Prolonged Effects of B-Type Natriuretic Peptide Infusion on Cardiac Remodeling After Sustained Myocardial Injury

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Running Title: BNP Therapy after Myocardial Injury

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Abstract

Brain natriuretic peptide (BNP) is an established first line therapy for acute decompensated heart failure (HF), but its efficacy in preventing left ventricular (LV) remodeling after myocardial injury is unknown. The goal of this study was to evaluate the effects of BNP therapy on remodeling following ischemic injury in an awake canine model. Dogs were chronically instrumented for hemodynamics. Ischemia was created by daily coronary embolization (Embo) (3.1 x 10^4 beads/day) for 3 wks; 60 minutes after the first embolization, BNP (100 ng/kg/min) (n=6) or saline (Control) (n=6) was continuously infused via a left atrial catheter for 3 wks. Hemodynamics and echocardiography were performed in an awake state at baseline, 3 wks after Embo plus BNP infusion, and 4 wks after stopping Embo plus BNP infusion. End-systolic elastance (Ees) and LV dP/dt were preserved throughout Embo plus BNP therapy versus Control therapy (Ees: 3.76 ± 1.01 vs. 1.41 ± 0.16 mmHg/ml; LV dP/dt: 2417 ± 96 vs. 2068 ± 95 mmHg/s, respectively, both p<0.05 vs. Control). LV end-diastolic dimension was significantly smaller in BNP-treated dogs compared to Control dogs (4.29 ± 0.10 vs. 4.77 ± 0.17 cm, respectively), and ejection fraction was maintained in treated dogs versus Control dogs (53 ± 1 vs. 46 ± 2%, respectively) (both p<0.05 vs. Control). COX-2 expression in terminal LV tissue was significantly reduced after BNP therapy. Treatment with continuous infusion BNP preserved LV geometry, improved systolic function, and prevented the progression of systolic HF after persistent ischemic injury.

Keywords: Natriuretic peptides, remodeling, heart failure
Recombinant B-type natriuretic peptide (BNP) has a wide array of pharmacologic effects by affecting cardiovascular, renal, and neurohormonal pathways. These effects are mediated primarily through production of cGMP, which occur after peptide binding to guanylyl cyclase-linked, natriuretic peptide receptors. By producing both arterial and venous dilation, BNP reduces left ventricular filling pressures and, myocardial work and oxygen consumption are reduced secondary to BNP-mediated coronary vasodilatation (29,30). BNP also alters renal hemodynamics by increasing glomerular filtration rate, directly promoting natriuresis and inhibiting aldosterone release (18). These acute hemodynamic changes cause a rapid reduction in clinical symptoms and have made it an effective first-line therapy in acutely decompensated congestive heart failure (CHF) (9,31,33).

An even more important clinical application of BNP lies in its potential ability to alter the natural history of heart failure following acute myocardial ischemia. Growing evidence suggests that natriuretic peptides affect multiple neurohormonal and anti-proliferative pathways involved in pathologic left ventricular (LV) remodeling. BNP opposes genes involved in fibrosis, proliferation, and inflammation via cGMP dependent protein kinase signaling (20). Likewise, studies in rats show that treatment in rats decreased infarct size after coronary occlusion (34). Clinically, intravenous atrial natriuretic peptide (ANP) given during coronary angioplasty for 24 hours improved LV ejection fraction and LV end diastolic volume index at 1 month (15); ANP administered for 48 hours after AMI decreased cardiac sympathetic nerve activity and improved cardiac function using nuclear imaging after two week follow-up (21).

These early studies imply that natriuretic peptide therapy may preserve LV geometry and enhance cardiac function after myocardial infarction. Currently, little animal or human data
exists addressing the specific use of BNP therapy after myocardial infarction to prevent the
development and progression of systolic heart failure. Clinical studies using ANP lack long-term
follow-up and have not provided a systematic/quantitative analysis of cardiac function and
biologic mechanisms. Moreover, ANP differs in structure and function from BNP - BNP has a
much longer half-life due to decreased affinity to neutral endopeptidases (24), greater potency
generating higher cGMP levels, and increased expression in ventricular tissue compared to ANP
(17). Finally, recombinant ANP is not approved for use in all countries, except Japan. As such,
the primary purpose of the present study was to evaluate the long-term effect of continuous,
intravenous BNP on myocardial function and remodeling after sustained coronary embolization-
induced, ischemic myocardial injury in an awake canine model. Additionally, pathways specific
to reverse remodeling, including inflammation and fibrosis, were examined from terminal
myocardial samples.
Methods

Study Design

The study consisted of three phases for a total period of over 7 weeks: 1) an initial instrumentation surgery, 2) induction of CHF due to sustained myocardial ischemia through daily coronary embolization for three weeks in addition to a continuous infusion of vehicle or BNP (Embo+Inf), and 3) a four week observation period after stopping embolization and infusion (Obs). Twelve mongrel dogs of both sexes (age: 2.0±0.5 yrs; weight: 25±6 kg) were chronically instrumented at the initial surgery and allowed to recover for 7-10 days. At the beginning of Embo+Inf, each dog was randomized to Control (n=6), who received a continuous infusion of vehicle, 0.9% normal saline (NS) or intravenous BNP (100 ng/kg/min) (Scios Inc., Fremont, CA) (n=6) at equivalent volumes starting 60 minutes after the first embolization using a portable infusion pump (CADD-Legacy PCA Pump Model 6500, Smith Medical MD Inc., St. Paul, MN, USA) and continuing throughout the 3 week Embo+Inf period. Hemodynamic measurements and echocardiograms were performed 10-12 days after instrumentation but immediately prior to Embo+Inf (Baseline), after three weeks of Embo+Inf, and after four weeks of Obs in an awake state. At the end of four weeks of Obs, the animals were sacrificed, and LV myocardial tissue was harvested for histology and immunohistochemistry. This study was approved by the institutional Animal Care and Use Committee of Columbia University, which conforms to the “Guide for Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH publication 85-23, revised 1996).

Surgical Instrumentation

The surgical procedures involved in the initial instrumentation surgery have been described in full previously (8). In short, a Konigsberg solid state pressure gauge (6.5,
Konigsberg Instruments Inc., Pasadena, CA) was placed through the LV apex for measurement of LV pressure, and fluid filled catheters were placed in the ascending aorta and the LV for measurement of aortic pressure and calibration of the Konigsberg pressure gauge, respectively. A pneumatic cuff was placed around the inferior vena cava (IVC) to vary loading conditions postoperatively. A thin silastic cannula was inserted into the left anterior descending coronary artery (LAD) for coronary embolization. Another Tygon catheter was chronically implanted into the right atrium, and the catheter was connected to a venous infusion pump for drug infusion. In addition, 6 sonomicrometric crystals (Sonometrics Corp, London, Canada) were placed on the anterior, posterior, apex, base, septal and free walls of the LV. The catheters and wires were run subcutaneously and externalized through the skin, the chest was closed in layers, and a temporary chest tube was placed. Dogs were allowed to recover fully from surgery and were trained to lie quietly on a laboratory table before experiments.

**Coronary Embolization-Induced Heart Failure**

This model has been described previously in detail (8). In brief, CHF was produced by daily intracoronary microembolization with 25,000 (90-120 μm in diameter) polymer bead injections through the previously implanted LAD cannula for three consecutive weeks. In order to avoid bias in cardiac function and hemodynamics due to variable degrees of coronary embolization, daily and total doses of polymer beads during the three-week coronary embolization period were comparable among Control and BNP-treated dogs. Embolization was begun after recovery from surgery, and immediately after baseline measurements on the same day.

**BNP Solution Preparation and Administration**
BNP solution was prepared every day using normal saline. The concentration of BNP for pump infusion was varied and based on the animal’s weight as the infusion rate was kept constant at 1 ml/hour. Thus, the concentration of BNP solution (ng/ml) for a particular animal was as follows: 100 (ng/kg/min) x body weight (kg) x 60 (min/hr) x 1 (hr/ml). At the start of infusion, the right atrial tunneled catheter was connected to the continuous infusion pump, which was attached to the back of the dogs using custom-made backpacks. New infusion pumps containing freshly prepared BNP solution or normal saline were connected every day to ensure stability of the solution.

The dose of continuous infusion BNP was chosen, in part, based on canine pharmacokinetics of the drug (internal data, data not shown), and, in part, to provide maximal benefit in this pilot experiment and to cause significantly increases in serum cGMP without obvious hemodynamic effects. Published clinical studies have utilized intravenous BNP doses ranging from 10 to 30 ng/kg/min (9,33). As such, the dose used in this experiment was more than double these studies, in order to ensure adequate dosing and maximum effect of downstream BNP signaling.

Hemodynamic Measurements and Echocardiography

Hemodynamics and echocardiography were measured as previously described (8). Aortic pressure was measured by attaching the previously implanted catheters to P23ID strain-gauge transducers (Statham Instruments, Inc, Oxnard, CA), and LV pressure was measured via a chronically implanted solid pressure gauge. Mean arterial pressure (MAP) was determined online by use of 3-Hz averaging filters (DA26, Medtron Engineering, Olivenhain, CA). Data was recorded on an eight-channel thermal writing chart recorder (30-V8808-10, Gould Electronics, East Rutherford, NJ), and periods of interest were digitized (a Gateway 2000 486 computer...
equipped with an analog-to-digital conversion system, Sonometric Corp., London, Ontario, Canada) for off-line analysis. Echocardiograms were performed using a Hewlett Packard Sonos 5500 at Baseline, after 3 weeks of Embo+Inf, and after 4 weeks of Obs, as previously described (8), and analyzed off-line by a blinded observer. LV mass was calculated using the formula of Devereaux.

**LV Pressure-Volume Relationship Analysis**

LV pressure and LV volume were measured at Baseline, after 3 weeks of Embo+Inf, and after 4 weeks of Obs via previously implanted solid state pressure gauges in the LV and sonomicrometric crystals. As previously described (8), LV pressure-volume relationship loops were measured at rest and during a transient preload reduction induced by IVC occlusion; the end-systolic pressure-volume relationship (ESPVR) and end-diastolic pressure-volume relationship (EDPVR) were then calculated.

The area between ESPVR and EDPVR at steady state, known as pressure-volume area, is an afterload independent index of pump function that is a predictor of myocardial consumption (39). LV stroke work (SW) was calculated as the integrated area of each PV loop at steady state. Both pressure-volume area and SW were normalized to echocardiographically-determined LV mass, to remove any confounding differences in ventricular size. LV mechanical efficiency was calculated as the ratio of stroke work to PVA (4, 39). Arterial elastance, $E_a$, was calculated as stroke volume/end-systolic pressure. All calculations were made using custom software (Matlab, v6.5).

**Assessment of Serum cGMP and BNP Levels**

Blood samples via the implanted aortic catheter for cGMP levels were drawn from two BNP-treated dogs in order to verify BNP-mediated cGMP signaling. In these two dogs, BNP
infusion was begun 1 hour prior to embolization, unlike the remaining BNP group. Blood samples were drawn at Baseline, 1 hour after initial BNP infusion but prior to start of Embolization, and then 1 hour after Infusion+Embolization. Two samples were drawn for each animal per time point.

Blood samples for BNP levels were also drawn in all Control and BNP-treated dogs at Baseline (prior to Embolization or Infusion), after 1 hour of Embolization alone, after 1 hour of Embo+Inf, after 1 week of Embo+Inf, after 3 weeks of Embo+Inf, and after 4 weeks of observation.

All blood samples were drawn while the dogs were laying quietly on the table, and serum was used for cGMP and BNP measurements. The levels of cGMP were measured using the cGMP EIA kit (Cayman Chemical) and BNP levels were measured using a radioimmunoassay kit (Phoenix Pharmaceuticals).

**Immunohistochemistry and Histology:** Fibrosis, Factor VIII, COX-2, and Macrophage Infiltration

For immunohistochemical staining, slides were deparaffined and rehydrated in PBS followed by blocking the endogenous peroxidase with 3% hydrogen peroxide. To avoid nonspecific reaction with primary antibody, slides were pretreated with 15% normal goat serum before incubation with primary antibodies. Slides were incubated with primary antibodies including anti-Factor VIII (DAKO, Carpinteria, CA, in 1:400), anti-Cox-2 (Novus Biologicals, Littleton, CO, in 1:500) and anti-macrophage, respectively. Normal mouse IgG was used as a negative control. The immunoreactivities were visualized by ABC reagents (Vector, Burlingame, CA) and dianinobenzidine, followed by counterstaining with hematoxylin. The image analysis was performed by using a Nikon E600 light microscope equipped with Spot
digital camera. Digital image software (Image Pro Plus 4.5.1, Silver Spring, MD) was used for image analysis of myocardial fibrosis, positive Factor VIII staining blood vessels, COX-2, as well as macrophage infiltration, was measured in 10 fields per slide and normalized by the number of covered fields. The image analysis was blinded to the person who performed the histopathologic evaluation.

Statistics

Values are reported as mean ± standard error. Continuous variables were compared using paired, two-tailed $t$-testing with Levene’s test for Equality of Variances. A paired Student’s $t$ test was used for subgroup comparisons made between measurements obtained at Baseline, Embo+Inf, and after Obs. Significance was adjusted for multiple comparisons using one-way ANOVA with post-hoc Bonferroni analysis, when necessary. Non-parametric analysis and analysis of covariance of ESPVR and EDPVR variables were performed using two-tailed Wilcoxon Signed Rank testing and ANCOVA, respectively, at the $p<0.05$ significance level. Analysis was performed using SPSS v.11.5 (Chicago, IL).

Results

Coronary Embolization

All 12 dogs survived the full study period. A trend towards a higher terminal heart weight was seen in Control dog vs. BNP dogs (211±5 vs. 194±16 g, respectively, $p=0.13$), although terminal mean body weight was similar (26.4±8.7 vs. 23.4±2.4 kg, $p=0.54$), as was the ratio of terminal heart to body weight (0.95±0.14 vs. 0.84±0.18, $p=0.21$). Embolization proceeded without mortality throughout the entire three week Embo period in all Control and
BNP-treated dogs. An average of 31,000 beads were delivered daily to dogs; a slightly higher cumulative bead total was delivered to BNP-treated dogs compared to Control dogs (BNP: 6.6 x 10^5 vs. Control: 6.3 x 10^5 beads over 3 week Embo period, p=0.57).

Hemodynamic Measurements and Echocardiography

Hemodynamics at baseline, after 3 weeks of Embo+Inf, and after 4 weeks of Obs are summarized in Table 1. Baseline hemodynamics and echocardiographic function were comparable prior to embolization in BNP and Control dogs. After 3 weeks of Embo+Inf, the heart failure state was effectively achieved by embolization in both BNP and Control groups, as shown by a 13-14% reduction in MAP, an over 50% increase in LV end-diastolic pressure, and significant decreases in LV dP/dt_max (Figure 1 Panel A) (all p<0.05 vs. Baseline).

However, at the conclusion of both Embo+Inf and Obs periods, a consistent pattern of decreased myocardial injury was obvious in BNP-treated dogs. BNP-treated dogs recorded a significantly higher LV dP/dt_max and EF after Embo+Inf and Obs versus Control dogs, (2466±120 vs. 2042±54 mmHg/s, 53 vs. 46%, respectively, both p<0.01 vs. Control) (Figures 1 Panel A and Figure 2 Panel A). Recovery of ventricular function corresponded with lessened remodeling by echocardiography: ventricular dimensions (LVEDD) did not change after embolization or observation after BNP therapy unlike Control dogs, which experienced an 8% increase in ventricular size (see Figure 2 Panel B). At the end of Obs, the reverse remodeling effects were even more pronounced - LVEDD in BNP-treated dogs was significantly smaller when compared to Control dogs (4.29±0.10 vs. 4.77±0.17 cm, respectively, p<0.05). Similar findings were present for end-systolic dimensions (data not shown). Finally, echocardiographically-derived LV mass progressively increased from 134±6 g to 165±14 g in Control dogs, suggestive of progressive pressure-volume overload stress (Figure 2 Panel C). In
contrast, BNP-treated dogs had no significant evidence of LV hypertrophy (LV mass: 131±6 to 137±8 g, Baseline vs. Obs), despite the aforementioned gains in contractile function.

**Pressure-Volume Analysis**

A complete summary of pressure-volume analyses is found in Table 2. Representative PV loops at steady-state and after IVC occlusion are displayed in Figure 3 Panels A-D. BNP therapy was associated with a maintained end-systolic pressure-volume relationship, as shown by an unchanged $E_{es}$ in BNP-treated dogs versus Control dogs after Embo+Inf and Obs (Figure 1 Panel B) ($p<0.05$ vs Control). ESPVR pressure-volume curves produced similar results, displaying an increase in systolic function in BNP dogs during Embo+Inf compared to a progressive decline in Control dogs; a slight decline in ESPVR was seen after cessation of BNP therapy during Obs in BNP dogs (Figure 4). BNP therapy was also associated with significantly improved myocardial efficiency during Embo+Inf compared with Control therapy alone ($p<0.01$ vs. Control) (Figure 1 Panel C). Analysis of myocardial energetics suggested improved cardiac performance with BNP therapy: trends to improved SW and reduced pressure-volume area at the end of Obs were seen. Lastly, arterial elastance was improved after BNP treatment versus Control ($p<0.05$ vs. Control). The EDPVR demonstrated no significant change.

**Serum cGMP and BNP Levels**

A summary of serum cGMP and BNP levels are displayed in Tables 3 and 4. cGMP levels rose almost ten-fold (27.2±8.8 to 187.1±13.9 pmol/ml, $p=0.001$) after 1 hour of BNP infusion, signifying effective downstream cGMP mediated signaling. After an hour of Embolization+Infusion, cGMP dropped slightly to 127.4±15.9 pmol/ml ($p=0.45$). Serum BNP levels also confirmed effective drug delivery - BNP levels more than doubled in BNP dogs (4.54±0.06 to 9.66±2.02 ng/ml) after 1 hour of Embolization from Baseline, but decreased to
5.20±0.66 ng/ml by 3 weeks of Embo+Inf (both $p<0.05$). BNP levels dropped further in BNP dogs during 4 weeks of Obs, in contrast to Control dogs.

**Immunohistochemistry and Histology**

**Fibrosis**

Extensive ischemic myocardial scar in our study was confirmed on fibrosis staining of the ventricular region at risk for ischemia in both Control and BNP dogs. No statistically significant difference was apparent between Control and BNP dogs (20.6 ± 8.8% and 22.8 ± 16.3), respectively, measured as percentage fibrosis of ischemic region, $p=0.81$.

**Factor VIII Expression**

A strong trend towards increased Factor VIII expression (important in the regulation of angiogenesis and measured as newly formed vessels per high power field) in the ischemic region was seen in dogs treated with BNP versus Control ($p=0.07$ vs. Control).

**COX-2 Expression**

Expression of COX-2, a marker of systemic inflammation, was significantly lower by immunohistochemical staining in ischemic myocardium treated with BNP compared to myocardium from Control therapy ($p<0.05$ vs. Control) (Figure 5). Similar to other histologic variables, this effect was limited to the ischemic regions.

**Macrophage Infiltration**

No change in macrophage infiltration in ischemic myocardium was seen between BNP and Control therapy dogs at experiment termination (50.2±14.7 vs. 53.2 ± 23.1, $p=0.91$).
Clinical studies thus far have focused on using BNP in the chronic heart failure population. The cardiovascular and renal effects after BNP administration in these studies, as well as its neurohormonal inhibitory properties, suggest that use after myocardial infarction may provide protection against the progression of heart failure. Accordingly, the purpose of the current experiment was to evaluate the long-term effects of continuous intravenous BNP infusion on myocardial performance and remodeling after sustained myocardial injury. We present strong evidence for the first time that short-term, continuous BNP infusion preserves systolic function, prevents LV remodeling, and improves cardiac performance specifically after sustained myocardial injury. BNP therapy also reduced expression of COX-2, thereby altering the inflammatory milieu. These study results justify further investigation addressing use after myocardial infarction. The application of BNP therapy to the acute myocardial infarction population could have important clinical implications on reducing the burden of heart failure on the healthcare system, currently estimated as 2% of health care expenditures (31).

Although concern has arisen recently regarding the safety of recombinant BNP (3), numerous earlier clinical reports have demonstrated its clinical benefit. In 2000, Colucci et al. (9) reported a reduction in pulmonary capillary wedge pressure, reduced dyspnea, and less fatigue in patients treated with 6 hours of intravenous nesiritide; these findings were later confirmed by the VMAC trial (33). BNP after cardiac surgery has been studied extensively; short-term intravenous infusion increased cardiac output (14), decreased pulmonary artery pressures (37), and increased urine output (13) in separate small case series. The Nesiritide Administered Peri-Anesthesia in Patients Undergoing Cardiac Surgery (NAPA) trial studied 272 patients randomized to nesiritide or placebo (28). Contrary to previous studies (36), parameters...
of renal function were improved, and 180-day mortality was lower than placebo. To our knowledge, only atrial natriuretic peptide has been used immediately after acute myocardial infarction (15, 21): ten days after a 24 hr infusion of ANP administered after first anterior wall acute myocardial infarction, ejection fraction in treated patients increased while LV dimensions were not changed (15), and ANP reduced sympathetic activity two weeks after myocardial infarction two weeks after myocardial infarction in a similar study (21). BNP offers a potentially superior alternative to ANP, as its slightly longer half-life ($t_{1/2} – 22$ min), peripheral intravenous route, easy dose-adjustability, rapid onset of action, and documented safety in previous studies (9,13,14,28,33,37) make it an attractive drug in post-AMI patients. Furthermore, BNP is a USA FDA approved drug and its safety profile has been partially established already.

**Effect of BNP on Myocardial Function**

Using an extreme myocardial ischemia model, our key findings are as below. First, systolic function was preserved throughout the period of embolization and BNP infusion, as evidenced by the increase in end-systolic elastance and relative preservation of LV $dP/dt_{max}$ compared to Control dogs. Ejection fraction throughout the experiment was higher in BNP-treated dogs. Second, stroke work, as measured by pressure-volume loop analysis, provided confirmation that BNP-treated hearts were capable of generating greater external work. Finally, and most importantly, these gains in systolic function were not at the expense of myocardial oxygen consumption, as there was no change in pressure-volume area; this is in direct contrast to dobutamine, which, like BNP, augments contractility but increases mortality versus BNP (38). The consequence of these favorable inotropic findings of BNP was that myocardial efficiency (4), or ratio of stroke work to pressure-volume area and a measure of global cardiac performance, of the BNP-treated hearts was significantly greater than Control hearts. These
results are also similar to animal findings by Chen et al. (7) in which subcutaneous BNP, given to dogs with pacing-induced heart failure, showed that cardiac output, pulmonary capillary wedge pressure and systemic vascular resistance were all improved after 10 days of therapy. Myocardial perfusion secondary to coronary vasodilation and cGMP-generated nitric oxide (10,23,29) may also help account for gains in systolic function, lower myocardial oxygen demand, and salvage at-risk myocardium thus reducing infarct size (12).

An increase in heart rate was observed during the infusion/embolization period in BNP-treated animals which may partially confound the true extent of BNP’s effect on contractile function in this study. Conflicting reports exist as to whether intravenous BNP affects heart rate (43,44), but high dose therapy has consistently increased heart rate in most studies (24) and in the setting of ventricular dysfunction (27). We speculate that a concurrent fall in mean arterial pressure, coupled with probable BNP-associated natriuresis, stimulated the baroreceptor reflex, thus driving an increase in heart rate (7,16). Systemic vascular resistance, likewise, falls, and preload is decreased due to significant venodilation; these results create favorable conditions for myocardial energetic and pump function, as reflected in the improvements in ejection fraction, LV dP/dt\text{max}, and end-diastolic pressures. However, the effect on heart rate occurred only during BNP infusion, and blood pressure and heart rate equalized among groups after infusion cessation during the 4 week observation period. Most importantly, the gains in cardiac function persisted after BNP infusion cessation, and indices of ventricular remodeling were, in many instances, less pronounced. Furthermore, end-systolic elastance (E\text{es}), a load-independent index, should not be altered with changes in volume status, and was increased in animals treated with BNP, thus supporting the hypothesis that BNP sustains contractile function. Finally, the ability of BNP infusion to interrupt the cycle of heart failure and persist after cessation may suggest intact
natriuretic receptor function, unlike the downregulation or unresponsiveness seen in congestive heart failure (32). The ability of BNP therapy to affect the course of heart failure before receptor desensitization occurs argues even more for its use after acute myocardial injury.

**Effect of BNP on LV Remodeling**

A substantial degree of LV remodeling was prevented by BNP therapy in the current study, and is an important beneficial long-term clinical outcome associated with therapy after sustained myocardial injury. Although the myocardial and vascular pressure-volume unloading secondary to BNP partially contribute to long-term prevention of ventricular remodeling, the biologic properties of BNP on inflammation, proliferation, and neurohormonal inhibition may also account for preservation of ventricular geometry. Using microarray analysis, BNP was previously shown to oppose TGF-β, and genes involved in inflammation (*COX2, IL6, TNF α-induced protein 6, TNF superfamily member 4*), fibrosis (*collagen I, fibronectin, CTGF, PAI-1, TIMP3*), and proliferation (*PDGF, IGF1, FGF18, IGFBP10*), via cGMP dependent protein kinase signaling (20). Our study confirmed a reduction in the inflammatory response mediator COX-2, which may help stabilize myocyte and vascular endothelium in response to ischemic stress. It is also known that BNP inhibits collagen synthesis, increases MMP-1, -2, and -3 (42) and limits cardiac fibroblast growth (6); this occurs likely as a mechanism to limit remodeling when secreted by cardiomyocytes and cardiac fibroblasts in response to stretch and infarction (5,42). Mice with disruption of the BNP gene display exaggerated myocardial fibrosis, hypertension, and upregulation of fibrotic genes (20,40). Although no change in fibrosis and only a trend to lower heart weight was seen in the current study after BNP therapy, anti-proliferative pathways may promote recovery of cells at risk in infarct border and at-risk regions (34). Second, BNP has been shown to block the expression of a number of key neurohormones
central to worsening HF, including endothelin-1, aldosterone, and norepinephrine (1,2,44), as well as inhibition of the renin-angiotensin-aldosterone system (25). Anti-fibrotic, anti-proliferative, and neurohormonal sympathetic inhibitory mechanisms share common molecular pathways, dependent on BNP-related cGMP signaling. Modulation of endogenous cGMP by other pharmacologic means, such as nitrates or bradykinin, has been shown to provide similar myocardial benefits in animal models, supporting the favorable biologic actions of BNP (19,26).

The clinical manifestation of attenuation of ventricular remodeling can be seen in our study results: cardiac dimensions did not change throughout embolization or four weeks after BNP treatment, unlike Control dog dimensions, which progressively increased even in the absence of embolization-induced injury in the observation period. Arterial elastance, representing ventriculo-vascular coupling and central arterial changes, demonstrated a beneficial effect after BNP therapy. Echocardiographically derived LV mass and ventricular dimensions were both lower in BNP-treated dogs, consistent with previous literature showing that natriuretic peptides decrease myocyte hypertrophy (41); in contrast, LV mass and ventricular dimensions significantly increased in Control dogs, signifying LV hypertrophy and late dilatation in response to pressure-volume overload. In Control dogs, as cardiac geometric dimensions increase, LV wall stress (governed by the law of Laplace) and, thus, myocardial oxygen consumption, should increase (4); this was also confirmed by pressure-volume analysis. This sustained increase in wall stress and stretch response feeds the vicious cycle of heart failure progression in Control dogs, activating compensatory sympathetic pathways ultimately leading to decompensated hemodynamics. BNP counteracts these pathologic mechanisms on multiple levels: hemodynamic, renal, and neurohormonal. As discussed earlier, improved hemodynamics maintain coronary and organ perfusion during acute injury. Renal mechanisms regulate volume
status and reduce myocardial workload, and the specific actions of BNP on cardiomyocyte proliferation, fibrosis, inflammation, and sympathetic response mediate cardiac and vascular remodeling over a long-term period.

**Coronary Embolization Model and Clinical Applicability**

The coronary-embolization model employed in the current experiment deserves mention. In order to create an extreme and sustained myocardial ischemia state and test whether BNP therapy can overcome the pathology associated with myocardial ischemia induced heart failure, the daily coronary embolization model was chosen: 1) to create hemodynamic derangement that is consistent with heart failure over a three-week time frame, 2) to use a well-known and established model of, specifically, ischemic injury with infarction (22, 35), and 3) to maintain constant and persistent stimulation for pathologic remodeling through daily embolization. In our experience, simple coronary ligation is not sufficient to create the level of heart failure required to mimic large human myocardial infarction; extensive coronary collaterals aid myocardial recovery in a short time in canines. Furthermore, the specific method of injury, an ischemic insult, was necessary to mimic an appropriate human clinical setting, and was the reason pacing induced heart failure was not used. The coronary embolization model fulfills these criteria, and awake measurements provide reliable and clinically relevant measurements. The study design was intended to replicate symptom onset to response by medical personnel, with induction of injury first, followed by administration of BNP after 60 minutes. The model does not replicate the physiology of reperfusion after revascularization or acute infarction – rather, it replicates remodeling that occurs after a severe prolonged infarction or repeated infarction without revascularization. The study findings, therefore, represent the most extreme form of acute
ischemic myocardial injury, and it is plausible that improvement in this model also translates to improvement in less severe ischemic injury.

**Conclusions**

In conclusion, acute myocardial injury was created in awake dogs using daily coronary embolizations. Continuous intravenous BNP therapy preserved systolic function and attenuated LV remodeling, supported by both energetic and biochemical mechanisms. These results provide a rationale for prolonged intravenous BNP treatment after myocardial infarction with the goal of prevention of the chronic heart failure state.
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Figure Legends

Figure 1. Hemodynamics and Pressure-Volume Analysis. In Panel A, LV dP/dt\textsubscript{max} was significantly depressed from Baseline after embolization+infusion (Embo+Inf) in both BNP and Control dogs; however, BNP-treated dogs experienced a significantly smaller decline throughout both Embo+Inf and the observation period (Obs). Panel B depicts a significant improvement in end-systolic elastance (E\textsubscript{es}) during Embo+Inf in BNP-treated dogs compared to Control dogs. Finally, myocardial efficiency was greatly enhanced during BNP therapy versus Control therapy (Panel C). * \( p < 0.05 \) vs. Baseline, † \( p < 0.05 \) vs. Control, ‡ \( p < 0.01 \) vs. Control.

Figure 2. Echocardiographic changes before and after BNP therapy. (Panel A) Ejection fraction in the BNP cohort was preserved when compared to the Control cohort throughout the experiment. Similarly, in Panel B, left ventricular end-diastolic dimension (LV EDD) was smaller in BNP-treated dogs after embolization+infusion (Embo+Inf) and observation (Obs). LV mass, as shown in Panel C, showed no change after BNP therapy during Embo+Inf and Obs, in contrast to Control therapy which demonstrated a significant increase in LV mass after embolization and after a period of observation. * \( p < 0.05 \) vs. Baseline, † \( p < 0.05 \) vs Embo+Inf, ‡ \( p < 0.05 \) vs Control, ¶ \( p < 0.005 \) vs Control.

Figure 3. Representative Pressure-Volume (PV) loops at steady state (Panels A, B) and after Inferior Vena Cava Occlusion (Panels C, D) in BNP-treated and Control dogs. After the embolization + infusion period (Embo+Inf), PV loops were shifted to the right and downwards in Control dogs along with an increase in cardiac dimensions, while PV loops after BNP therapy...
demonstrated a slight decrease in cardiac dimensions during embolization without shifting relative location.

**Figure 4.** Changes in End-Systolic Pressure Volume Relationship (ESPVR). In Panel A, an increase in contractile function from Baseline to Embo/Inf is seen in BNP dogs, as demonstrated by a shift to the left, while a slight decline is seen during Obs. Panel B shows a progressive decline in ESPVR in Control dogs from Baseline to Embo+Inf through Obs, as shown by shifting of the curves downwards and to the right. *p* < 0.05 vs. Baseline, †*p* < 0.05 vs Embo+Inf

**Figure 5.** COX-2 expression. Panel A depicts Control and BNP slides stained for COX-2 expression at 200X and the area outlined in green at 400X magnification. COX-2 expression in ischemic myocardium was significantly higher in BNP-treated dogs compared to Control (*p* < 0.05 vs. Control) (Panel B).
### Table 1. Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Embo+Inf</th>
<th>Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>75.5 ± 2.5</td>
<td>65.5 ± 3.6</td>
<td>72.0 ± 4.9</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>76.0 ± 3.6</td>
<td>93.5 ± 5.7§</td>
<td>83.5 ± 5.4</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>95.5 ± 2.2</td>
<td>88 ± 4.4*</td>
<td>87 ± 4.3</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>99.8 ± 1.6</td>
<td>86.7± 5.0*</td>
<td>93.7 ± 3.2</td>
</tr>
<tr>
<td><strong>LVESP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>119.5 ± 2.4</td>
<td>111.7 ± 2.4</td>
<td>113.6 ± 4.4</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>124.0 ± 1.9</td>
<td>107.3 ± 4.8*</td>
<td>114.3 ± 3.9</td>
</tr>
<tr>
<td><strong>LVEDP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>12.9 ± 1.8</td>
<td>20.4 ± 1.3*</td>
<td>18.3 ± 0.9*</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>11.3 ± 1.7</td>
<td>17.5 ± 2.9*</td>
<td>17.6 ± 2.7*</td>
</tr>
</tbody>
</table>

* p<0.05 vs. Baseline, † p<0.05 vs. Control, ‡ p<0.01 vs. Control, § p<0.005 vs. Control

Values are Mean±SE. MAP-mean arterial pressure, LVESP-left ventricular end-systolic pressure, LVEDP-left ventricular end-diastolic pressure
Table 2. Pressure-Volume Analysis

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Embo+Inf</th>
<th>Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0 (ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>-4.32 ± 5.06</td>
<td>-21.24 ± 4.22*</td>
<td>-1.50 ± 8.78</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>19.39 ± 18.48</td>
<td>2.64 ± 6.65†</td>
<td>4.58 ± 5.25</td>
</tr>
<tr>
<td>α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>0.028 ± 0.010</td>
<td>0.018 ± 0.004</td>
<td>0.036 ± 0.010</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>0.052 ± 0.015</td>
<td>0.038 ± 0.009</td>
<td>0.035 ± 0.005</td>
</tr>
<tr>
<td>β</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>2.74 ± 1.60</td>
<td>2.86 ± 1.00</td>
<td>0.49 ± 0.36</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>2.54 ± 2.10</td>
<td>5.70 ± 4.36</td>
<td>0.50 ± 0.12</td>
</tr>
<tr>
<td>SW/LV Mass (mmHg*ml/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>28.4 ± 2.0</td>
<td>23.3 ± 4.9</td>
<td>25.2 ± 2.3</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>24.8 ± 4.5</td>
<td>32.6 ± 13.0</td>
<td>22.8 ± 2.0</td>
</tr>
<tr>
<td>PVA/LV Mass (mmHg*ml/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>51.7 ± 3.0</td>
<td>52.4 ± 8.1</td>
<td>49.2 ± 5.4</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>49.7 ± 5.1</td>
<td>50.2 ± 15.1</td>
<td>46.3 ± 2.9</td>
</tr>
<tr>
<td>Ea (mmHg/ml/m²)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control (n=6)</td>
<td>3.56 ± 0.69</td>
<td>3.17 ± 0.29</td>
<td>2.67 ± 0.21</td>
</tr>
<tr>
<td>BNP (n=6)</td>
<td>5.39 ± 1.48</td>
<td>4.13 ± 0.32†</td>
<td>3.50 ± 0.22†</td>
</tr>
</tbody>
</table>

* p<0.05 vs. Baseline, † p<0.05 vs. Control, ‡ p<0.01 vs. Control

Values are Mean±SE. V0-volume intercept, α-myocardial stiffness, β-scaling constant, SW-stroke work, PVA-pressure-volume area, Ea-arterial elastance
Table 3. cGMP Levels in BNP-treated Animals (n=2)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 hr after Inf</th>
<th>1 hr after Inf+Embo</th>
</tr>
</thead>
<tbody>
<tr>
<td>cGMP (pmol/ml)</td>
<td>27.2 ± 8.8</td>
<td>187.1 ± 13.9*</td>
<td>127.4 ± 15.9†</td>
</tr>
</tbody>
</table>

Inf-Infusion, Embo-Embolization

* p<0.05 vs. Baseline, † p<0.05 vs. 1 hr after Inf+Embo
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Embo 1 hr</th>
<th>Embo+Inf 1</th>
<th>Embo/Inf 1</th>
<th>Embo/Inf</th>
<th>Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (n=6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.88 ± 0.37</td>
<td>19.57 ± 6.46†</td>
<td>-</td>
<td>2.88 ± 0.26</td>
<td>2.92 ± 0.87</td>
<td>3.36 ± 0.31</td>
</tr>
<tr>
<td><strong>BNP (n=6)</strong></td>
<td>4.54 ± 0.06*</td>
<td>9.66 ± 2.02†</td>
<td>9.33 ± 2.61</td>
<td>9.83 ± 0.72*</td>
<td>5.20 ± 0.66¶</td>
<td>4.38 ± 0.08*</td>
</tr>
</tbody>
</table>

* p<0.05 vs. Control, † p<0.05 vs. Baseline, ¶ p<0.05 vs. Embo/Inf 1 wk

Embo-Embolization, Inf-Infusion, Obs-Observation
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5