

**Actions of a Novel Synthetic Natriuretic Peptide on  
Hemodynamics and Ventricular Function in the Dog**

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**Abstract:**

Dendroaspis natriuretic peptide (DNP) is a recently discovered peptide with structural similarity to known natriuretic peptides. DNP possesses potent renal actions and has potential as a novel pharmacological agent. 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 ( $141\pm 6$  to  $109\pm 7$  mmHg,  $p<0.05$ ) and pulmonary capillary wedge pressure ( $5.8\pm 0.3$  to  $3.4\pm 0.2$  mmHg,  $p<0.05$ ). Cardiac output decreased and systemic vascular resistance increased with DNP and placebo. DNP-like immunoreactivity and cGMP concentration increased without changes in other natriuretic peptides. In conscious dogs, DNP decreased LV end-systolic pressure ( $120\pm 7$  to  $102\pm 6$  mmHg,  $p<0.05$ ) and volume ( $32\pm 6$  to  $28\pm 6$  ml,  $p<0.05$ ) and LV end diastolic volume ( $38\pm 5$  to  $31\pm 4$  ml,  $p<0.05$ ) but not arterial elastance. LV end systolic elastance increased ( $6.1\pm 0.7$  to  $7.4\pm 0.6$  mmHg/ml,  $p<0.05$ ) and Tau decreased ( $31\pm 2$  to  $27\pm 1$  ms,  $p<0.05$ ). The effects of synthetic DNP on hemodynamics, LV function and second messenger generation suggest a role as a cardiac unloading and lusitropic peptide with therapeutic potential.

**Key words :** Natriuretic Peptides, Systolic Function, Diastolic Function

## Introduction

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 which are mediated by the second messenger 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 have demonstrated that the natriuretic peptides possess direct inotropic and lusitropic myocardial actions in the dog (9, 25). 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 lib. On the day of the acute experiment, dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), intubated and mechanically ventilated with supplemental oxygen (Harvard respirator, Amersham, MA) at 16 cycles per minute. 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 or vehicle infusion. Supplemental doses of pentobarbital sodium (12.5 to 25 mg) were given as needed during the experiment.

A 60 minute 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., Inc., Mountain View, CA) was administered as an intravenous infusion at 10ng/kg/min to 6 dogs, and hemodynamics were repeated after 30 minutes. Then the DNP infusion rate was increased to 50ng/kg/min with hemodynamics repeated after a further 30 minutes. We have previously reported renal response to the DNP infusion and its effect on mean

arterial pressure(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 7 dogs served as time matched controls and received vehicle (saline) only.

Cardiovascular parameters measured included mean arterial pressure (MAP), right atrial pressure (RAP), mean pulmonary artery pressure (PAP), cardiac output (CO), pulmonary capillary wedge pressure (PCWP). Cardiac output 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  $MAP-RAP/CO$ .

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 utilizes 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 inter-assay coefficients of variation were  $10\pm 2\%$  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 6 chronically instrumented conscious normal dogs. These dogs were anesthetized with Pentothal Sodium (20 mg/kg) and isoflurane (0.5-2.5%) and ventilated with supplemental oxygen. A left lateral thoracotomy was performed and the pericardium 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) 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 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 minutes duration to account for respiratory variation, were made over 5 minutes. 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 minutes at 100ng/kg/min. At the end of the 30 minute 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 second recording period to account for respiratory variation. LV volume was calculated as a modified ellipsoid model using the equation  $V_{LV} = (\pi/6)SA^2LA$ , 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 ( $\tau$ ). The dP/dt to 5 mmHg above LV end-diastolic pressure (EDP) was used to calculate  $\tau$ . Because the DNP resulted in changes in LV EDP, the less load-sensitive method of Raff and Glantz (15) was used to calculate  $\tau$ . This method calculates as the negative inverse of the slope of dP/dt versus 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  $ESP = Ees(Ves - V_0)$ , where Ees is slope of the linear ESP volume relationship, representing the LV end-systolic elastance;  $Ves$  is volume at end

systole; and  $V_0$  is intercept with the volume axis. The Ees is sensitive to changes in contractile state but relatively insensitive to changes in loading conditions. Arterial elastance, a relatively preload insensitive measure of afterload, was calculated as ESP divided by stroke volume (23).

Results are expressed as Mean  $\pm$  SEM. Data was assessed by one way ANOVA with Student-Newman-Keuls post hoc test for within group comparisons and with two way analysis of variance for repeated measures with Student-Newman-Keuls post hoc test for comparison between groups. Statistical significance was accepted as  $p < 0.05$ .

## Results

The effects of synthetic DNP or placebo infusion on systemic and pulmonary hemodynamics in anesthetized normal dogs are shown in Table 1. Compared to 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 where as 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 change from baseline with each dose of synthetic DNP versus the corresponding placebo time control for all hemodynamic parameters are shown in figure 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. The changes in CO and 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, while plasma concentration of ANP, BNP and CNP were unchanged (Table 2).

The effects of DNP infusion in the 6 conscious dogs instrumented for assessment of LV function are shown in Table 3. DNP infusion resulted in decreases in LV afterload as evidenced by significant decreases in LVESP and LVESV. There was no significant change in Ea although a trend toward an increase in this measure of arterial tone was observed. There were decreases in preload as evidenced by a significant decrease in LVEDV and a trend towards a decrease in LVEDP. Contractility was modestly but significantly enhanced as evidenced by an increase in Ees (figure 2). The time constant of isovolumic relaxation ( $\tau$ ) decreased significantly, suggesting improvement in LV relaxation. Plasma DNP-like immunoreactivity ( $14.5 \pm 3.0$  vs  $1347 \pm 241$  pg/ml,  $P < 0.05$ ) and cGMP concentration ( $7.0 \pm 1.3$  vs  $50 \pm 5$  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 results 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 decrease in LVEDV in the LV function study (conscious dogs). These findings are consistent

with the actions of the other members of natriuretic peptide family which have also been reported to decrease venous return and indices of preload(9). The more impressive reduction in LVEDV than LVEDP in the conscious dogs likely reflects the curvilinear nature of the LV end diastolic pressure-volume relationship where by 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. While 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 *pre-constricted* arterial strips (8). Further, 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 systemic vascular resistance, an effect thought to be due to reflex mediated increases in arterial tone associated with the marked reduction in venous return and cardiac output (16, 20). This effect is blunted in heart failure where decreases in SVR are more consistently reported (5). In the current study, LVESP and LVESV 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 suggest 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 beta blockade that may have allowed unopposed reflex increases in alpha adrenergic activity to overcome any direct arterial vasodilation.

### ***Effect of synthetic DNP on diastolic function***

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 LVESP and LVESV contribute to the improvement in LV relaxation. In vivo studies have 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 the 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 have reported increases in contractility with ANP and BNP infusion in normal dogs and we now report an increase in contractility with DNP infusion. While in vitro data have 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 which utilize similar technology but different doses (bolus administration) and study protocol (no atrial pacing or beta blockade) (13). While the end systolic pressure volume relationship may be curvilinear in the normal dog, our study was performed in the presence of beta adrenergic blockade and there was good overlap of the end systolic pressures before and

after DNP infusion (figure 2) suggesting that this factor was not responsible for the observed increase in Ees. While we did not administer synthetic DNP to dogs with heart failure in the current study, we have previously reported that the known natriuretic peptides do not alter contractility in heart failure and might expect similar findings with synthetic DNP.

### ***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 (the 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.

While DNP-like immunoreactivity has been detected in mammalian species, its gene remains to be cloned. Therefore, the current studies have more relevance to cardiovascular therapy than physiology. Indeed, the plasma concentrations of DNP achieved with these infusions are pharmacological. Most importantly however, these pharmacological actions are beneficial and further studies are warranted to define the myocardial actions of synthetic DNP in the presence of cardiovascular disease.

In conclusion, 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. Although the presence and biological significance of DNP in mammalian species remains to be established, these findings demonstrate biological properties with therapeutic potential.

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**Figure legends:**

**Figure 1.** Change from baseline (Delta) in mean arterial pressure (MAP), pulmonary artery pressure (PAP), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO) and systemic vascular resistance (SVR) in normal anesthetized dogs in response to infusion of synthetic DNP or the corresponding placebo time controls. 10 - change from baseline after 30 minute infusion of DNP at 10ng/kg/min or placebo; 50 - change from baseline after a further 30 minutes of DNP at 50ng/kg/min or placebo. DNP group (n=6) open bars, placebo group (n=7) black bars. \*P<0.05 vs placebo.

**Figure 2.** Representative variably loaded pressure volume loops in one dog before (Baseline) and after (DNP) synthetic DNP infusion at 100 ng/kg/min for 30 minutes. 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 LV contractility.



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

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

Abbreviations. Baseline, baseline recordings; DNP 10, recordings after infusion of DNP 10ng/kg/min for 30 minutes; DNP 50, recordings after a further 30 minutes of exogenous DNP at 50ng/kg/min; 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.

\* P<0.05 vs baseline, † P<0.05 vs DNP 10 or PLAC30 respectively.

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

	<b>Baseline</b>	<b>DNP 10</b>	<b>DNP 50</b>
<b>DNP (pg/ml)</b>	5.9±2.4	269±74	3240±1658*†
<b>ANP (pg/ml)</b>	17.4±0.7	17.6±0.4	18.1±1.2
<b>BNP (pg/ml)</b>	10.1±1.4	12.9±2.6	12.6±2.0
<b>CNP (pg/ml)</b>	9.7±0.5	9.1±0.5	9.5±0.9
<b>cGMP (pmol/ml)</b>	10.6±1.3	38±7*	74±5*†

Abbreviations: DNP, dendroaspis natriuretic peptide; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide, cGMP, cyclic guanosine monophosphate; otherwise as in Table 1. \* P<0.05 vs baseline, † P<0.05 vs DNP30.

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

	<b>Baseline</b>	<b>DNP</b>
<b>LVESP (mmHg)</b>	120±7	102± 6*
<b>LVESV (ml)</b>	32±6	28±6*
<b>Ea (mmHg/ml)</b>	13±2	16±5
<b>LVEDP (mmHg)</b>	5.7±1.3	3.7±0.9
<b>LVEDV (ml)</b>	38±5	31±4*
<b>Ees (mmHg/ml)</b>	6.1±0.7	7.4±0.6*
<b>Tau (ms)</b>	31±2	27±1*

Baseline, baseline recordings; DNP, recordings after 30 minute infusion of DNP 100ng/kg/min 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

