

Actions of a novel synthetic natriuretic peptide on hemodynamics and ventricular function in the dog

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Lainchbury, John G., Ondrej Lisy, John C. Burnett, Jr., Donna M. Meyer, and Margaret M. Redfield. Actions of a novel synthetic natriuretic peptide on hemodynamics and ventricular function in the dog. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R993–R998, 2002; 10.1152/ajpregu.00388.2001.—Dendroaspis natriuretic peptide (DNP) is a recently discovered peptide with structural similarity to known natriuretic peptides. DNP has been shown to possess potent renal actions. Our objectives were to define the acute hemodynamic actions of DNP in normal anesthetized dogs and the acute effects of DNP on left ventricular (LV) function in conscious chronically instrumented dogs. In anesthetized dogs, DNP, but not placebo, decreased mean arterial pressure (141 ± 6 to 109 ± 7 mmHg, $P < 0.05$) and pulmonary capillary wedge pressure (5.8 ± 0.3 to 3.4 ± 0.2 mmHg, $P < 0.05$). Cardiac output decreased and systemic vascular resistance increased with DNP and placebo. DNP-like immunoreactivity and guanosine 3',5'-cyclic monophosphate concentration increased without changes in other natriuretic peptides. In conscious dogs, DNP decreased LV end-systolic pressure (120 ± 7 to 102 ± 6 mmHg, $P < 0.05$) and volume (32 ± 6 to 28 ± 6 ml, $P < 0.05$) and LV end-diastolic volume (38 ± 5 to 31 ± 4 ml, $P < 0.05$) but not arterial elastance. LV end-systolic elastance increased (6.1 ± 0.7 to 7.4 ± 0.6 mmHg/ml, $P < 0.05$), and Tau decreased (31 ± 2 to 27 ± 1 ms, $P < 0.05$). The effects on hemodynamics, LV function, and second messenger generation suggest synthetic DNP may have a role as a cardiac unloading and lusitropic peptide.

natriuretic peptides; systolic function; diastolic function

DENDROASPIS NATRIURETIC PEPTIDE (DNP) is a recently discovered 38-amino acid peptide, isolated from the *Dendroaspis augusticeps* snake, with structural similarity to atrial, brain, and C-type natriuretic peptide (ANP, BNP, and CNP) (4, 18). DNP-like immunoreactivity has been reported in human atria and plasma and is increased in the plasma of patients with congestive heart failure (17). Recently, DNP-like immunoreactivity has been reported in canine plasma and myocardium, and synthetic DNP has been shown to be markedly natriuretic in dogs (10). Indeed, the potent renal actions of synthetic DNP suggest its potential use

in the treatment of cardiovascular disease states such as congestive heart failure.

The natriuretic peptides have natriuretic and vasodilating properties that are mediated by the second messenger guanosine 3',5'-cyclic monophosphate (cGMP). Studies with synthetic DNP to date indicate that it, too, produces natriuresis, causes relaxation in rodent aorta and rodent and canine coronary arteries, and augments formation of cGMP from aortic endothelial cells (4, 18). Most recently, we demonstrated that the natriuretic peptides possess direct inotropic and lusitropic myocardial actions in the dog (9, 24). However, the effects of synthetic DNP on systemic hemodynamics and left ventricular (LV) function in vivo are poorly defined.

The aim of the current study was to observe the acute in vivo effects of synthetic DNP on systemic hemodynamics and LV function in normal dogs. We hypothesized that exogenously infused synthetic DNP would result in reductions in preload and afterload, together with improvements in ventricular systolic and diastolic function in normal dogs.

METHODS

Experiments were performed in male mongrel dogs. Dogs weighed between 18 and 24 kg and were fed standard dog chow (Lab Canine Diet 5006, Purina Mills, St. Louis, MO) with free access to drinking water. The study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic and conducted in accordance with the Animal Welfare Act.

Thirteen normal anesthetized dogs were studied to assess the effects of acute DNP administration on systemic and pulmonary hemodynamics. On the night before the acute protocol, the animals were fasted and allowed access to water ad libitum. On the day of the acute experiment, dogs were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and mechanically ventilated with supplemental oxygen (Harvard respirator, Amersham, MA) at 16 cycles/min. A flow-directed balloon-tipped thermodilution catheter (Ohmeda, Criticath, Madison, WI) was advanced to the pulmonary artery via the external jugular vein for cardiac hemodynamic measurement. The femoral artery was cannulated for blood pressure monitoring and blood sampling. The femoral vein was also cannulated for infusion of active drugs

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or vehicle infusion. Supplemental doses of pentobarbital sodium (12.5 to 25 mg) were given as needed during the experiment.

A 60-min equilibration period followed the instrumentation of the dogs. At the completion of the equilibration period, baseline hemodynamic recordings were made and plasma was collected for hormonal determination. After the baseline recordings, synthetic DNP (DNP 1–38, Phoenix Pharm, Mountain View, CA) was administered as an intravenous infusion at $10 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to six dogs, and hemodynamics were repeated after 30 min. Then the DNP infusion rate was increased to $50 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with hemodynamics repeated after a further 30 min. We previously reported renal response to the DNP infusion and its effect on mean arterial pressure (MAP) (10) but did not report the hemodynamic actions of the infusion and did not compare the effects of the infusion to that of an infusion of vehicle. Thus an additional seven dogs served as time-matched controls and received vehicle (saline) only.

Cardiovascular parameters measured included MAP, right atrial pressure (RAP), mean pulmonary artery pressure (PAP), cardiac output (CO), and pulmonary capillary wedge pressure (PCWP). CO was determined by thermodilution in triplicate and averaged (Cardiac Output model 9510-A computer, American Edwards Laboratories, Irvine, CA). MAP was assessed via direct measurement from the femoral arterial catheter. Systemic vascular resistance (SVR) was calculated as $(\text{MAP} - \text{RAP})/\text{CO}$.

After each hemodynamic determination, arterial blood was collected in heparin and EDTA tubes and immediately placed on ice. After centrifugation at 2,500 rpm at 4°C , plasma was decanted and stored at -20°C until analysis. After plasma extraction, DNP-like immunoreactivity, ANP, BNP, and CNP were measured by radioimmunoassay as previously described (1, 2, 4, 22). The assay for DNP uses a rabbit anti-DNP antibody and has no cross-reactivity with ANP, BNP, or CNP. Recovery for the DNP assay is $83 \pm 1\%$, and intra- and interassay coefficients of variation were 10 ± 2 and $12 \pm 2\%$, respectively. Plasma for cGMP was measured by RIA using the method of Steiner et al. (21).

To determine the effects of DNP on LV function, we studied six chronically instrumented conscious normal dogs. These dogs were anesthetized with pentobarbital sodium (20 mg/kg) and isoflurane (0.5–2.5%) and ventilated with supplemental oxygen. A left lateral thoracotomy was performed, and the pericardium was widely opened. A solid-state micromanometer pressure transducer (Konigsberg Instruments, Pasadena, CA) and a silicon fluid-filled catheter for transducer calibration were inserted through the LV apex.

Piezoelectric ultrasound dimension crystals (Sonometrics, London, Ontario, Canada) were implanted on opposing anterior and posterior endocardial surfaces of the left ventricle to measure the internal short-axis dimension and at the basal epicardial and apical endocardial surfaces to measure the LV long-axis dimension. Hydraulic occluders were placed on the proximal superior and inferior vena cavae (In Vivo Metrics, Heladsburg, CA). A pacing wire was sutured to the left atrial free wall to control heart rate during the acute experiments. All wires, leads, and catheters were exteriorized to the dorsal neck. Animals received prophylactic antibiotics postoperatively for 2 wk. The LV catheter was flushed weekly with heparinized saline to maintain patency.

Studies were performed after full recovery from the thoracotomy (10–14 days) with the animals awake and standing quietly in a sling. The LV fluid-filled catheter was connected to a pressure transducer calibrated with a mercury manometer, and the signal from the micromanometer was adjusted

to match that of the fluid-filled catheter. LV dimensions were measured using the implanted ultrasonic crystals (3 MHz) and a sonomicrometer. The analog signals of pressure and dimension were processed with an on-line analog-to-digital converter at 250 Hz and recorded continuously on a computerized data-collection and -analysis system, which allowed on-line display of all parameters (CA Recorder version 1.1, Data Integrated Scientific Systems, Pinckney, MI).

Dogs were given propranolol (2 mg/kg iv) and paced via the atrial pacemaker lead at ~ 20 beats/min above their intrinsic heart rate to block effects of sympathetic activation and to control heart rate throughout the experimental protocol. Fifteen minutes after the administration of propranolol and commencement of atrial pacing, baseline recordings were made. Three steady-state recordings, each of 20-s duration to account for respiratory variation, were made over 5 min. After the steady-state recordings were completed, at least three sets of variably loaded pressure-volume loops were generated by transient occlusion of the cavae. Hemodynamic variables were allowed to return to baseline between each caval occlusion. After collection of the baseline data, DNP was infused intravenously for 30 min at $100 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. At the end of the 30-min infusion, steady-state and variably loaded pressure-volume loop recordings were repeated as described above. Venous blood samples were collected for measurement of plasma DNP concentrations and cGMP at baseline and at the end of infusion.

Data were analyzed using the SPECTRUM software program (Wake Forest University School of Medicine). Steady-state recordings were averaged over the 20-s recording period to account for respiratory variation. LV volume was calculated as a modified ellipsoid model using the equation $V_{\text{LV}} = (\pi/6)SA^2LA$, where V_{LV} is volume of LV, SA is short-axis LV dimension, and LA is long-axis LV dimension. This method of volume calculation gives consistent measures of LV volume despite changes in loading conditions and inotropic state (3). Calculated rate of increase of LV pressure over time (dP/dt) was derived from LV pressure by the five-point Lagrangian fit (11). The rate of LV relaxation was analyzed by determining the time constant of the isovolumic fall of LV pressure (τ) from the peak $-dP/dt$ to 5 mmHg above LV end-diastolic pressure (EDP). The method of Raff and Glantz (15) was used to calculate τ . This method calculates as the negative inverse of the slope of dP/dt vs. pressure. Only caval occlusions that produced a fall in end-systolic pressure (ESP) of at least 30 mmHg were analyzed. Premature beats and two subsequent beats were excluded from the analysis. The LV ESP and volume data during the fall in LV pressure caused by each caval occlusion were fit using the least-squares technique to the equation $\text{ESP} = E_{\text{es}}(V_{\text{es}} - V_0)$, where E_{es} is slope of the linear ESP volume relationship, representing the LV end-systolic elastance; V_{es} is volume at end systole; and V_0 is intercept with the volume axis. The E_{es} is sensitive to changes in the contractile state but relatively insensitive to changes in loading conditions. Arterial elastance (E_a), a relatively preload-insensitive measure of afterload, was calculated as ESP divided by stroke volume (23).

Results are expressed as means \pm SE. Data were assessed by one-way ANOVA with Student-Newman-Keuls post hoc test for within-group comparisons and with two-way ANOVA for repeated measures with Student-Newman-Keuls post hoc test for comparison between groups. Statistical significance was accepted as $P < 0.05$.

Table 1. Hemodynamic response to synthetic DNP ($n = 6$) or placebo ($n = 7$) in normal anesthetized dogs

	DNP			Placebo		
	Baseline	DNP 10	DNP 50	Baseline	Placebo 30	Placebo 60
MAP, mmHg	141 ± 6	128 ± 8*	109 ± 7*†	146 ± 6	141 ± 6	141 ± 6
PAP, mmHg	16.4 ± 1.0	14.9 ± 0.9*	12.4 ± 0.4*†	17.4 ± 1.6	16.1 ± 0.9	15.9 ± 1.1
PCWP, mmHg	5.8 ± 0.3	4.3 ± 0.3*	3.5 ± 0.2*†	4.6 ± 0.6	4.7 ± 0.8	3.7 ± 0.3
RAP, mmHg	2.3 ± 0.5	1.5 ± 0.3	0.8 ± 0.5*	2.9 ± 0.4	2.7 ± 0.3	2.6 ± 0.2
HR, beats/min	113 ± 12	118 ± 5	119 ± 8	122 ± 13	121 ± 9	121 ± 9
CO, l/min	4.0 ± 0.2	3.3 ± 0.3*	2.4 ± 0.1*†	5.5 ± 0.4	5.2 ± 0.4*	4.4 ± 0.4*†
SVR, RU	36 ± 6	39 ± 8	46 ± 11*	27 ± 4	29 ± 6	33 ± 7*†

Values are means ± SE. Baseline, baseline recordings; DNP 10, recordings after infusion of dendoaspis natriuretic peptide 10 ng·kg⁻¹·min⁻¹ for 30 min; DNP 50, recordings after a further 30 min of exogenous dendoaspis natriuretic peptide at 50 ng·kg⁻¹·min⁻¹; Placebo 30 and Placebo 60, time-matched placebo recordings; MAP, mean arterial pressure; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; HR, heart rate; CO, cardiac output; SVR, systemic vascular resistance; RU, resistance units. * $P < 0.05$ vs. baseline, † $P < 0.05$ vs. DNP 10 or placebo 30, respectively.

RESULTS

The effects of synthetic DNP or placebo infusion on systemic and pulmonary hemodynamics in anesthetized normal dogs are shown in Table 1. Compared with baseline, synthetic DNP infusion resulted in dose-related decreases in MAP, PAP, and PCWP. These parameters were unchanged with placebo infusion. The higher dose of synthetic DNP reduced RAP, whereas no change in RAP was seen with placebo infusion. CO decreased and SVR increased significantly with DNP and placebo infusions. Heart rate was unchanged in both DNP and placebo groups.

The changes from baseline with each dose of synthetic DNP vs. the corresponding change with placebo control for the hemodynamic parameters MAP, RAP, PAP, and PCWP are shown in Fig. 1. The change in MAP, PAP, and RAP with the higher dose of synthetic DNP was significantly greater than with the time-matched placebo infusion, and the decrease in PCWP was significantly greater than with placebo at both

doses of DNP. A larger percentage fall in CO was seen with DNP infusion than with placebo, but the difference did not reach statistical significance. Changes in SVR with synthetic DNP were not significantly different than those observed with the time-matched placebo infusion. Plasma DNP and cGMP concentrations increased with synthetic DNP infusion, whereas plasma concentration of ANP, BNP, and CNP was unchanged (Table 2).

The effects of DNP infusion in the six conscious dogs instrumented for assessment of LV function are shown in Table 3. DNP infusion resulted in significant decreases in the measures of LV afterload, LV ESP, and LV end-systolic volume (ESV). There was no significant change in Ea. There were decreases in preload as evidenced by a significant decrease in LV end-diastolic volume (EDV) and a trend toward a decrease in LV EDP. Stroke volume fell slightly but significantly, whereas heart rate (controlled by atrial pacing) was stable. Contractility was modestly but significantly en-

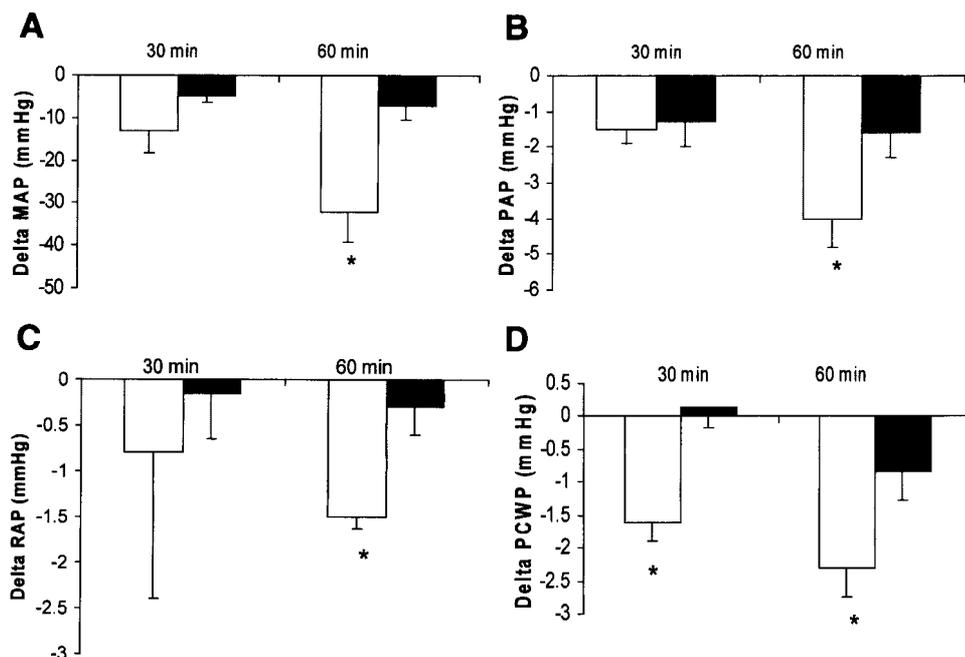


Fig. 1. Change from baseline (Delta) in mean arterial pressure (MAP; A), pulmonary artery pressure (PAP; B), right atrial pressure (RAP; C), and pulmonary capillary wedge pressure (PCWP; D) in normal anesthetized dogs in response to infusion of synthetic dendoaspis natriuretic peptide (DNP) or the corresponding placebo time controls. 30 Min: change from baseline after 30-min infusion of DNP at 10 ng·kg⁻¹·min⁻¹ or placebo; 60 min: change from baseline after a further 30 min of DNP at 50 ng·kg⁻¹·min⁻¹ or placebo. DNP group ($n = 6$): open bars; placebo group ($n = 7$): black bars. * $P < 0.05$ vs. placebo.

Table 2. Plasma natriuretic peptide and cGMP response to infusion of DNP in 6 normal anesthetized dogs

	Baseline	DNP 10	DNP 50
DNP, pg/ml	5.9 ± 2.4	269 ± 74	3,240 ± 1,658*†
ANP, pg/ml	17.4 ± 0.7	17.6 ± 0.4	18.1 ± 1.2
BNP, pg/ml	10.1 ± 1.4	12.9 ± 2.6	12.6 ± 2.0
CNP, pg/ml	9.7 ± 0.5	9.1 ± 0.5	9.5 ± 0.9
cGMP, pmol/ml	10.6 ± 1.3	38 ± 7*	74 ± 5*†

Values are means ± SE. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; cGMP, cyclic guanosine monophosphate. * $P < 0.05$ vs. baseline, † $P < 0.05$ vs. DNP 30.

hanced as evidenced by an increase in Ees (Fig. 2). The time constant of isovolumic relaxation (τ) decreased significantly, suggesting improvement in LV relaxation. Plasma DNP-like immunoreactivity (14.5 ± 3.0 vs. $1,347 \pm 241$ pg/ml, $P < 0.05$) and cGMP concentration (7.0 ± 1.3 vs. 50 ± 5 pmol/ml, $P < 0.05$) increased with synthetic DNP infusion.

DISCUSSION

This study reports the in vivo effects of synthetic DNP on cardiovascular hemodynamics and LV systolic and diastolic function in anesthetized and conscious dogs. Administration of synthetic DNP resulted in reductions in cardiac preload in association with improvements in diastolic function and a small but significant increase in systolic performance.

Effect of synthetic DNP on preload. In the current study, synthetic DNP infusion resulted in marked decreases in preload as shown by the decreases in RAP and PCWP in the hemodynamic study (anesthetized dogs) and by a decrease in LV EDV in the LV function study (conscious dogs). These findings are consistent with the actions of the other members of the natriuretic peptide family that have also been reported to decrease venous return and indexes of preload (9). The more impressive reduction in LV EDV than LV EDP in

Table 3. Myocardial and hemodynamic response to synthetic DNP infusion in 6 conscious chronically instrumented dogs

	Baseline	DNP
LVESP, mmHg	120 ± 7	102 ± 6*
LVESV, ml	26 ± 3	22 ± 3*
Ea, mmHg/ml	13 ± 2	16 ± 5
LVEDP, mmHg	5.7 ± 1.3	3.7 ± 0.9
LVEDV, ml	38 ± 5	31 ± 4*
Ees, mmHg/ml	6.1 ± 0.7	7.4 ± 0.6*
Tau, ms	31 ± 2	27 ± 1*
Stroke volume, ml	12 ± 3	10 ± 2*
HR, beats/min	130 ± 6	131 ± 5

Values are means ± SE. DNP, recordings after 30-min infusion of DNP $100 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ IV; LVESP, left ventricular end-systolic pressure; LVESV, left ventricular end-systolic volume; Ea, arterial elastance; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; Ees, end-systolic elastance; Tau, time constant of isovolumic left ventricular relaxation. * $P < 0.05$.

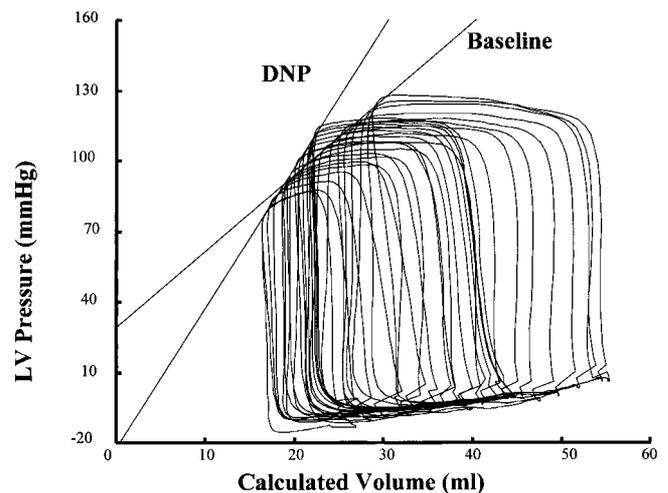


Fig. 2. Representative variably loaded pressure volume loops in 1 dog before (Baseline) and after (DNP) synthetic DNP infusion at $100 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min. Infusion of synthetic DNP resulted in an increase in the slope of the line (Ees) connecting the end-systolic pressure and volume points consistent with an increase in left ventricular (LV) contractility.

the conscious dogs likely reflects the curvilinear nature of the LV EDP-volume relationship whereby a marked change in volume may occur with little change in pressure in the normal ventricle. The changes in preload are accentuated in the anesthetized state where reductions in filling pressures were observed.

Effect of synthetic DNP on afterload. We did not observe evidence of arterial vasodilation with synthetic DNP infusion. Although the natriuretic peptides are considered to be a vasoactive system, the effects of the natriuretic peptides on arterial tone in vivo in normal subjects are somewhat controversial. In vitro studies show that the natriuretic peptides cause relaxation in precontracted arterial strips (8). Furthermore, prolonged systemic infusion (14) or isolated forearm infusions (6) in vivo suggest that the natriuretic peptides cause arterial relaxation. However, acute short-term administration in normal dogs or humans may not demonstrate decreases in SVR, an effect thought to be due to reflex-mediated increases in arterial tone associated with the marked reduction in venous return and CO (16, 20). This effect is blunted in heart failure where decreases in SVR are more consistently reported (5). In the current study, LV ESP and LV ESV were significantly reduced by DNP infusion, demonstrating a reduction in LV afterload. However, the lack of decreases in SVR in the anesthetized study or Ea in the conscious study suggests that the reduction in LV afterload is mediated primarily by the reduction in preload and is not related to arterial vasodilation. Interpretation of the lack of change in Ea must take into account the presence of β -blockade that may have allowed unopposed reflex increases in α -adrenergic activity to overcome any direct arterial vasodilation.

Effect of synthetic DNP on LV relaxation. Improvement in LV relaxation as reflected in a reduction in Tau was found with DNP infusion. The method of

calculating Tau is relatively load insensitive, but we cannot exclude that the reductions in LV ESP and LV ESV contribute to the improvement in LV relaxation. In vivo studies found effects of other natriuretic peptides on myocardial relaxation and postulated that these effects are mediated by the second messenger cGMP (9, 13, 24). In vitro studies suggest that the effect on relaxation is, at least in part, mediated by a direct myocardial effect as cGMP, the second messenger for natriuretic peptides and DNP, has a dose-related effect to enhance myocardial relaxation in vitro (12, 19).

Effect of synthetic DNP on contractility. In the current study, synthetic DNP produced a small but significant increase in Ees, a relatively load-insensitive index of contractility. We reported increases in contractility with ANP and BNP infusion in normal dogs, and we now report an increase in contractility with DNP infusion. Although in vitro data suggested that cGMP may have positive inotropic effects (12), it should be noted that others have not demonstrated a positive inotropic effect with ANP in vivo in studies that use similar technology but different doses (bolus administration) and study protocol (no atrial pacing or beta blockade) (13). Although the ESP-volume relationship may be curvilinear in the normal dog, our study was performed in the presence of β -adrenergic blockade, and there was good overlap of the ESPs before and after DNP infusion (Fig. 2), suggesting that this factor was not responsible for the observed increase in Ees.

Effect of synthetic DNP on the natriuretic peptide second messenger cGMP. The actions of DNP were clearly associated with increases in plasma cGMP, and this supports the results of in vitro studies demonstrating that the actions of DNP are modulated by the natriuretic peptide second messenger cGMP through activation of a particulate guanylate cyclase-coupled receptor (7). In addition, despite infusion of high doses of DNP, no increase in the plasma concentrations of the other natriuretic peptides was seen. This suggests that the actions of DNP were mediated by interaction with receptors and not through displacement of the other natriuretic peptides from clearance mechanisms. Whether synthetic DNP activates the known natriuretic peptide receptors (NPR-A and NPR-B receptors) or whether additional guanylyl cyclase-linked receptors may mediate its effects is unclear and was not addressed by the current study.

DNP-like immunoreactivity has been detected in mammalian species, but the presence of DNP as an endogenous peptide in mammalian species remains to be established. Therefore, these current studies may have more relevance to cardiovascular pharmacology than physiology. Indeed, the plasma concentrations of DNP achieved with these infusions are pharmacological. In normal humans, plasma DNP-like immunoreactivity was reported at concentrations of 6.3 ± 1.0 pg/ml ($n = 19$) (17).

Perspectives

The current study establishes the preload-reducing, lusitropic, and inotropic actions of synthetic DNP in normal dogs, and that these actions are associated with increases in the natriuretic peptide second messenger cGMP. Further work is required to establish the presence of DNP as an endogenous peptide in mammalian species, and the gene remains to be identified. Importantly, our study suggests that investigating the therapeutic potential of this peptide in cardiovascular disease and particularly heart failure is worthwhile.

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