

Therapeutic Actions of a New Synthetic Vasoactive and Natriuretic Peptide, Dendroaspis Natriuretic Peptide, in Experimental Severe Congestive Heart Failure

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Abstract—Dendroaspis natriuretic peptide (DNP), a recently discovered peptide, shares structural similarity to the other known natriuretic peptides, ANP, BNP, and CNP. Studies have reported that DNP is present in human and canine plasma and atrial myocardium and increased in plasma of humans with congestive heart failure (CHF). In addition, synthetic DNP is markedly natriuretic and diuretic and is a potent activator of cGMP in normal animals. To date, the ability of synthetic DNP to improve cardiorenal function in experimental CHF is unknown. Synthetic DNP was administered intravenously at 10 and 50 ng · kg⁻¹ · min⁻¹ in dogs (n=7) with severe CHF induced by rapid ventricular pacing for 10 days at 245 bpm. In addition, we determined endogenous DNP in normal (n=4) and failing (n=4) canine atrial and ventricular myocardium. We report that administration of synthetic DNP in experimental severe CHF has beneficial cardiovascular, renal, and humoral properties. First, DNP in CHF decreased cardiac filling pressures, specifically right atrial pressure and pulmonary capillary wedge pressure. Second, DNP increased glomerular filtration rate in association with natriuresis and diuresis despite a reduction in mean arterial pressure. Third, DNP increased plasma and urinary cGMP and suppressed plasma renin activity. Fourth and finally, we report that DNP immunoreactivity is present in canine atrial and ventricular myocardium and increased in CHF. These studies report the acute intravenous actions of synthetic DNP in experimental severe CHF and suggest that on the basis of its beneficial properties, DNP may have potential as a new intravenous agent for the treatment of decompensated CHF. (*Hypertension*. 2001;37:1089-1094.)

Key Words: heart failure ■ natriuretic peptides ■ cyclic guanosine monophosphate ■ renin ■ endopeptidase

As the prevalence of congestive heart failure (CHF) has increased during the past decade, so has hospitalization for acutely decompensated CHF.¹ This has resulted in a continued search for new therapeutic agents to treat CHF, including intravenous agents for acutely decompensated CHF, which is characterized by markedly elevated cardiac filling pressure with pulmonary congestion, impaired glomerular filtration rate (GFR) with sodium retention, and activation of the renin-angiotensin-aldosterone system.

Recently, a new member of the natriuretic peptide family, Dendroaspis natriuretic peptide (DNP), has been reported.² DNP, originally isolated from the venom of the *Dendroaspis angusticeps* (green mamba snake), is a 38-amino-acid peptide that contains a 17-amino-acid disulfide ring structure with 15-residue C-terminal extension. This peptide, which shares structural similarity to atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), potentially vasorelaxes isolated precontracted rodent aorta and canine coronary arteries and augments the formation of 3',5'

cyclic guanosine monophosphate (cGMP) in aortic endothelial and smooth muscle cells.^{2,3} We have reported that DNP immunoreactivity is present in human plasma and atrial myocardium and is elevated in the plasma of humans with CHF.⁴

The therapeutic potential of DNP is supported by recent studies in normal animals in which intravenous administration of synthetic DNP had potent natriuretic and diuretic properties, which were associated with marked increases in plasma and urinary cGMP.⁵ DNP, in contrast to the other known natriuretic peptides, may have unique characteristics that support its development as a new intravenous agent for acutely decompensated severe CHF. These properties include an extended C-terminus, which may result in greater resistance to neutral endopeptidase (NEP), the degradative enzyme of the natriuretic peptides.⁶ DNP also has greater potency in cGMP generation compared with ANP, BNP, or CNP⁷ and demonstrates a lack of marked plasma elevation in CHF,⁴ which could have relevance to receptor sensitivity. However, to date, the biological actions of synthetic DNP in heart failure are unknown.

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The goal of the present study was 2-fold. First, we sought to define the cardiorenal and neurohumoral actions of intravenously administered synthetic DNP in a well-characterized canine model of severe CHF. We hypothesized that acute intravenous synthetic DNP would have beneficial actions on cardiorenal and neurohumoral function in this experimental severe CHF, which would support its potential role as a new intravenous agent in decompensated CHF. In addition, we defined the presence and concentration of DNP in normal and failing canine atrial and ventricular myocardium.

Methods

Animals

Studies were performed in male mongrel dogs ($n=11$) in which severe CHF was induced by rapid ventricular pacing at 245 bpm for 10 days. We evaluated the cardiovascular, renal, and humoral actions of exogenously administered synthetic DNP ($n=7$). We also determined the presence and concentration of DNP in the heart in the presence ($n=4$) and absence ($n=4$) of severe experimental CHF. All studies conformed to the guidelines of the American Physiological Society and were approved by the Mayo Clinic Animal Care and Use Committee.

Canine Model of Severe CHF and Acute Protocol

An epicardial lead (Medtronic) was implanted on the right ventricle through a left thoracotomy with a 1- to 2-cm pericardiotomy as previously reported.^{8,9} After a 14-day postoperative recovery period, severe CHF was produced by rapid ventricular pacing at 245 bpm for 10 days, which in previous studies resulted in a profile similar to severe CHF in humans.^{8,9} On the night before the acute experiment, CHF animals ($n=7$) were given 300 mg of lithium carbonate for the assessment of renal tubular function. On the 11th day of pacing, dogs were anesthetized with pentobarbital sodium 15 mg/kg IV. After tracheal intubation, dogs were mechanically ventilated (Harvard respirator) with 4 L/min of supplemental oxygen. A flow-directed balloon-tipped thermodilution catheter (Ohmeda, Criticath) was used through the external jugular vein for cardiac hemodynamic measurements. Through left lateral flank incision, the left ureter was cannulated for timed urine collection, and a calibrated electromagnetic flow probe was placed around the left renal artery (model FM 5010, King) for monitoring of renal blood flow. The right femoral vein was cannulated with 2 catheters (PE-240) for infusion of inulin and synthetic DNP (DNP 1-38, Phoenix Pharmaceuticals, Inc). The right femoral artery was cannulated for direct arterial blood pressure measurement and arterial blood sampling. A priming dose of inulin dissolved in isotonic saline solution was injected, followed by a constant infusion of 1 mL/min to achieve a steady-state plasma inulin concentration between 40 and 60 mg/dL. The dogs were placed in dorsal suspension and allowed to equilibrate for 60 minutes without intervention.

After an equilibration period, a 30-minute baseline clearance (baseline) was performed. This was followed by a 15-minute lead-in period during which DNP infusion at $10 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was begun intravenously, after which the second 30-minute clearance period was performed. After the second clearance, intravenous infusion of DNP was increased to $50 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. After a 15-minute lead-in period with this dose of DNP, a 30-minute clearance was performed. At the end of the third clearance, the DNP infusion was stopped. A 30-minute washout period was followed by a 30-minute postinfusion clearance.

Determination of Cardiac DNP

Presence and concentration of endogenous DNP in the left atrial and ventricular myocardium were evaluated in the presence ($n=4$) and absence ($n=4$) of experimental severe CHF. Severe CHF was induced by rapid ventricular pacing at 245 bpm for 10 days as described above in a group of dogs not exposed to exogenous DNP.

Dogs were euthanized, and hearts were harvested. Left atrial and ventricular sections were taken from the full thickness of the free wall and immediately fixed in 10% buffered formalin for immunohistochemical studies. Sections of free wall from left atrium and ventricle also were taken, immediately frozen in liquid nitrogen, and stored at -80°C until further processing for radioimmunoanalysis (RIA). Immunohistochemical staining for DNP was performed in the left atrial and ventricular myocardium from normal and CHF dogs. Immunohistochemical studies were performed by the indirect immunoperoxidase method as described previously.⁵ After fixation in formalin was completed, these tissues were embedded in paraffin and sections 5 μm thick were cut and mounted on glass slides treated with silica. Subsequently, the slides were stained for DNP and normal goat serum as a control and reviewed.

Atrial and ventricular tissue for endogenous DNP determined by RIA was pulverized, boiled for 5 minutes in 10 vol of 1 mol/L acetic acid and 20 mmol/L hydrochloric acid solution, and homogenized at high speed (PT 1200, Polytron). The homogenate was ultracentrifuged for 30 minutes at 15 000 rpm at 4°C , and the supernatant was stored at -20°C until RIA. Before centrifugation was begun, a sample of the homogenate was taken for measurement of tissue protein content according to the folin-phenol method of Lowry. Immunoreactive DNP in tissue was measured as picograms per milliliter homogenate and normalized for protein content.

Analytical Methods

Plasma and urine electrolytes, including lithium, were measured by flame-emission spectrophotometer (IL943, Flame Photometer, Instrumentation Laboratory). Plasma and urine inulin concentrations were measured by the anthrone method, and GFR was measured by clearance of inulin. Lithium clearance technique was used to estimate proximal and distal fractional reabsorption of sodium. Proximal fractional reabsorption of sodium was calculated by the formula $[1 - (\text{Lithium Clearance}/\text{GFR})] \times 100$. Distal fractional reabsorption of sodium was calculated by the formula $[(\text{Lithium Clearance} - \text{Sodium Clearance})/\text{Lithium Clearance}] \times 100$. Plasma and urinary cGMP were measured by RIA by use of the method of Steiner et al.¹⁰ Urine for cGMP measurement was heated to 90°C before being stored at -20°C to inhibit degradative enzymatic activity. Plasma and urinary DNP levels were determined by use of a specific and sensitive RIA for DNP before, during, and after DNP administration.^{4,5} This assay was also used to determine DNP concentration in left atria and ventricles.^{4,5} Specificity of RIA for DNP was tested by use of the following methodology. First, we performed cross-reactivity studies to determine the effect of ANP on DNP RIA. Addition of 500 pg of ANP to the DNP RIA resulted in zero detection. Second, a DNP RIA displacement curve with dilution of cardiac extracts was performed. Measurement of DNP immunoreactivity in atrial extracts was performed without dilution and with dilutions of 1:2, 1:4, and 1:8. This resulted in a curve that paralleled the standard curve (Figure 1). Third and finally, we tested the specificity of the DNP immunostaining. DNP antiserum was preabsorbed in the immunohistochemical study by incubating antiserum with DNP peptide overnight. Atrial and ventricular myocardium was stained with both preabsorbed and unabsorbed antiserum at the same dilution. Preabsorbed antiserum showed no staining and was similar to the nonimmune control. Plasma ANP, BNP, and plasma renin activity were determined by RIA.^{5,11}

Statistical Analysis

Results of quantitative studies are expressed as mean \pm SEM. Statistical comparisons within groups were performed by use of repeated measures ANOVA followed by a post hoc Dunnett test. Statistical comparison between groups was performed by the unpaired Student *t* test. Statistical significance was accepted at $P < 0.05$.

Results

Cardiovascular and Renal Hemodynamics and Tubular Function

Cardiovascular hemodynamics before, during, and after DNP administration is reported in Table 1. Synthetic DNP resulted

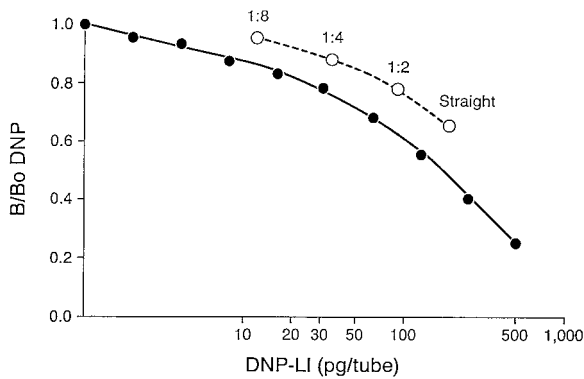


Figure 1. DNP-RIA displacement curve using diluted atrial extracts. Curve of ●; represents DNP standard. Curve of ○; parallel to standard curve represents atrial extracts diluted at 1:2, 1:4, and 1:8. DNP-LI indicates DNP-like immunoreactivity; B/Bo, (average net counts of standard or sample/average net counts of zero standard)×100.

in a decrease in mean arterial pressure (MAP) during the administration of high dose DNP, which returned to baseline during postinfusion clearance. Both doses of synthetic DNP decreased the markedly elevated right atrial pressure, pulmonary capillary wedge pressure, and pulmonary artery pressure. Cardiac output did not change with DNP, whereas SVR decreased during low-dose infusion. In addition, pulmonary vascular resistance tended to decrease during low-dose infusion.

In severe CHF, high-dose DNP increased GFR despite the reduction in MAP with an increase in urinary sodium excretion and urine flow (Figure 2A through C). These renal actions were associated with a marked increase in urinary cGMP excretion (Figure 2D). As shown in Table 1, renal blood flow did not change during DNP infusion and proximal fractional reabsorption of sodium decreased, whereas distal fractional reabsorption of sodium remained unchanged.

Neurohumoral Function

Plasma DNP and urinary DNP excretion before, during, and after DNP infusion are reported in Table 2. Administration of

synthetic DNP increased plasma DNP and urinary DNP excretion in a dose-dependent manner. During postinfusion clearance, plasma DNP remained elevated. High-dose DNP increased plasma cGMP, which remained increased during postinfusion clearance. Plasma ANP and BNP remained unchanged during DNP infusion. Low-dose DNP was associated with a reduction in plasma renin activity (Table 2).

Cardiac DNP

Immunohistochemistry for DNP revealed positive staining in the atrial and ventricular myocardium of both normal and CHF dogs (Figure 3). DNP immunoreactivity was observed within the cytoplasm of cardiomyocytes and was distributed widely in the peripheral cytoplasm, with immunoreactivity present also in the perinuclear region. DNP immunoreactivity was more intense in the atria than in the ventricles of both normal and CHF dogs, with more intense staining in CHF dogs. Atrial and ventricular concentrations of DNP were increased in CHF compared with normal dogs (atrial DNP from 0.4 ± 0.1 to 2.9 ± 0.5 pg/mg of protein, $P=0.002$, and ventricular DNP from 0.5 ± 0.2 to 2.1 ± 0.3 pg/mg of protein, $P=0.0014$) and thus paralleled immunohistochemical findings.

Discussion

The present study reports for the first time cardiorenal and neurohumoral actions of synthetic DNP in experimental severe CHF. Here, we report that exogenous administration of synthetic DNP in CHF results in beneficial cardiorenal and neurohumoral actions. Specifically, DNP in severe experimental CHF decreased the markedly elevated cardiac filling pressures characterizing this model and severe CHF. DNP increased GFR, sodium excretion, and urine flow despite the reduction in MAP. This DNP-mediated natriuretic response was secondary to a reduction in proximal tubular reabsorption of sodium together with an increase in filtered load of sodium. The renal actions were further associated with reductions in plasma renin activity during administration of low-dose DNP. In addition, DNP increased plasma and

TABLE 1. Cardiovascular and Renal Hemodynamics and Tubular Function

Function	Baseline	DNP-10	DNP-50	Post-DNP
MAP, mm Hg	101 ± 7	95 ± 6	$89 \pm 7^*$	94 ± 9
RAP, mm Hg	9.2 ± 1.4	$7.1 \pm 1.1^*$	$6.5 \pm 1.2^*$	8.6 ± 0.7
PCWP, mm Hg	26.7 ± 2.1	$23.0 \pm 2.0^*$	$21.6 \pm 1.5^*$	$23.9 \pm 1.5^*$
PAP, mm Hg	32.8 ± 3	$28.9 \pm 2^*$	$27.1 \pm 2^*$	$29.9 \pm 2^*$
CO, L/min	2.3 ± 0.1	2.6 ± 0.1	2.2 ± 0.2	$1.8 \pm 0.1^*$
SVR, mm Hg · L ⁻¹ · min ⁻¹	41 ± 4	$35 \pm 3^*$	38 ± 3	$47 \pm 3^*$
PVR, mm Hg · L ⁻¹ · min ⁻¹	2.7 ± 0.4	2.3 ± 0.3	2.6 ± 0.4	$3.3 \pm 0.3^*$
RBF, mL/min	112 ± 10	117 ± 9	121 ± 16	93 ± 15
PFRNa, %	89.0 ± 3.1	$86.0 \pm 3.6^*$	$70.3 \pm 9.1^*$	$79.2 \pm 5.1^*$
DFRNa, %	99.2 ± 0.4	99.4 ± 0.1	97.9 ± 0.5	$97.1 \pm 0.9^*$

Baseline indicates 30-min baseline clearance; DNP-10, 30-min clearance with 10 ng/kg IV infusion of DNP; DNP-50, 30-min clearance with 50 ng/kg IV infusion of DNP; Post-DNP, 30-min postinfusion clearance after a 30-min washout period; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; PAP, pulmonary artery pressure; CO, cardiac output; PVR, pulmonary vascular resistance; RBF, renal blood flow; PFRNa, proximal fractional reabsorption of sodium; and DFRNa, distal fractional reabsorption of sodium.

* $P < 0.05$ vs baseline.

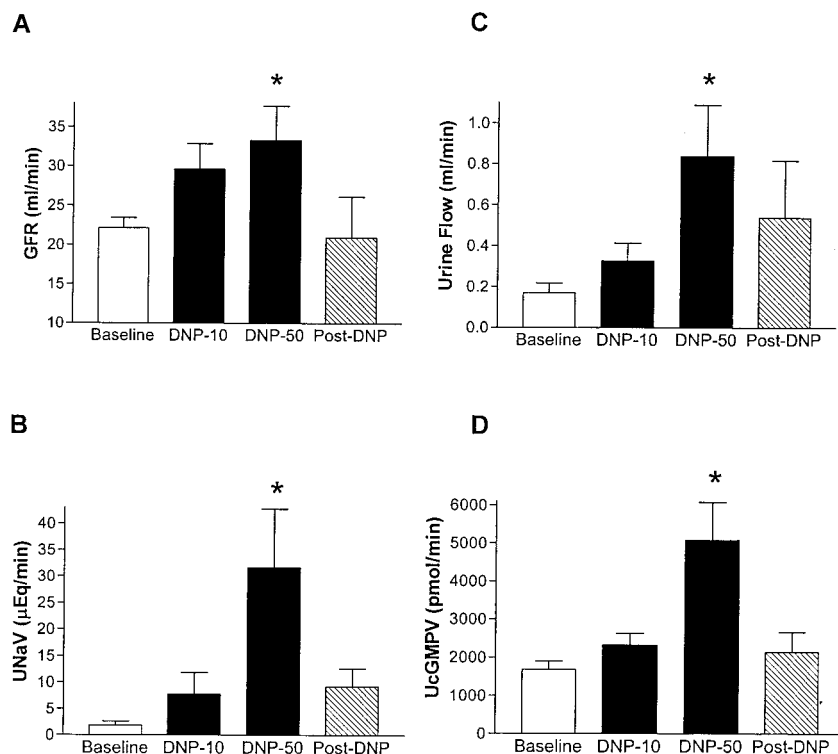


Figure 2. Renal response to synthetic DNP in experimental severe CHF. A, GFR; B, urinary sodium excretion (UNaV); C, urine flow; D, urinary excretion of cGMP (UcGMPV). □ represents baseline; ■, infusion of low- (10 ng/kg; DNP-10) and high-dose (50 ng/kg; DNP-50) DNP; and ▨, postinfusion clearance (Post-DNP). Values are mean ± SEM. * $P < 0.05$ vs baseline.

urinary cGMP, as would be predicted to occur if this peptide was structurally similar to the other known natriuretic peptides and functioned through the same second-messenger system.

Acute administration of synthetic DNP in severe experimental CHF decreased MAP, which returned to baseline levels in the 30-minute post-DNP clearance period, and reduced elevated cardiac filling pressures. Such actions are considered clinically beneficial to reduction of pulmonary congestion. These effects were probably mediated by acute vascular mechanisms, given that at a low dose of DNP, they occurred immediately and before observed natriuretic and diuretic responses. The profound effect to lower filling pressures may be due to venodilation in addition to diuresis. The increase in SVR after a significant decrease in SVR with a tendency to decrease pulmonary vascular resistance during administration of low-dose DNP may be explained by activation of compensatory vasoconstrictive mechanisms. However, we caution that the present study made use of a small number of animals, which limits interpretations of more-vari-

able dose-related changes in vascular resistance and cardiac output.

A hallmark of severe heart failure is a reduction in GFR and renal resistance to natriuretic peptides.^{12–14} Synthetic DNP increased GFR in the present experimental model of severe CHF, an action not observed in normal animals. In the absence of an increase in renal blood flow, glomerular actions of synthetic DNP may be explained by afferent arteriolar dilatation and efferent arteriolar constriction or a direct action to increase the coefficient for filtration. This increase in GFR is even more significant inasmuch as it occurred during a reduction in renal perfusion pressure with administration of high-dose DNP.

In addition to enhancing GFR, high-dose DNP was natriuretic and diuretic, despite the presence of intense baseline sodium retention. Of note, DNP-mediated natriuresis and diuresis occurred despite significant reductions in MAP. The natriuretic response to DNP may be best explained by a decrease in proximal reabsorption of sodium in addition to an increase in filtered load of sodium. The mechanism for the

TABLE 2. Neurohumoral Function

Function	Baseline	DNP-10	DNP-50	Post-DNP
DNP, pg/mL	13 ± 2	463 ± 146*	1060 ± 177*	442 ± 85*
UDNPV, pg/min	28 ± 7	339 ± 155	1713 ± 876*	770 ± 360
cGMP, pmol/mL	35 ± 6	46 ± 6	70 ± 10*	63 ± 7*
ANP, pg/mL	359 ± 55	296 ± 42	299 ± 42	333 ± 46
BNP, pg/mL	127 ± 23	203 ± 40	193 ± 48	136 ± 31
PRA, ng · mL ⁻¹ · h ⁻¹	11.9 ± 1.0	9.0 ± 1.6*	10.1 ± 1.5	8.9 ± 1.4*

UDNPV indicates urinary DNP excretion; and PRA, plasma renin activity.

* $P < 0.05$ vs baseline.

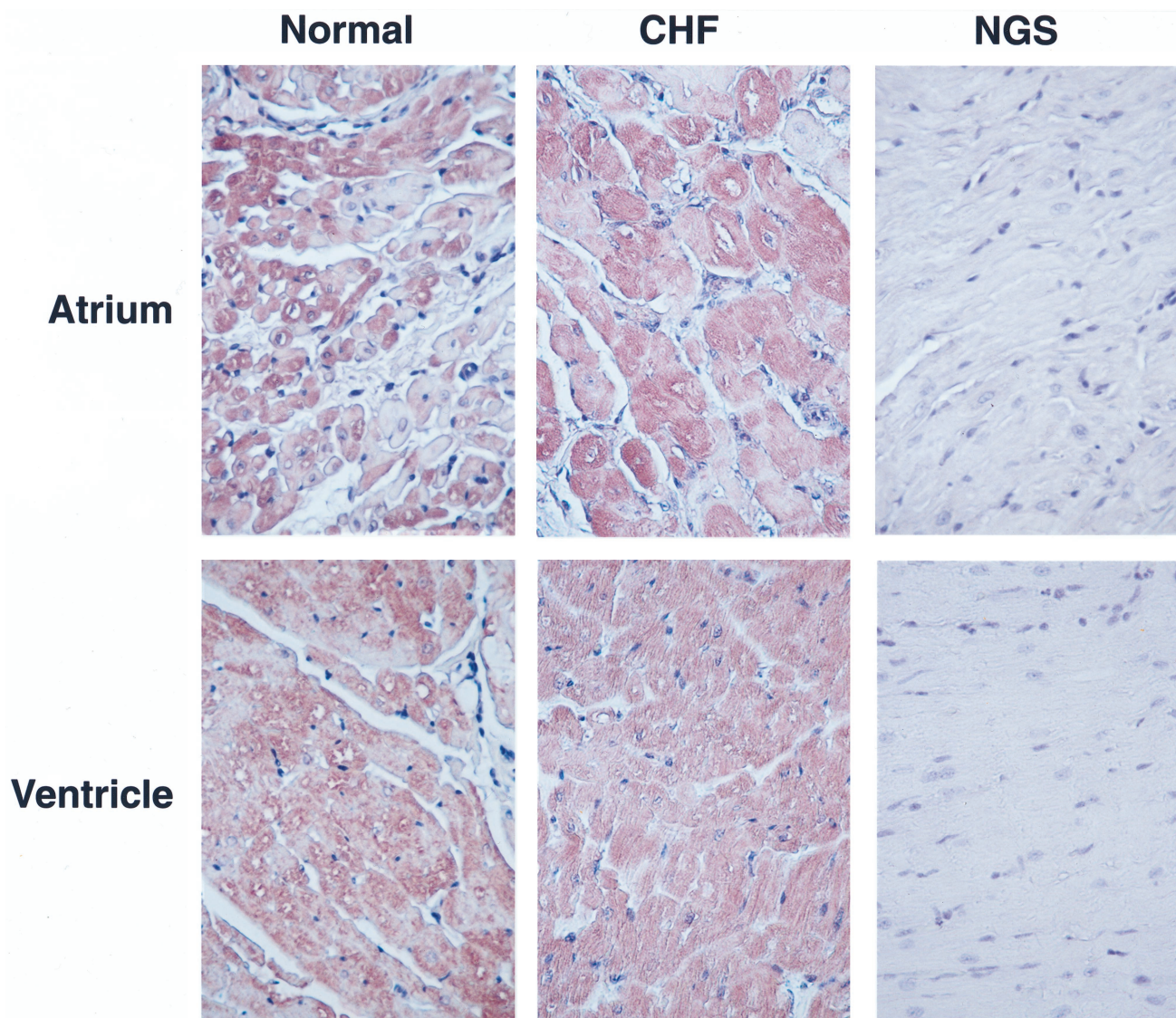


Figure 3. Immunohistochemical staining for DNP in canine normal and CHF atrium and ventricle. Representative sample of atrial and ventricular myocardium from normal ($n=4$) and CHF dogs ($n=4$) not exposed to exogenous DNP. NGS indicates normal goat serum (magnification $\times 400$).

decrease in proximal tubular reabsorption in CHF may be secondary to antagonism of angiotensin II in proximal tubules, as reported with ANP in studies in isolated perfused proximal tubules.¹⁵ The actions of synthetic DNP on plasma renin activity are unknown. In the present study, DNP at a low dose decreased plasma renin activity. Such renin inhibitory actions, although modest, occurred despite the presence of known renin-stimuli such as reductions in atrial pressure and renal perfusion pressure.

Infusion of DNP markedly increased urinary excretion of DNP. In previous studies, ANP infusion has resulted in either no increase or a modest increase in urinary ANP excretion because of rapid ANP degradation by NEP.⁸ The present observation is consistent with DNP being more resistant to degradation by renal NEP.⁶ Cardiorenal actions of DNP were associated with an increase in plasma and urinary cGMP, the second messenger of the natriuretic peptides. Importantly, renal hyporesponsiveness to ANP is

characterized by an attenuated cGMP response to ANP in experimental severe CHF.^{8,9} Thus, DNP differs inasmuch as urinary as well as plasma cGMP increases in response to exogenous DNP in this model of severe CHF. It is presently unclear as to whether DNP interacts with natriuretic peptide receptor (NPR)-A or with an unknown receptor, and further studies will be required to address this question.

In recent studies, we reported the presence of DNP in canine and human plasma and atrial myocardium with elevated concentrations in plasma in humans with CHF.^{4,5} An additional part of the present study confirmed and extended these reports and demonstrated endogenous DNP concentrations in atrial and ventricular myocardium and its elevations in CHF. Although we were able to detect DNP immunoreactivity in plasma and cardiac tissue, cloning and sequencing of mammalian DNP will be required to demonstrate conclusively DNP existence in mammalian species.

The present study provides a rationale for the potential therapeutic efficacy of synthetic DNP as a new intravenous compound for acutely decompensated CHF. Synthetic DNP has unique cardiorenal actions that go beyond those of conventional vasodilators. Data suggest that DNP may differ from the other known natriuretic peptides in that it exhibits greater resistance to degradation by NEP, marked activation of the cGMP system, and potent vasoactive properties. Another unique characteristic of synthetic DNP in CHF, which supports its therapeutic role, is the ability to increase GFR. The importance of this phenomenon is supported by recent reports that reevaluated data from the Second Prospective Randomized Study of Ibopamine on Mortality and Efficacy (PRIME II) and Studies Of Left Ventricular Dysfunction (SOLVD). Hillege et al¹⁶ reported that preservation of GFR is the most important determinant of enhanced survival in patients with severe CHF. Dries et al¹⁷ evaluated the SOLVD study and reported that even moderate degrees of renal insufficiency are independently associated with an increased risk of all-cause mortality in patients with heart failure. Thus, a therapy capable of improving renal function may also play a role in delaying disease progression.

In conclusion, synthetic DNP in a model of severe CHF reduced markedly elevated cardiac filling pressures, enhanced GFR, and resulted in natriuresis, diuresis, and renin inhibition. These actions were also associated with the ability of synthetic DNP to activate the cGMP second-messenger system. Further studies are now needed to elucidate the cardiorenal and neurohumoral actions of acutely administered synthetic DNP in human decompensated CHF.

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