doses calculated with a continuously infused dose and a coronary blood flow at steady state was 2.8 ± 2.0 and 5.6 ± 3.3 ng/ml in the 12 dogs, respectively.

Study 3. Effects of natriuretic peptide receptor stimulation with exogenous intracoronary BNP in CHF dogs. Nine dogs with CHF were prepared as previously described, and baseline measurements were made. The same two doses of BNP were infused for 30 min. The data were collected at the end of each infusion. The concentration of infused BNP in the left circumflex coronary artery at each of the two doses calculated with a continuously infused dose and a coronary blood flow at steady state was 4.7 ± 0.7 and 9.5 ± 1.6 ng/ml, respectively.

After all the acute experimental protocols, the dogs were killed by KCl infusion, the hearts were harvested, and the LV weights were measured after the right ventricular free wall,
increased and peak administration of HS-142–1. Change in contractile duration of LVP.

1 After administration of BNP contractile duration increased. Compared with control, apparent in left ventricular pressure (LVP) tracings at control and after administration of brain natriuretic peptide (BNP; high dose) in normal dog.

Data analysis. The manometric LV pressure tracing and \( \frac{dP}{dt} \) were digitized using the digitizing pad (Summagraphics) interfaced with the personal computer system (IBM). To assess the rate of LV relaxation, the time constant of LV relaxation was calculated using two methods as previously described (39, 40), assuming zero-pressure asymptote (TL) or a variable asymptote (TD). Because cGMP has been reported to induce an early onset of relaxation as evidenced by an abbreviation of twitch duration in isolated myocytes (28, 29), the duration between the points at peak \( \frac{dP}{dt} \) and peak \( \frac{-dP}{dt} \) (\( \Delta d \)) was measured to assess contractile duration (Fig. 1). Averaged values of three consecutive cardiac cycles were used for the quantitative analysis.

Systemic vascular resistance (SVR, mmHg·min·l\(^{-1}\)) was defined as mean arterial pressure minus right atrial pressure divided by cardiac output. Pulmonary vascular resistance (PVR, mmHg·min·l\(^{-1}\)) was defined as mean pulmonary artery pressure minus pulmonary capillary wedge pressure divided by cardiac output. Coronary vascular resistance (CVR, mmHg·min·ml\(^{-1}\)) was defined as mean aortic pressure minus right atrial pressure divided by coronary blood flow. The LV weight corrected for body weight was determined as LV mass index.

Blood for humoral analysis was placed in EDTA tubes and immediately placed on ice. After centrifugation at 2,500 rpm and 3°C, the plasma was decanted and stored at −20°C until analysis. Plasma BNP was determined by radioimmunoassay using antibody to a human BNP as previously described (7). Plasma cGMP was measured by a specific radioimmunoassay as previously described (7).

Statistical analysis. Values are expressed as means ± SD. The serial changes in measured variables after drug infusion within each study protocol were tested with a repeated measures analysis of variance (ANOVA) followed by Fisher’s least significant difference test. The difference between the data of the two different groups was compared by ANOVA followed by Fisher’s least significant difference test. Bivariate correlation between two variables was performed with simple least-squares linear regression analysis. Results were considered significant at \( P < 0.05 \). All calculations were performed with the STATVIEW II (Abacus) statistical program.

RESULTS

In CHF dogs LV end-systolic (45 ± 3 in CHF dogs vs. 26 ± 1 mm in normal dogs, \( P < 0.05 \)) and end-diastolic (52 ± 3 in CHF dogs vs. 41 ± 1 mm in normal dogs, \( P < 0.05 \)) diameters were greater, ejection fraction was reduced (25 ± 5 in CHF dogs vs. 59 ± 2% in normal dogs, \( P < 0.05 \)) and LV mass index was higher (4.9 ± 0.7 in CHF dogs vs. 4.3 ± 0.5 g/kg in normal dogs, \( P < 0.05 \)) compared with the normal dogs.

Study 1. Effects of natriuretic peptide receptor antagonism with HS-142–1 in CHF. The paced heart rate in this protocol was 87 ± 19 beats/min. Intracoronary infusion of the lowest dose of HS-142–1 induced prolongation of the time constant (TL and TD) and \( \Delta d \) (the duration between the points at peak \( \frac{dP}{dt} \) and peak \( \frac{-dP}{dt} \) (Table 1). Cardiac output increased in all dogs, although there was no statistical significance, and the changes in cardiac output induced by the lowest dose of HS-142–1 tended to correlate with those in \( \Delta d \) (\( r = 0.87, P = 0.06 \)). There was no change in peak \( \frac{dP}{dt} \), aortic, LV systolic, or pulmonary artery pressures or in PVR or SVR. There were no changes in filling pressures. There was a decrease in coronary blood flow and an increase in CVR. There were no changes in intrinsic heart rate at this or any dose. These hemodynamic effects were associated with a small but significant decrease in plasma cGMP concentration without changes in hematocrit.

The further incremental infusion of HS-142–1 induced further prolongation of the time constant and \( \Delta d \) (Fig. 1A) without further changes in coronary blood flow or CVR. There was no change in peak \( \frac{dP}{dt} \). Cardiac output modestly decreased at the peak dose with modest increases in aortic and LV systolic pressures and SVR. There were moderate increases in pulmonary artery pressure and PVR. There were minimal but consistent and thus statistically significant increases in right atrial and pulmonary capillary wedge pressures but no significant increase in LV end-diastolic pressure. There was progressive reduction in plasma cGMP concentrations but no change in hematocrit. Throughout the infusion of HS-142–1, significant ST-T change was not observed on electrocardiogram.
Table 2. Effects of exogenous BNP in normal dogs

<table>
<thead>
<tr>
<th>BNP</th>
<th>Control</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAoP, mmHg</td>
<td>89 ± 11</td>
<td>90 ± 10</td>
<td>93 ± 8*</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>18 ± 4</td>
<td>16 ± 4</td>
<td>16 ± 3*</td>
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<tr>
<td>MPCWP, mmHg</td>
<td>4 ± 2</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>MRAP, mmHg</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>SVR, mmHg·min−1</td>
<td>24 ± 6</td>
<td>28 ± 7*</td>
<td>30 ± 5*</td>
</tr>
<tr>
<td>PVR, mmHg·min−1</td>
<td>3.9 ± 1.24</td>
<td>3.32 ± 0.84*</td>
<td>3.12 ± 0.64*</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>112 ± 8</td>
<td>114 ± 10</td>
<td>111 ± 5</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>196 ± 22</td>
<td>188 ± 24*</td>
<td>183 ± 27*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>6 ± 6</td>
<td>6 ± 5</td>
<td>6 ± 5</td>
</tr>
<tr>
<td>Peak + dP/dt, mmHg/s</td>
<td>2,232 ± 287</td>
<td>2,229 ± 288</td>
<td>2,187 ± 246</td>
</tr>
<tr>
<td>Δd, ms</td>
<td>27 ± 5</td>
<td>25 ± 4*</td>
<td>23 ± 4*</td>
</tr>
<tr>
<td>Tl, ms</td>
<td>31 ± 8</td>
<td>26 ± 7*</td>
<td>24 ± 8*</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td>57 ± 30</td>
<td>59 ± 35</td>
<td>60 ± 33</td>
</tr>
<tr>
<td>CVR, mmHg·min−1</td>
<td>2.0 ± 1.2</td>
<td>2.3 ± 1.9</td>
<td>2.0 ± 1.3</td>
</tr>
<tr>
<td>Intrinsic HR, beats/min</td>
<td>39 ± 26</td>
<td>39 ± 25</td>
<td>39 ± 26</td>
</tr>
<tr>
<td>BNP, pg/ml</td>
<td>3 ± 2.7</td>
<td>3 ± 2.7</td>
<td>3 ± 2.7</td>
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<tr>
<td>cGMP, pmol/ml</td>
<td>4 ± 1.8</td>
<td>4 ± 1.8</td>
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</table>

Values are means ± SD; n = 6 dogs. All hemodynamic indexes except intrinsic heart rate were recorded under consistent heart rate with artificial atrial pacing. BNP, plasma concentration of brain natriuretic peptide; β-adrenoceptor antagonist (−), in absence of β-adrenergic receptor antagonist; β-adrenoceptor antagonist (+), in presence of β-adrenergic receptor antagonist, BNP low dose, 1.25 ng·kg−1·min−1; BNP high dose, 2.5 ng·kg−1·min−1. All other abbreviations as in Table 1. *P < 0.05 vs. control. †P < 0.05 vs. BNP low dose. ‡P < 0.05 vs. control.
There was a slight increase in mean aortic pressure and a slight decrease in pulmonary artery pressure with the high dose of BNP, but LV systolic pressure was not increased. There was a small increase in SVR but no change in PVR. Cardiac filling pressures did not change. The paced heart rate was 128 ± 20 beats/min. Plasma concentration of BNP and cGMP increased without changes in hematocrit.

In the presence of esmolol infusion, BNP shortened the time constant (TL and TD) and Δd, decreased cardiac output, and increased SVR as in the absence of esmolol. In contrast, in the presence of esmolol, the high dose of BNP significantly decreased peak +dp/dt and there were no changes in mean aortic or pulmonary artery pressure. As in the absence of esmolol, there was no change in LV systolic pressure, PVR, filling pressures, coronary blood flow, CVR, or intrinsic heart rate. The paced heart rate was 111 ± 11 beats/min. Plasma concentrations of BNP and cGMP increased without changes in hematocrit.

In pooled data obtained in the absence and presence of esmolol, the changes in cardiac output correlated with those in Δd (r = 0.68, P < 0.01, n = 24).

Study 3. Effects of natriuretic peptide receptor stimulation with exogenous BNP in CHF dogs. BNP significantly shortened the time constant (TL and TD) but did not change Δd or cardiac output (Table 3). The high dose of BNP significantly decreased peak +dp/dt. There were no changes in aortic, pulmonary, or LV systolic pressures or in SVR or PVR. There was no change in filling pressures. The paced heart rate was consistent at 109 ± 17 beats/min, and the intrinsic heart rate decreased. Coronary blood flow and CVR were unchanged. Plasma concentrations of BNP and cGMP increased without changes in hematocrit. The BNP-induced changes in the time constant (TL and TD) and cGMP concentration were not different between the normal dogs and the CHF dogs and were in a direction opposite to those of HS-142-1 in Table 1.

DISCUSSION

The current study reports that antagonism of the intracardiac natriuretic peptide system with HS-142-1 delayed the onset of and decreased the rate of LV isovolumic relaxation in dogs with CHF, whereas stimulation of the intracardiac natriuretic peptide receptors with BNP in normal dogs had the opposite effect, speeding the onset and rate of LV isovolumic relaxation. The effects of exogenous BNP in the normal dog did not appear to be mediated via sympathetic nervous system stimulation. Whereas the effect of exogenous BNP to abbreviate contractile duration was blunted in the presence of CHF, the effect to accelerate the rate of LV isovolumic relaxation was preserved. Antagonism of the intracardiac natriuretic peptide receptors in CHF had no effect on peak +dp/dt, although the highest dose of BNP slightly decreased peak +dp/dt in CHF dogs and in normal dogs in the presence of β-adrenergic receptor blockade. Antagonism of the intracardiac natriuretic peptide system in CHF dogs resulted in a non-dose-dependent decrease in coronary blood flow, whereas exogenous BNP had no effect on coronary blood flow in normal dogs or in CHF dogs. Antagonism of systemic natriuretic peptide receptors with high doses of HS-142-1 in CHF resulted in a mild systemic and moderate pulmonary vasoconstriction, effects associated with a decrease in cardiac output.

Effects of endogenous natriuretic peptide system on LV function in CHF. The current in vivo data do suggest that physiologically relevant effects on LV function are produced by the endogenous activation of the intracardiac natriuretic peptides in CHF. These effects are demonstrated by natriuretic peptide receptor antagonism in CHF and by exogenous administration of BNP to normal dogs to mimic the myocardial levels present in CHF. Because the changes in contractile duration induced by HS-142-1 and BNP correlated with those in cardiac output in the absence of changes in heart rate or filling pressures, these data suggest that part of the effect of the natriuretic peptides on cardiac output is related to an effect on LV function, an effect that may be overlooked by conventional assessment of myocardial function (29). Interestingly, there were no significant effects on contractile duration with BNP infusion in the CHF dogs, consistent with the absence of a decrease in cardiac output with BNP infusion in human CHF (41). The absence of further effects on contractile duration with exogenous BNP in CHF suggests that the effect on contractile duration becomes maximal with pathophysiologic myocardial concentrations and is not enhanced by the suprapathophysiological myocardial concentrations produced by intracoronary BNP in CHF.

Whereas the changes in the time constant of LV relaxation observed with low-dose HS-142-1 in the CHF dogs and with exogenous BNP in the normal and
CHF dogs could also be seen with changes in loading conditions, there was no change in LV systolic or diastolic pressures observed with low-dose HS-142–1 or BNP infusion. The changes in the time constant by a magnitude similar to those seen in the current study require decreases in LV end-diastolic pressure of >10–20 mmHg in a normal or CHF dog (12). Thus it is very unlikely that minor changes in LV diastolic volume unassociated with changes in filling pressure produced the observed effects on the time constant. In the CHF dogs the higher doses of HS-142–1 did produce a small increase in LV systolic pressure and SVR, and this increase could contribute to the increases in the time constant observed. However, because these changes were small, it is unlikely that the pressure changes are the sole mediators of the change in the time constant.

Although the increase in the time constant observed in CHF dogs after infusion of low-dose HS-142–1 occurred in the setting of a decrease in coronary blood flow, this decrease seems unlikely to be responsible for the increase in the time constant for several reasons. The decrease in coronary blood flow was small and likely compensated for by enhanced oxygen extraction. There was no decrease in peak +dP/dt or increase in LV end-diastolic pressure to indicate expected effects of ischemia on inotropic function or diastolic distensibility, and there were no electrocardiographic changes to suggest ischemia. The progressive increases in the time constant with increasing doses of HS-142–1 occurred without further changes in coronary blood flow, and the decreases in the time constant produced by exogenous BNP were unassociated with alterations in coronary blood flow.

Whereas the effects of cGMP on myocardial contractility remain controversial, in vitro studies in isolated myocytes or papillary muscles have demonstrated that analogs of cGMP, the second messenger for the natriuretic peptides, produce effects on indexes of myocyte contraction and relaxation. Among the most consistently described effects are a slight decrease in peak twitch amplitude without changes in shortening velocity (17, 22, 29), an early onset of relaxation (17, 29), and an increase in the resting diastolic cell length (29). Whereas the effects of cGMP are complex and probably concentration dependent (18), recent studies suggest that cGMP reduces the sensitivity of the myofilaments to calcium (8, 29). In vivo, reduced calcium sensitivity would be expected to result in an abbreviation of contraction and an acceleration of the rate of LV isovolumic relaxation with a mild decrease in peak LV systolic pressure and without a decrease in the velocity of pressure development. Thus the natriuretic peptides, which are produced in the heart, have receptors on the myocyte, and are potent stimulators of cGMP production, may have an autocrine-paracrine role to modulate myocardial performance. Because the changes in the time constant of LV relaxation and contractile duration observed in the current study occurred without apparent alterations in loading conditions under the biconorary infusion of the antagonist and agonist of the natriuretic peptide system, they suggest an effect on intrinsic inactivation that would be consistent with the reported effect of cGMP on sensitivity of the myofilaments to calcium (2).

To date, in vivo studies with exogenously administered ANP have not demonstrated a physiologically significant effect on LV function (9, 27). There are several potential reasons for this discrepancy. Previous studies have suggested that BNP may be more potent than ANP in vivo (41). Previous studies have used doses of ANP that had effects on loading conditions, did not control for potential sympathetic nervous system stimulation, used unbalanced intracoronary infusion, employed brief (10 min) ANP infusions, and did not specifically examine contractile duration. Aside from these methodological considerations, although both the natriuretic peptides and nitric oxide stimulate cGMP production, they do so at different intracellular sites and thus may have different effects on myocyte function. Alternatively, the effects on myocyte function may be difficult to observe in vivo because of the many factors that affect myocyte function in vivo and that may mask the natriuretic peptide-induced effects on LV function. Furthermore, the effects of cGMP may depend on the prevailing level of adenosine 3’,5’-cyclic monophosphate, which may vary depending on experimental conditions (29).

Relationship of observed effects to natriuretic peptide receptors. Stevens et al. (32) demonstrated that HS-142–1 reversed the hypotensive effect of ANP infusion in the normal anesthetized dog when the HS-142–1 was administered during the ANP infusion. The HS-142–1 also antagonized the renal effects of the ANP infusion. In a related study, Stevens et al. (31) demonstrated that in a model of acute CHF in the dog produced by acute rapid ventricular pacing and associated with hypotension, activation of ANP and cGMP and maintenance of urinary sodium excretion, administration of HS-142–1 reversed the hypotensive effects of rapid ventricular pacing, lowered cGMP levels, and produced sodium retention. These two studies in the dog indicate that HS-142–1 does antagonize natriuretic peptide receptors in the canine cardiovascular and renal systems.

Studies in various species have demonstrated that HS-142–1 blocks ANP- and BNP-mediated cGMP generation in a variety of tissues and that its in vivo effects are specific to blockade of natriuretic peptide-induced cGMP generation. Imura et al. (11) showed that HS-142–1 produced a dose-dependent inhibition of ANP and porcine-, rat- and human-BNP-stimulated cGMP production in bovine aortic smooth muscle cells and bovine aortic endothelial cells. Furthermore, these investigators demonstrated that HS-142–1 produced a dose-dependent blockade of the vasorelaxation produced by ANP and BNP in bovine aortic rings. This blockade was overcome by increasing concentrations of ANP and BNP. The blockade of the vasorelaxation by HS-142–1 was specific to the natriuretic peptide receptors because HS-142–1 had no effect on the vasorelaxation produced by numerous other vasodilators, including sodium nitroprusside, isoproterenol, papaverine, or forskolin, and HS-142–1 alone did not produce vasocon-
striction (11). Whereas no studies have specifically looked at canine myocytes, Liu et al. (16) demonstrated that HS-142–1 blocked ANP-mediated coronary vasodilation in the anesthetized dog. Nachshon et al. (21) did demonstrate that HS-142–1 reduced cGMP production in rat atrial myocytes, and previous in vitro studies by Meulemans et al. (17) demonstrated effects of ANP on isolated cat and rat papillary muscle. Finally, Zhang et al. (42) demonstrated first in in vitro studies using lung fibroblasts that HS-142–1 blocked cGMP production produced by ANP and BNP and then demonstrated in vivo that HS-142–1 blocked the renal response to ANP and BNP in the rat. These studies demonstrate that the effects of HS-142–1 to block natriuretic peptide induced-cGMP generation, and subsequent biological effects are consistent between tissues in the same species and between species. In the current study HS-142–1 had significant effects on myocardial and vascular function in a model of CHF characterized by activation of the natriuretic peptides. These effects were associated with reductions in cGMP levels, and although BNP and HS-142–1 were not administered simultaneously in the same animal, BNP produced opposing effects to HS-142–1 when administered to normal dogs and dogs with CHF. This study, when interpreted in the context of the large body of data that demonstrates the tissue and species consistency of the agonistic and antagonistic effects of the natriuretic peptides and HS-142–1 on natriuretic peptide receptors, provides evidence that natriuretic peptide receptor stimulation by endogenous activation or exogenous administration of the natriuretic peptides has significant effects on myocardial function.

Effect of BNP on heart rate. In the CHF dogs, exogenous BNP produced a decrease in intrinsic heart rate. However, HS-142–1 had no effect on heart rate in CHF dogs and exogenous BNP had no effect on heart rate in the normal dogs. These results suggest that only at the very high myocardial concentrations of BNP produced by exogenous BNP in the setting of endogenous activation of BNP, BNP had a negative chronotropic effect. The mechanism of this effect is unclear but could be related to stimulation of vagal afferents, sympathetic withdrawal, or a direct effect of cGMP (1).

Effect of natriuretic peptides on coronary vascular function. Previous studies have reported that ANP (5), BNP (25), and CNP (38) can produce coronary vasodilation. We have observed decreases in coronary blood flow with intravenous administration of HS-142–1 in the normal dog (35). The current study extends the previous results by demonstrating the coronary vasoconstrictive effects of antagonism of the natriuretic peptide receptors in CHF. These results suggest that the endogenous natriuretic peptide system modulates coronary vascular tone in CHF as well as in normals. The coronary blood flow was measured with an electromagnetic flow probe in this study. Although this methodology is subject to drift and instability, HS-142–1 promptly decreased the flow in all the dogs at the same point in the experiment, which suggests that the directional changes with the HS-142–1 infusion are consistent, whereas the quantitative measure of absolute flow may not be accurate with electromagnetic flow probe measurements in the absence of repeated measurement of zero-flow throughout the experiment. Although exogenous BNP did not affect coronary circulation in this study as it has in a previous study (25), this may be partly explained by the much smaller dose used in the current study. Our findings would suggest that it is primarily the natriuretic peptide B receptor that mediates the observed effect of HS-142–1 on coronary blood flow because A-receptor stimulation at pathophysiological (intracoronary BNP in normal dogs) and supraphysiological (intracoronary BNP in CHF dogs) concentrations did not increase coronary blood flow, whereas A- and B-receptor antagonism of the pathophysiological levels present in CHF consistently decreased coronary blood flow. Because there is currently no specific blocker for just the A or B receptor, this remains speculative.

Effect of high dose HS-142–1 on SVR and PVR. Infusion of high doses of HS-142–1 resulted in a mild increase in SVR and a moderate increase in PVR, which is likely due to systemic spillover with antagonism of peripheral and pulmonary A and B receptors. The vasoconstriction observed with high-dose HS-142–1 confirms the role of the natriuretic peptides as endogenous vasodilators in CHF. This effect may be due to direct effects of the natriuretic peptides on vascular smooth muscle and/or due to effects on other vasoactive peptides. Stevens et al. (30) demonstrated that administration of HS-142–1 to dogs with mild LV dysfunction resulted in activation of the renin-angiotensin system, and Wada et al. (36) have reported that HS-142–1 administration in dogs with pacing-induced CHF results in increases in circulating endothelin levels. Although not assessed in the current study, increases in angiotensin II or endothelin may have contributed to the vasoconstriction evident with high-dose HS-142–1 administration in CHF. The increment in BNP observed with the intracoronary human BNP was likely inadequate to produce vasodilation as previously reported with BNP in humans with CHF (41).

Physiological significance of current study. The natriuretic peptide system is activated in the presence of heart failure, and its effects as a circulating hormonal system have been investigated in previous studies by our laboratory and others. However, the interest in their autocrine-paracrine roles in the heart has been growing after the recent demonstration of the presence of natriuretic peptide receptors in the myocyte. Although previous in vitro studies have suggested significant effects of cGMP on myocardial function, little is known about the physiological significance of these effects in vivo. Although previous studies measured several indexes of cardiac function during the systemic infusion of natriuretic peptides, the infused dose was so high that changes in systemic hemodynamics resulted, which may have contributed to the controversy about the effects of the natriuretic peptides on ventricular function. Thus we chose an intracoronary infusion of low doses, and thus we believe that our study gives an important insight into this interesting issue from a
more physiological perspective. Furthermore, the magnitude of the changes in the time constant of LV relaxation is of the order observed with antagonism or natriuretic peptide antagonist of microbial origin, of atrial natriuretic peptide-induced relaxation of isolated rabbit aorta through the blockade of guanylyl cyclase-linked receptors. Mol. Pharmacol. 42: 982–990, 1992.


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