

Differential effects of natriuretic peptides and NO on LV function in heart failure and normal dogs

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Hart, Chari Y. T., Eugenia L. Hahn, Donna M. Meyer, John C. Burnett, Jr., and Margaret M. Redfield. Differential effects of natriuretic peptides and NO on LV function in heart failure and normal dogs. *Am J Physiol Heart Circ Physiol* 281: H146–H154, 2001.— β -Adrenergic hyporesponsiveness in congestive heart failure (CHF) is mediated, in part, by nitric oxide (NO). NO and brain natriuretic peptide (BNP) share cGMP as a second messenger. Left ventricular (LV) function and inotropic response to intravenous dobutamine (Dob) were assessed during sequential intracoronary infusion of saline, HS-142-1 (a BNP receptor antagonist), and HS-142-1 + *N*^G-monomethyl-L-arginine (L-NMMA) in anesthetized dogs with CHF due to rapid pacing and in normal dogs during intracoronary infusion of saline, exogenous BNP, and sodium nitroprusside (SNP). In CHF dogs, intracoronary HS-142-1 did not alter the inotropic response to Dob [percent change in first derivative of LV pressure (% Δ dP/dt) $47 \pm 4\%$ saline vs. $54 \pm 7\%$ HS-142-1, *P* = not significant]. Addition of intracoronary L-NMMA to HS-142-1 enhanced the response to Dob (% Δ dP/dt $73 \pm 8\%$ L-NMMA + HS-142-1, *P* < 0.05 vs. HS-142-1). In normal dogs, intracoronary SNP blunted the inotropic response to Dob (% Δ dP/dt $93 \pm 6\%$ saline vs. $71 \pm 5\%$ SNP, *P* < 0.05), whereas intracoronary BNP had no effect. In CHF dogs, the time constant of LV pressure decay during isovolumic relaxation increased with intracoronary HS-142-1 (48 ± 4 ms saline vs. 58 ± 5 ms HS-142-1, *P* < 0.05) and further increased with intracoronary L-NMMA (56 ± 6 ms HS-142-1 vs. 66 ± 7 ms L-NMMA + HS-142-1, *P* < 0.05). Endogenous BNP and NO preserve diastolic function in CHF, whereas NO but not BNP inhibits β -adrenergic responsiveness.

inotropy; cGMP; lusitropy

THE CLINICAL SYNDROME of congestive heart failure (CHF) is characterized by left ventricular (LV) remodeling, systolic and diastolic dysfunction, and a diminished inotropic response to catecholamine stimulation. Associated with these structural and functional abnormalities is the activation of biochemical mediators that may influence myocardial function. The natriuretic peptides (NP) are markedly activated within the heart in CHF and mediate their actions via their second

messenger, cGMP (4). While some studies have suggested that nitric oxide (NO), which also stimulates cGMP production, is activated in CHF, this remains controversial (6, 48).

The NP family is a group of structurally similar but genetically distinct peptides that includes atrial (ANP) (7) and brain natriuretic peptide (BNP) (28), of myocardial cell origin, and C-type natriuretic peptide (CNP) (5), of endothelial cell origin. NP exert effects by binding to the NP-A and NP-B receptors on the cell surface, which are linked to particulate guanylyl cyclase. cGMP is the sole second messenger of the NP family (20). There are NP-A and NP-B receptors on ventricular myocytes, suggesting the potential for an autocrine/paracrine effect of NP on LV function in CHF (24, 30). Indeed, studies (51) suggest that NP may exert a prolusitropic effect.

NO is synthesized from the amino acid L-arginine in a reaction catalyzed by the enzyme family of NO synthase (NOS) (22, 29, 34). NO activates a guanylyl cyclase distinct from that associated with the NP receptor, soluble guanylyl cyclase (16), by binding with the heme-moiety. Although NO stimulates cGMP production in vascular smooth muscle, NO effects may also be mediated by modulation of other heme-containing proteins, other nitrosylation events, and/or oxidation events (39, 49). NOS has been shown to be present in cardiac myocytes (38). Previous *in vivo* studies (14) have reported that endogenous NO attenuates β -adrenergic responsiveness in humans with LV dysfunction.

Because NO and NP share cGMP as a second messenger, NP may also modulate β -adrenergic responsiveness in CHF. However, the effect of NP on β -adrenergic responsiveness has not been investigated. In addition, the effect of endogenous NO on lusitropic function *in vivo* in CHF has not been reported. The purpose of this study was to determine the effects of endogenous NP and NO on systolic and diastolic LV function *in vivo* in a canine model of CHF. Thus we performed global intracoronary infusion of the NP-A and -B receptor antagonist HS-142-1 alone and in the

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presence of the NOS inhibitor N^G -monomethyl-L-arginine (L-NMMA) in dogs with CHF produced by rapid right ventricular pacing. In addition, to confirm the specificity of our findings, we examined the effect of intracoronary infusion of BNP and a NO donor in normal dogs.

METHODS

All experimental procedures were designed in accordance with National Institutes of Health guidelines and approved by the Mayo Institutional Animal Care and Use Committee.

Animal Model of Tachycardia-Induced Congestive Heart Failure

CHF was induced in adult male mongrel dogs ($n = 7$) weighing 22.0–24.4 kg (23.5 ± 0.3 kg) by rapid right ventricular pacing. Programmable cardiac pacemakers (Intermedics) were implanted using an epicardial lead under general anesthesia [consisting of 4% methohexital solution (0.5 ml/kg) and 0.5–2.5% isoflurane]. Torbugesic (0.2–0.4 mg/kg every 4–6 h) as needed was used for postoperative analgesia. Postoperative antibiotics (500 mg Cephtabs every 12 h) were administered for 3 days. After a recovery period of 2 wk, rapid ventricular pacing was initiated at 180 beats/min for 10 days, followed by 200 beats/min for 7 days, 210 beats/min for 7 days, and finally 220 beats/min for 7 days to produce a model of progressive LV dysfunction (42).

Animal Preparation for Acute Experiments

Normal and CHF dogs were fasted the night before the acute experiment. On the day of the acute experiment, the dog was anesthetized with a bolus dose of fentanyl (Johnson Matthey) at 0.25 mg/kg and midazolam (Roche) at 0.75 mg/kg given over 5–10 min. The animal was immediately intubated and supported with artificial ventilation (Harvard Apparatus Respirator Pump) using room air supplemented with O_2 and maintained on a continuous intravenous infusion of fentanyl ($0.18 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and midazolam ($0.59 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) titrated to effect. In the CHF dogs, the pacemaker was reprogrammed to 70 beats/min during induction and subsequently deprogrammed once atrial pacing had begun. The heart was exposed via a left thoracotomy. A femoral vein was cannulated for administration of dobutamine. A femoral artery was cannulated for monitoring arterial pressure and blood sampling. A pressure transducer (Konigsberg Instruments; Pasadena, CA) was inserted into the apex of the LV via an apical stab wound and calibrated with a fluid-filled pigtail catheter (USCI). The proximal portions of the left circumflex (LCx) and left anterior descending (LAD) coronary arteries were isolated. Right-angle needles (27 gauge) connected to polyethylene tubing (Intramedic, 0.38-mm internal diameter, 1.09-mm outer diameter) were inserted into both coronary arteries and stabilized with Nexaband (Veterinary Products Laboratories) after retrograde flow was confirmed. Normal saline was infused to maintain patency. A temporary pacing lead was placed on the left atrial appendage for atrial pacing to maintain a constant heart rate. A 3-lead electrocardiogram was monitored. The LV pressure and the first derivative of the LV pressure (dP/dt) were monitored using the CA recorder data acquisition and recording system (Data Integrated Scientific Systems; Pinckney, MI).

Experimental Protocols

Study 1: effects of intracardiac NP receptor antagonism and intracardiac NOS inhibition on LV systolic and diastolic function in experimental CHF. Seven dogs with CHF were used for this protocol. After surgical preparation and equilibration, baseline hemodynamic measurements were made during intracoronary infusion of saline, and the inotropic response to intravenous dobutamine was then assessed. Dobutamine was infused at a dose that increased dP/dt by $\geq 50\%$ ($10\text{--}15 \text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) until the maximum dP/dt change was stable for 5 min. Dobutamine was then discontinued, and, after a 15-min recovery period, new steady-state readings were obtained. HS-142-1 (Kyowa-Hakko) was then infused into the LCx and LAD coronary arteries with a bolus dose of 250 $\mu\text{g}/\text{kg}$ (125 $\mu\text{g}/\text{kg}$ in each coronary artery), followed by a maintenance infusion of 20 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in each coronary artery) for the remainder of the experimental protocol. After the initial 30 min of HS-142-1, steady-state readings were obtained, and intravenous dobutamine was then infused at the same dose and continued until the change in maximum dP/dt was stable. Dobutamine was then stopped, and hemodynamic parameters were allowed to recover over a 15-min period. After this period, steady-state readings were again obtained, and the NOS inhibitor L-NMMA (Calbiochem; La Jolla, CA) was then added to the HS-142-1 intracoronary infusion at 15 $\mu\text{mol}/\text{min}$ (7.5 $\mu\text{mol}/\text{min}$ in each coronary artery). After 15 min of this combined intracoronary infusion, steady-state readings and the response to dobutamine were assessed. Hemodynamic data and blood samples were collected at the end of each infusion. All steady-state data were obtained during constant heart rate by atrial pacing at 10–20 beats greater than the intrinsic heart rate. All dobutamine data were obtained at a constant heart rate determined by the maximal heart rate achieved during the first dobutamine infusion. Because of the blunted chronotropic response to dobutamine observed in the CHF dogs, the dobutamine atrial pacing rate did not differ from the nondobutamine pacing rate.

Study 2: effects of exogenous intracoronary BNP and the NO donor sodium nitroprusside on LV systolic function and diastolic function in normal dogs. Five normal dogs were used for this protocol. After surgical preparation and equilibration, baseline hemodynamic parameters were collected. During the surgical preparation, a test dose of dobutamine ($10 \text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was given to determine the maximum heart rate achieved. Because of the marked chronotropic response to dobutamine, atrial pacing was maintained at that rate throughout the protocol to avoid wide swings in heart rate. During intracoronary saline infusion, intravenous dobutamine was started at a rate of $10 \text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and continued until dP/dt had stabilized. The dobutamine infusion was stopped until hemodynamic parameters returned to baseline. Steady-state readings were obtained, and human BNP (Phoenix Pharmaceuticals) was then infused into the LCx and LAD coronary arteries at $50 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (25 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in each coronary artery) for 30 min. Steady-state readings and responses to intravenous dobutamine ($10 \text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) were assessed. Intracoronary BNP was then replaced with saline (*saline 1*) for a 20-min recovery period, and new steady-state readings were obtained. Subsequently, the NO donor sodium nitroprusside (SNP; Abbott Laboratories; Chicago, IL) was infused at 8 $\mu\text{g}/\text{min}$ in the coronary arteries (4 $\mu\text{g}/\text{min}$ in each coronary artery) for 30 min. Steady-state recordings and the response to dobutamine

were assessed. The SNP infusion was then replaced with saline again (*saline 2*; used as a time control), and, after a 20-min recovery period, steady-state readings and the response to dobutamine were assessed. Hemodynamic measurements and blood samples were collected at the end of each drug infusion.

After completion of the acute experimental protocols, the animals were killed by pentobarbital sodium overdose (Sleep Away; 2 ml plus 1 ml per additional 5 kg over the initial 5 kg) consistent with the guidelines of the Panel on Euthanasia of the American Veterinary Medical Association.

Intracoronary drug infusions. BNP, SNP, and HS-142-1 were each dissolved in normal saline for their individual intracoronary infusions. L-NMMA was dissolved in HS-142-1 solution for the combined intracoronary infusion at the end of the second protocol. Normal saline alone was infused to maintain patency of the coronary catheters when a study drug was not used. All intracoronary infusions were set at a constant rate of 0.25 ml/min. The dosages of BNP, HS-142-1, L-NMMA, and SNP were adapted from previous studies (14, 36, 51) whereby sufficient intracardiac activity was demonstrated while avoiding confounding systemic vascular effects.

Plasma cGMP analysis. Blood samples were collected in EDTA tubes and immediately placed in 4°C for centrifugation at 2,500 rpm for 10 min. The plasma was stored at -20°C until analysis. Plasma cGMP was measured by a specific radioimmunoassay as described by Stein et al. (40).

Myocardial cGMP analysis. To determine if measurement of the plasma cGMP reflected myocardial concentrations, tissue sampling of the LV was performed in a separate group of dogs before and after each intracoronary drug infusion (saline, BNP, and SNP in normal dogs, $n = 5$). Transmural biopsies were obtained from the anterolateral free wall of the LV using a stainless steel drill bit (4-mm internal diameter) mounted on an electrical hand-piece unit (ROTEX 782) with variable rotations per minute. The biopsy specimen (~150–200 mg) was immediately ejected into liquid nitrogen and stored at -80°C until analysis. Complete sampling time averaged <5 s.

The frozen tissue was crushed and boiled in 1 M acetic acid and 20 mM hydrochloric acid for 5 min. The samples were homogenized, a 10- μ l aliquot was taken for protein analysis by the Lowry method (25), and the average of duplicate determinations was expressed as milligrams of protein. The remainder of the homogenate was centrifuged for 30 min at 15,000 rpm at 4°C. The supernatant was removed for cGMP radioimmunoassay by the method of Steiner et al. (40). Results were expressed as picomoles of cGMP per milligram of protein.

Coronary sinus sampling. Anesthetized dogs ($n = 3$) were instrumented with LCx and LAD needles and a catheter in the coronary sinus. Coronary sinus blood was sampled at baseline and after incremental 20-min infusions of 2, 4, and 8 μ g SNP/min. After a 1-h recovery period, the infusions were repeated. A total of five dose-response infusions were performed in three dogs.

Data acquisition and analysis. Data was acquired and recorded utilizing an electronic real-time biological data acquisition system, CA Recorder 1.1 (Data Integrated Scientific Systems). Signals were digitized with a maximum sampling frequency of 250 Hz. Steady-state cardiovascular data were analyzed by Spectrum 1.0 (Wake Forest University). Steady-state data include hemodynamic parameters obtained during drug infusions in the absence of intravenous dobutamine infusions. All steady-state data acquisitions and those ob-

tained during all dobutamine infusions were made at a constant atrial pacing rate. The effect on systolic function with β -adrenergic receptor stimulation was determined by quantifying the percent increase in maximum dP/dt with each dobutamine infusion. The time constant of LV pressure decay during isovolumic relaxation (τ) was quantified by two methods. The data obtained from a high-fidelity manometer of LV pressure during the period from peak $-dP/dt$ to 5 mmHg above LV end-diastolic pressure (LVEDP) was used for measurements of τ assuming a zero pressure asymptote (47). The same period was used to compute a linear regression of dP/dt against LV pressure during isovolumic relaxation, and τ was defined as the negative inverse of the slope ($-1/T$). LV end-systolic pressure (LVESP) was defined as the pressure at the peak rate of fall of pressure (peak $-dP/dt$). LVEDP was defined as the pressure at the time of the initial upward deflection on the dP/dt trace and verified by simultaneous examination of the LV pressure trace as the point after the a wave (atrial contraction) and just before the rise in LV pressure.

Statistical Analysis

Data are averaged and reported as means \pm SE. Student's *t*-test was used for comparison of baseline hemodynamic parameters in normal and CHF dogs. The serial changes in measured variables after drug infusions within each study protocol were tested with a repeated-measures ANOVA followed by Bonferroni and Student-Newman-Keuls post hoc tests for multiple comparisons. Results were considered statistically significant in all analyses at $P < 0.05$.

RESULTS

Baseline Hemodynamics in CHF and Normal Dogs

Baseline hemodynamic parameters and systolic and diastolic function are shown in the presence and absence of CHF in Table 1. In the presence of CHF, the systolic function parameters of LVESP and $+dP/dt$ were significantly decreased, and LV filling pressures (as evident by LVEDP) were significantly elevated compared with the normal dogs. In addition, the CHF group demonstrated abnormal LV relaxation, having a significantly higher τ and a decreased $-dP/dt$.

Table 1. Baseline hemodynamics for normal and CHF dogs before intracoronary drug infusions

	Normal Dogs	CHF Dogs
LVP _{max} , mmHg	113 \pm 3	99 \pm 3*
LVEDP, mmHg	8 \pm 1	20 \pm 2†
LVESP, mmHg	101 \pm 4	73 \pm 4*
Peak $+dP/dt$, mmHg/s	2264 \pm 203	834 \pm 38‡
Peak $-dP/dt$, mmHg/s	-1825 \pm 113	-927 \pm 33‡
$\tau_{dP/dt}$, ms	25 \pm 3	49 \pm 4‡
τ Weiss, ms	28 \pm 2	44 \pm 1‡

Data are means \pm SE; $n = 5$ normal and 7 congestive heart failure (CHF) dogs. LVP_{max}, maximum left ventricular (LV) systolic pressure; LVEDP and LVESP, LV end-diastolic and end-systolic pressure, respectively; dP/dt , first derivative of LV pressure rise; $\tau_{dP/dt}$, time constant of LV pressure decay during isovolumic relaxation; τ Weiss, time constant of LV pressure decay during isovolumic relaxation assuming a zero pressure asymptote. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

Table 2. Preload and afterload effects during intracoronary infusion of saline, HS-142-1, and L-NMMA in CHF dogs and intracoronary infusion of saline, BNP, and SNP in normal dogs

	Normal Dogs				CHF Dogs			
	Saline	BNP	Saline 1	SNP	Saline	HS-142-1	HS-142-1 Recovery	L-NMMA + HS-142-1
LVESP, mmHg	101 ± 4	97 ± 3	98 ± 2	90 ± 2*	76 ± 4	76 ± 5	76 ± 5	75 ± 6
LVEDP, mmHg	8 ± 1	8 ± 1	7 ± 1	8 ± 1	19 ± 3	19 ± 2	19 ± 2	20 ± 2

Data are means ± SE; $n = 5$ normal dogs and 6 CHF dogs. *Saline*, baseline infusion of saline; *saline 1*, infusion of saline before SNP infusion. BNP, brain natriuretic peptide; SNP, sodium nitroprusside; L-NMMA, N^G -monomethyl-L-arginine. * $P < 0.05$ vs. baseline saline (*saline*) but not vs. *saline 1*.

Effect of NP and NO on Systolic Function in the Absence of Dobutamine

In the presence of CHF, antagonism of intracardiac BNP and NO by sequential intracoronary infusion of HS-142-1 and HS-142-1 + L-NMMA had no effect on afterload or preload, because LVESP and LVEDP did not change with either infusion (Table 2). There was also no effect on systolic function, because $+dP/dt$ did not change (Fig. 1A). In the normal dogs, intracoronary BNP infusion did not alter preload, afterload, or systolic function, because there were no significant changes in LVEDP, LVESP (Table 2), or $+dP/dt$ (Fig. 1B), respectively. Intracoronary SNP in the normal dogs did not alter LVEDP and $+dP/dt$; however, a decrease in LVESP was observed during the SNP infusion compared with the baseline saline (*saline*) infusion, but the decrease during SNP compared with the pre-SNP saline control (*saline 1*) was not significant (Table 2).

Effect of NP and NO on the Inotropic Response to β -Adrenergic Stimulation with Intravenous Dobutamine

In the presence of CHF, antagonism of intracardiac NP by intracoronary HS-142-1 did not have an effect on the percent change in $+dP/dt$ in response to β -adrenergic stimulation with intravenous dobutamine. However, the addition of intracardiac NO antagonism by intracoronary L-NMMA to the HS-142-1 infusion did enhance the inotropic response to intravenous dobut-

amine (Fig. 2A). In the normal dogs, intracoronary SNP infusion, but not intracoronary BNP infusion, blunted the inotropic response to intravenous dobutamine (Fig. 2B).

Second Messenger (cGMP) Response to NP and NO Antagonism and Exogenous Administration

The plasma concentration of cGMP was measured during intracoronary drug infusions before intravenous dobutamine administration. In the presence of CHF, plasma concentrations of cGMP decreased in response to intracardiac NP antagonism by intracoronary HS-142-1 infusion (Fig. 3A). Plasma cGMP levels tended to decrease further when L-NMMA was added to the HS-142-1 infusion. In the normal dogs, plasma concentration of cGMP increased in response to intracoronary BNP infusion. In contrast, plasma cGMP concentrations (Fig. 3B) were not different from baseline during intracoronary SNP infusion. Local production of cGMP in response to NP and NO administration in the normal dog paralleled the response in the circulation. Myocardial tissue levels of cGMP increased in the presence of intracardiac NP but were not significantly changed from baseline during intracoronary SNP administration (Fig. 4). Furthermore, in a separate experiment, coronary sinus cGMP levels were not changed in response to intracoronary infusion of SNP. The cGMP concentration at baseline and after infusion of 2, 4, and 8 $\mu\text{g}/\text{min}$ of SNP were 3.0 ± 0.7 , 2.9 ± 0.7 ,

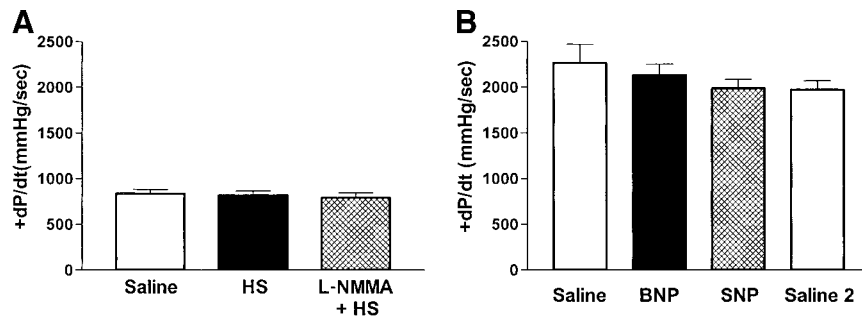


Fig. 1. Studies done in the absence of dobutamine. A: in congestive heart failure (CHF) dogs ($n = 7$), systolic function [rise in the first derivative of left ventricular (LV) pressure ($+dP/dt$)] was similar during intracoronary infusion of saline, the natriuretic peptide receptor antagonist HS-142-1 (HS), and the nitric oxide (NO) synthase (NOS) inhibitor N^G -monomethyl-L-arginine (L-NMMA) combined with HS-142-1 (L-NMMA + HS). B: in normal dogs ($n = 5$), systolic function ($+dP/dt$) was similar during intracoronary infusion of saline, exogenous brain natriuretic peptide (BNP), the exogenous NO donor sodium nitroprusside (SNP), and saline again as a time control (*saline 2*). Differences were not statistically significant. Data are shown as means ± SE.

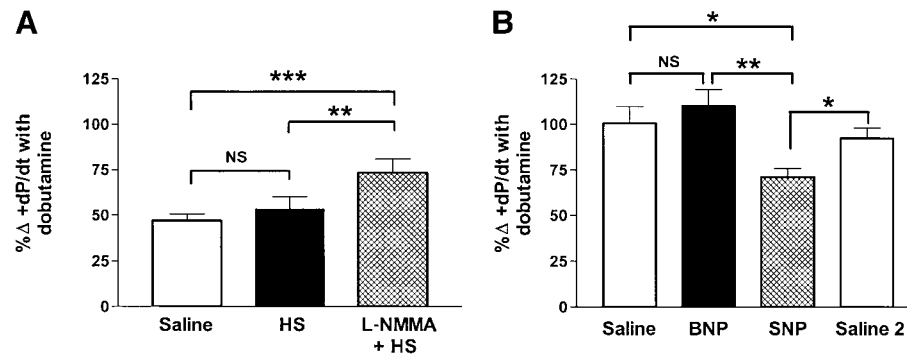


Fig. 2. Effect of intracoronary infusions on the inotropic response [percent change in $+dP/dt$ ($\% \Delta +dP/dt$)] to intravenous dobutamine in CHF ($n = 7$; A) and normal ($n = 5$; B) dogs. In CHF, the $\% \Delta +dP/dt$ with dobutamine during intracoronary infusion of HS-142-1 was similar to that observed during intracoronary infusion of saline. The $\% \Delta +dP/dt$ with dobutamine during intracoronary infusion of HS-142-1 + L-NMMA was greater than that observed during intracoronary infusion of saline or HS-142-1 alone. In the normal dogs, the $\% \Delta +dP/dt$ with dobutamine during intracoronary infusion of BNP was similar to that observed during intracoronary infusion of saline. The $\% \Delta +dP/dt$ with dobutamine during intracoronary infusion of SNP was lower than that observed during intracoronary infusion of saline, BNP, or the time control (saline 2). Data are shown as means \pm SE. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NS, not significant. Abbreviations are as in the Fig. 1 legend.

3.0 ± 0.7 , and 3.2 ± 0.8 pmol/ml [$P =$ not significant (NS)].

Effect of NP and NO on Diastolic Function in the Absence and Presence of Dobutamine

In the CHF model, intracoronary infusion of HS-142-1 was associated with an increase in τ (Fig. 5A) in the absence of dobutamine. The addition of L-NMMA to the HS-142-1 infusion further increased τ (Fig. 5A). Responses were similar whether τ was calculated assuming a zero asymptote or a nonzero asymptote. In the presence of CHF, the lusitropic response (percent decrease in τ) during intravenous dobutamine, was similar during intracoronary infusion of saline, HS-142-1 alone, and the combination of L-NMMA + HS-142-1 (data not shown). In the normal dogs, neither intracoronary BNP nor SNP significantly altered τ in the absence of dobutamine (Fig. 5B), and neither infusion altered the percent decrease in τ in the presence of dobutamine (data not shown).

DISCUSSION

In severe CHF, we found that antagonism of endogenous intracardiac NP or NO did not alter basal systolic function. Similarly, intracoronary administration

of exogenous NP and NO to normal dogs did not alter basal systolic function. Antagonism of cardiac NO in severe CHF enhanced the inotropic response to β -adrenergic stimulation, but NP antagonism had no effect on β -adrenergic responsiveness. Likewise, in the normal dogs, exogenous NO, but not exogenous NP, blunted the inotropic response to β -adrenergic stimulation. Antagonism of endogenous intracardiac NP and NO in severe CHF was associated with progressive impairment in diastolic function, as indicated by increases in τ . These findings suggest that endogenous NP and NO enhance diastolic performance in severe CHF, potentially via their common second messenger, cGMP. In contrast, because only NO modulates β -adrenergic responsiveness, this effect may be mediated by cGMP-independent mechanisms or cGMP-dependent subcellular interactions that are unique to NO-stimulated cGMP.

Activation of NP and NO in CHF

Previous studies (4, 26, 42, 46, 50) in human and animal models of CHF have demonstrated that NP are activated in CHF. Whether or not NO production is enhanced in the presence of CHF remains controversial. A number of studies (32, 33, 42, 48) have sug-

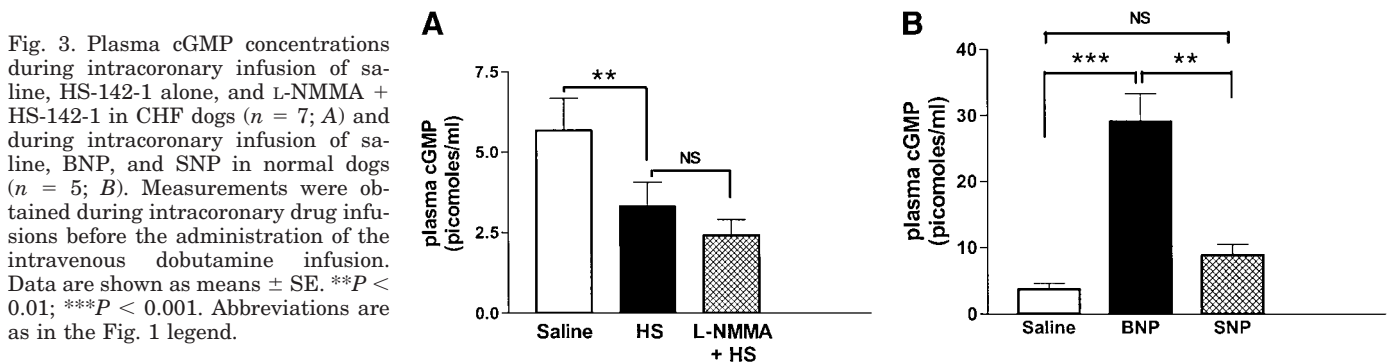


Fig. 3. Plasma cGMP concentrations during intracoronary infusion of saline, HS-142-1 alone, and L-NMMA + HS-142-1 in CHF dogs ($n = 7$; A) and during intracoronary infusion of saline, BNP, and SNP in normal dogs ($n = 5$; B). Measurements were obtained during intracoronary drug infusions before the administration of the intravenous dobutamine infusion. Data are shown as means \pm SE. ** $P < 0.01$; *** $P < 0.001$. Abbreviations are as in the Fig. 1 legend.

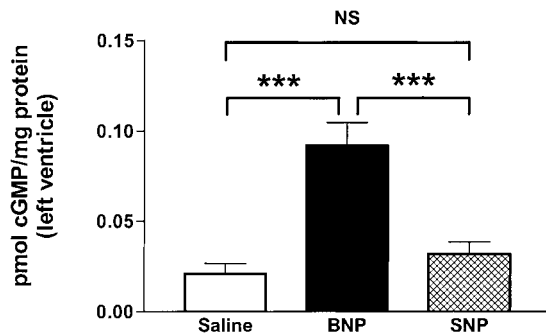


Fig. 4. Myocardial cGMP levels during intracoronary infusion of saline, BNP, and SNP in normal dogs ($n = 5$). Data are shown as means \pm SE. *** $P < 0.001$.

gested that vascular endothelial NO production may be enhanced in early CHF but blunted in severe CHF. However, myocardial production of NO may be divergently regulated because cytokine activation occurs in severe CHF and has been postulated to cause the increase in inducible NOS demonstrated in the failing myocardium (2, 6, 8, 11, 15, 18, 23, 44, 45). Our findings suggest that cardiac NOS activity is important in CHF because antagonism of NOS does result in effects on ventricular relaxation and β -adrenergic responsiveness. Because the effects of NO antagonism on β -adrenergic responsiveness are demonstrated to be absent in the normal myocardium (12, 13), we postulate that NO may be activated in CHF. However, this effect could be related to other conditions unique to CHF, and it remains unclear if myocardial NO production is increased in CHF.

Effect of NP and NO on the Inotropic Response to Dobutamine

NO donors blunt β -adrenergic responsiveness without an effect on basal systolic function in normal isolated myocytes (1, 3). Furthermore, in failing myocytes, NOS inhibition significantly augmented the inotropic response to β -adrenergic stimulation but did not alter inotropic responsiveness in normal myocytes (52). Studies (14) of humans with LV dysfunction have also demonstrated that antagonism of intracardiac NOS activity with intracoronary L-NMMA enhanced the inotropic response to intravenous dobutamine. The effects of the NP system on β -adrenergic responsiveness have not been previously investigated. Because the effects of

NO on β -adrenergic responsiveness were postulated to be mediated by cGMP and because NP are known potent stimulators of cGMP, we postulated that endogenous or exogenous NP should also modulate β -adrenergic responsiveness. In the current study, neither endogenous nor exogenous NP altered β -adrenergic responsiveness to dobutamine while the previously reported effects of endogenous and exogenous NO on β -adrenergic responsiveness were confirmed.

The mechanisms for this divergent effect on stimulated systolic function are unclear. It may be that NO is a more powerful stimulator of intracellular cGMP; however, this is not suggested by the plasma, myocardial, and coronary sinus levels of cGMP reported in the current study. Furthermore, previous *in vitro* studies (43) have demonstrated that NP are much more potent stimulators of cGMP than NO donors in isolated glomeruli. An alternative explanation is that cGMP may be compartmentalized, as reported by a previous *in vitro* study by Stasch et al. (41), where discordant effects of ANP and SNP on cGMP concentrations in aortic tissue were observed. Whereas ANP caused cGMP production in the aortic tissue and its surrounding bath solution, SNP increased cGMP in the tissue but not in the solution. The authors concluded that cGMP produced by the activation of soluble guanylyl cyclase may not be extruded from the cell. We therefore measured both plasma and myocardial cGMP concentrations to determine if compartmentalization of cGMP produced by SNP would be seen, as in the Stasch et al. *in vitro* study (41). In the current study, no compartmentalization was suggested, because SNP did not produce detectable increases in cGMP concentrations in the plasma, myocardium, and coronary sinus. The deficient cGMP response to SNP is in contrast to the *in vitro* findings of Paolucci et al. (35), where SNP in the isolated rat heart potently stimulated effluent cGMP formation. In the current study, the reason for the absence of cGMP production in response to SNP *in vivo* is unclear, and further studies performed over a more extensive concentration range of SNP may be needed. Indeed, cGMP concentrations may not be linearly related to its cellular effects. Both cGMP-stimulated and -inhibited phosphodiesterases exist that may modulate β -adrenergic responsiveness differently at different concentrations of cGMP. Furthermore, the potential for interaction with postreceptor components of the

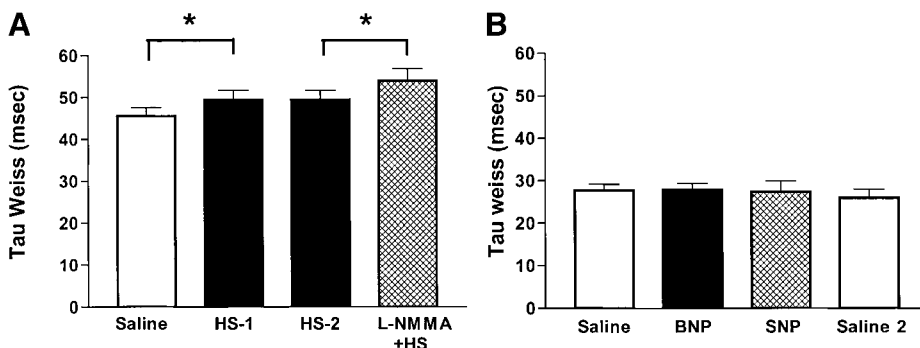


Fig. 5. Effects of intracoronary infusions in the absence of dobutamine on the time constant of LV pressure decay during isovolumic relaxation (τ). A: in CHF dogs ($n = 7$), intracoronary natriuretic peptide receptor HS-142-1 (HS-1) increased τ from that recorded with intracoronary saline infusion. Addition of L-NMMA further increased τ from that with HS-142-1 alone (HS-2). B: in normal dogs ($n = 5$), neither intracoronary BNP nor SNP altered τ . Data are shown as means \pm SE. * $P < 0.05$. τ Weiss, measurements of τ assuming a zero pressure asymptote.

adrenergic signaling system may be subject to subcellular compartmentalization of cGMP and not directly related to concentration.

Alternatively, while not addressed in these experiments, recent studies suggest that NO might alter myocardial cell function via modification of regulatory proteins other than soluble guanylyl cyclase. Indeed, there is preliminary *in vitro* evidence that NO may alter enzymes involved in oxidative phosphorylation or energy transport by creatinine kinase (9, 49). In another *in vitro* study (44), cytokine-induced NO formation caused a reduction in cellular ATP and myocyte contractility, observed to be a result of direct inhibition by NO of mitochondrial enzyme activity rather than by an indirect effect mediated through cGMP. These metabolic changes were blocked by the NOS inhibitor L-NMMA, but a cGMP analog demonstrated no effect on energy depletion. However, further studies are needed to define the importance of the cGMP-mediated and cGMP-independent effects of NO on myocardial function *in vivo*. The effects of these NO-mediated changes may only become apparent in states of increased myocardial cell demand such as that provided by β -adrenergic stimulation.

While the exact mechanism of NO in the regulation of myocardial function and its interaction with other regulatory pathways remain to be elucidated, the current data confirm previous studies that have documented the importance of the NO- β -adrenergic interaction. Indeed, Kanai and colleagues (17) recently reported that β -adrenergic agonists stimulate NO release from ventricular cardiomyocytes, supporting a favorable role of NO in conserving cardiomyocyte energy during increased myocardial demand.

Effect of NP and NO on Diastolic Function

In contrast to its effects on systolic function, *in vitro* studies (37) have demonstrated that cGMP has a monophasic dose-related effect to improve diastolic function. We (51) have previously shown *in vivo* that both endogenous NP in CHF and exogenous administration of NP to normal dogs results in enhanced LV relaxation, as evidenced by decreases in the time constant of isovolumic relaxation and by earlier onset of relaxation. Likewise, systemic infusion of exogenous NP in conscious chronically instrumented normal and CHF dogs produces improvements in relaxation (21, 31). The effects of NO on diastolic function *in vivo* have not been extensively investigated. In humans without CHF, exogenous intracoronary infusion of NO has been reported to improve lusitropic function (36); however, the effect of endogenous NO on lusitropic function *in vivo* in CHF has not been reported. In the current study, antagonism of endogenous NP and NO in dogs with severe CHF incrementally impaired diastolic function, as evidenced by the progressive increase in τ . Previous studies (10) in the mouse have demonstrated that L-NMMA (but not α -agonists) impairs relaxation in wild-type but not endothelial NOS-deficient mice,

suggesting that NO does facilitate relaxation independent of effects on vascular tone.

Neither NP nor NO altered τ in normal dogs. The absence of an effect in normal dogs may be related to the high pacing rate used in the normal dogs. Because β -adrenergic stimulation causes marked increases in heart rate in normal dogs, we paced the normal dogs at the maximal heart rate achieved with dobutamine throughout the entire study to avoid dramatic fluctuations in heart rate throughout the study. Because rapid atrial pacing has been shown to augment LV lusitropism in the normal organism (27), this effect may have masked our previously demonstrated effects of exogenous NP on diastolic function in normal dogs (51). We were able to use much more physiological heart rates throughout the entire study in the CHF group, where the chronotropic response to dobutamine was blunted.

Thus we believe that these previous studies as well as the current findings strongly suggest that endogenous NP and NO act to preserve diastolic function in the presence of CHF. We postulate that the observed effects of NO and the NP on diastolic function are related to their common second messenger, cGMP. However, we acknowledge that the effects are not linearly related to plasma cGMP levels as measured.

Study Limitations

This study was performed in the open-chest anesthetized dog, and these conditions may affect our findings.

Coronary blood flow was not measured in this study due to technical limitations. In a previous study (51), administration of the NP receptor antagonist in CHF dogs decreased coronary blood flow; however, this was not a dose-dependent effect. The effect of BNP on τ was dose dependent. Therefore, decreases in coronary blood flow are unlikely to account for the impairment in LV relaxation observed. In addition, if the observed increase in τ in the CHF dogs was due to ischemia from a reduction in coronary blood flow, an enhanced inotropic response to β -adrenergic stimulation in the presence of the NOS inhibitor would not have been likely.

In our study, the observed divergent effect of NP and NO on the production of their shared second messenger and the inotropic response to dobutamine suggested a non-cGMP mechanism responsible for the role of NO in the modulation of β -adrenergic responsiveness. Simultaneous comparison of NP and NO, activators of particulate and soluble guanylyl cyclase, respectively, provides some information regarding the differential mechanisms involved. However, further studies into the non-cGMP mechanisms of NO are needed.

Because of the need for multiple dobutamine challenges, it was not technically feasible to perform dose-response studies with our protocol. Therefore, we utilized doses of agonists and antagonists previously reported to be active but cannot provide dose-response curves for the observed effects.

In summary, this *in vivo* study suggests that endogenous NP and NO modulate LV function in severe

congestive CHF, where they act to preserve diastolic function, potentially through their common second messenger, cGMP. In contrast, these data suggest that NO has a unique effect on β -adrenergic responsiveness in CHF, which was not observed with NP. Consequently, these data importantly suggest a variance in the signaling mechanisms concerning the regulation of myocardial systolic and diastolic function. Finally, as NP did not impair basal or stimulated systolic function, these data have implications concerning clinical use of BNP infusion or augmentation of endogenous NP with vaso-peptidase inhibition. Our findings suggest that these emerging heart failure therapies will not induce further decompensation in the already failing myocardium but may improve LV diastolic function.

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