

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Circulation 2004;109:1680-1685; originally published online Mar 15, 2004;

DOI: 10.1161/01.CIR.0000124064.00494.21

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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Brain Natriuretic Peptide Enhances Renal Actions of Furosemide and Suppresses Furosemide-Induced Aldosterone Activation in Experimental Heart Failure

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Background—The renal actions of brain natriuretic peptide (BNP) in congestive heart failure (CHF) are associated with increased diuresis and natriuresis, preserved glomerular filtration rate (GFR), and lack of activation of the renin-angiotensin-aldosterone system (RAAS). In contrast, diuretic-induced natriuresis may be associated with reduced GFR and RAAS activation. The objective of this study was to test the hypothesis that exogenous BNP enhances the renal diuretic and natriuretic actions of furosemide (Fs) and retards the activation of aldosterone in a model of CHF.

Methods and Results—CHF was produced in 2 groups of dogs by ventricular pacing. One group received continuous (90-minute) intravenous Fs ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). A second group (Fs+BNP) received 45-minute intravenous coinfusion of Fs ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and low-dose ($2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP followed by 45-minute coinfusion of Fs ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and high-dose ($10 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP. Fs increased urinary flow, but the effect of Fs+BNP was greater. Similarly, urinary sodium excretion was higher in the Fs+BNP group. Although GFR tended to decrease in the Fs group, it increased in the Fs+BNP group (35 ± 3 to $56 \pm 4^*$) (* indicates $P < 0.05$ versus baseline) ($P < 0.0001$ between groups). Plasma aldosterone increased with Fs (41 ± 10 to $100 \pm 11^*$ ng/dL) but was attenuated in the Fs+BNP group (44 ± 11 to 54 ± 9 ng/dL low-dose and to 47 ± 7 ng/dL high-dose) ($P = 0.0007$ between groups).

Conclusions—Fs+BNP has more profound diuretic and natriuretic responses than Fs alone and also increases GFR without activation of aldosterone. Coadministration of BNP and loop diuretic is effective in maximizing natriuresis and diuresis while preserving renal function and inhibiting activation of aldosterone. (*Circulation*. 2004;109:1680-1685.)

Key Words: diuretics ■ natriuretic peptide, brain ■ renin-angiotensin system ■ glomerular filtration rate

Congestive heart failure (CHF) is characterized by avid sodium and water retention. Despite advances in therapy, CHF continues to be associated with high morbidity and mortality, and its prevalence is increasing. Loop diuretics are powerful natriuretic agents and are considered first-line therapy in the management of CHF with volume overload. Several studies support the important and positive impact of diuretics in cardiovascular disease. Indeed, the use of diuretics in the Systolic Hypertension in the Elderly Program (SHEP) trial reduced the development of CHF in elderly individuals with hypertension.¹ Despite their widespread use in CHF, studies have reported detrimental actions of diuretics.²⁻⁴ Over time and in severe CHF, sensitivity to diuretics may be reduced, requiring an increase in the dose administered. Investigators have shown that even low-dose loop diuretics can reduce glomerular filtration rate (GFR) and activate the renin-angiotensin-aldosterone system (RAAS) acutely and chronically.⁵ The importance of GFR is underscored by recent studies that have reported that GFR is a key

predictor of mortality in CHF,⁶ whereas activation of the RAAS has deleterious effects on cardiac remodeling, leading to accelerated morbidity and mortality.⁷ Therefore, new therapeutic strategies are warranted to minimize theoretical side effects of conventional diuretics and to potentiate their renal actions.

Human brain natriuretic peptide (BNP) has been approved by the Food and Drug Administration for treatment of acutely decompensated CHF. BNP is a 32-amino-acid peptide with natriuretic, vasodilating, lusitropic, sympathetic, and renin- and aldosterone-inhibiting properties.^{8,9} BNP is structurally similar to atrial natriuretic peptide (ANP) but genetically distinct. BNP is primarily of myocardial cell origin, with recent reports of its production in cardiac fibroblasts,¹⁰ and it plays a role in the control of sodium excretion and blood pressure. Both ANP and BNP are released by the heart in CHF and have a compensatory role in cardiorenal homeostasis.^{11,12} Studies have established that BNP binds to the natriuretic peptide receptor A, which, via 3',5'-cGMP, medi-

Received March 5, 2003; de novo received September 29, 2003; revision received December 8, 2003; accepted December 10, 2003.

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Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/01.CIR.0000124064.00494.21

ates its biological actions.^{13,14} Compared with ANP, BNP has emerged as the more potent peptide on the basis of its greater natriuretic actions, decreased susceptibility for degradation by neutral endopeptidase 24.11, and enhanced ability to augment cGMP.¹⁵

Although the beneficial action of exogenous BNP has been reported in experimental CHF and normal human subjects,¹⁶ the renal actions of BNP in human CHF are still controversial. Marcus et al¹⁷ reported beneficial renal actions of BNP, specifically increased sodium excretion, in CHF patients. However, Jensen et al¹⁸ observed a blunted natriuretic response without an increase in GFR in CHF after BNP administration. In addition, in the Vasodilation in the Management of Acute Congestive heart failure (VMAC) study, in which BNP was administered in patients with acute CHF, the authors reported a greater increase in creatinine in those patients receiving BNP, although this subgroup had more severe CHF compared with nitroglycerin groups.¹⁹ To date, the effects of exogenous BNP on cardiorenal and neurohumoral function during coadministration of a loop diuretic remain unknown.

The present study tested the hypothesis that BNP enhances the renal actions of furosemide (Fs) in experimental overt CHF. We hypothesized that exogenous BNP in combination with Fs would maintain GFR and suppress the RAAS while enhancing Fs-mediated diuretic and natriuretic actions. On the basis of the present findings, more studies in humans are warranted.

Methods

The present study was performed in accordance with the Animal Welfare Act and approval of the Mayo Clinic Animal Care and Use Committee.

Pacing-Induced Overt CHF

Severe CHF was induced in dogs (weight, 18 to 24 kg) by rapid ventricular pacing at 240 bpm for 10 days.²⁰

Acute Protocol

After 10 days of pacing, dogs were anesthetized with pentobarbital (15 mg/kg IV), intubated, and ventilated with oxygen. A thermodilution catheter was inserted via the right jugular vein for hemodynamic measurements. The femoral vein was cannulated for infusions, and the femoral artery was cannulated for arterial pressure measurements and blood sampling. Pressures were recorded and analyzed digitally (Sonometrics). Via a left lateral flank incision, the ureter was cannulated for urine sampling, and the renal artery was equipped with a flow probe (Carolina Medical). Cardiac output was measured by thermodilution (Cardiac output computer model 9510-A, American Edwards).

After preparation, a 60-minute equilibration period was started with intravenous inulin bolus, followed by continuous inulin infusion to achieve a steady-state plasma inulin concentration between 40 and 60 mg/dL. A 30-minute baseline clearance was performed that consisted of hemodynamic measurements, arterial blood sampling, and urine collection. After baseline measurements, dogs were randomly assigned to 1 of 2 groups. Group 1 (n=6) received continuous (90-minute) intravenously administered Fs ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), and group 2 (n=5) received a combination of Fs plus BNP. The second group (Fs+BNP) received a 45-minute intravenous coinfusion of Fs ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and low-dose ($2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP followed by 45-minute intravenous coinfusion of Fs ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and high-dose ($10 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP. Thus, the high dose of BNP was a later time measurement consistent with a dose-

response design. Hemodynamic and humoral data were measured for 30 minutes after a 15-minute lead-in period after starting Fs or both (low- and high-dose) Fs+BNP infusions. After a 30-minute washout period, a 30-minute recovery clearance was performed.

Hormone and Electrolyte Analysis

Plasma and urine cGMP and plasma renin activity (PRA) were measured by radioimmunoassay.²¹ Aldosterone was measured by microassay.²² Plasma norepinephrine (NE) was measured at Mayo Clinic Endocrinology Laboratory.²³ Urinary and plasma inulin were measured by the anthrone method and electrolytes by flame spectrophotometer. GFR was determined by inulin clearance.

Statistical Analysis

Results are expressed as mean \pm SEM. Data were assessed by 1-way ANOVA for comparisons within groups followed by post hoc Dunnett's test. Two-way ANOVA was used for comparisons between groups, followed by a Bonferroni posttest. Student's unpaired *t* test was performed for single comparisons between groups (Graph-Pad Prism software 3.0). Statistical significance was accepted at a value of $P < 0.05$.

Results

Baseline Hemodynamic and Humoral Parameters

No differences in cardiorenal, vascular hemodynamic, and humoral parameters were observed at baseline between groups.

Systemic Hemodynamics With Treatment

Table 1 reports systemic hemodynamics. Fs plus high-dose BNP reduced right atrial pressure (RAP) and pulmonary capillary wedge pressure, whereas Fs alone did not. Reduction of cardiac output (CO) was not sustained in the Fs+BNP group, whereas it was persistent in the Fs group. Systemic vascular resistance (SVR) was increased in the Fs but not in the Fs+BNP group.

Renal Hemodynamics, Urinary Flow, and Sodium Excretion With Treatment

Fs+BNP increased renal blood flow, whereas it did not change with Fs alone (Table 1). GFR tended to decrease in the Fs group, whereas it increased in the Fs+BNP group, thus resulting in a significant difference between groups (Figure 1A). Fs significantly increased urinary flow; however, the increase in urinary flow following Fs+BNP infusion was significantly higher (Figure 1B). Similarly, urinary sodium excretion was significantly higher in the Fs+BNP group (Figure 1C).

Humoral Parameters With Treatment

PRA significantly increased with Fs, whereas its increase was not significant with Fs+BNP (Figure 2A). Similarly, aldosterone increased with Fs but did not change in the Fs+BNP group (Figure 2B). No changes in plasma cGMP were observed in the Fs group, whereas plasma cGMP was significantly elevated in the Fs+BNP group with high-dose BNP (Figure 2C). Urinary cGMP excretion was significantly reduced after Fs administration, whereas it increased in the Fs+BNP group with high-dose BNP (Figure 2D). Table 2 reports plasma NE in the Fs alone and Fs+BNP groups. No difference in plasma NE concentration was observed between the 2 groups at baseline. NE increased in both groups after Fs infusion.

TABLE 1. Cardiorenal Hemodynamics

	Baseline	Low Dose	High Dose	Recovery
MAP, mm Hg				
Fs	105±8	108±8	100±7	96±7
Fs+BNP	104±1	107±3	98±3	102±4
RAP, mm Hg				
Fs	6.2±1.6	5.5±1.6	5.2±1.7	5.0±1.9
Fs+BNP	6.0±1.2	4.9±1.2	4.2±1.1*	4.9±0.9
PAP, mm Hg				
Fs	21±3	20±3	21±3	22±3
Fs+BNP	23±2	21±3	19±3	22±2
PCWP, mm Hg				
Fs	16±3	14±2	14±2	15±2
Fs+BNP	18±2	15±2	13±3*	14±2
CO, L/min				
Fs	2.0±0.1	1.9±0.2	1.6±0.2*	1.6±0.2*
Fs+BNP	2.1±0.1	2.0±0.1	1.7±0.1*	1.9±0.1
SVR, mm Hg · L ⁻¹ · min ⁻¹				
Fs	49±5	55±5	61±6*	64±7*
Fs+BNP	48±2	53±3	56±3	57±5
RVR, mm Hg · L ⁻¹ · mm ⁻¹				
Fs	0.81±0.16	0.77±0.15	0.82±0.17	1.04±0.27
Fs+BNP	0.71±0.16	0.61±0.16	0.51±0.10	0.63±0.13
RBF, mL/min				
Fs	151±35	168±40	148±36	128±38
Fs+BNP	170±36	220±53*	224±51*	187±43

MAP indicates mean arterial pressure; RAP, right atrial pressure; CO, cardiac output; SVR, systemic vascular resistance; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; RVR, renal vascular resistance; and RBF, renal blood flow. Values are expressed as mean±SEM.

* $P<0.05$ vs Baseline.

Discussion

This study defines the acute cardiorenal and humoral responses to coadministration of Fs and BNP in experimental CHF. We observed that Fs+BNP had favorable cardiovascular hemodynamic actions compared with Fs alone. Fs+BNP increased GFR and resulted in more profound diuresis and natriuresis responses without renin and aldosterone activation.

The cardiorenal and humoral responses to Fs observed in the present study are consistent with reports in experimental and human CHF. Specifically, Fs resulted in persistent reduction of CO and increase of SVR, whereas the reduction in CO with Fs+BNP was transient and returned to baseline after discontinuation of the infusion. Fs increased urine flow and urinary sodium excretion, tended to reduce GFR, promoted sustained activation of PRA and aldosterone, and reduced urinary cGMP excretion. The increases in PRA, aldosterone, and catecholamines, specifically NE, observed in the Fs group are consistent with observations that documented activation of the RAAS and sympathetic system in response to Fs in patients with CHF.⁵

Although we did not observe significant differences between the Fs alone and Fs+BNP group in mean arterial

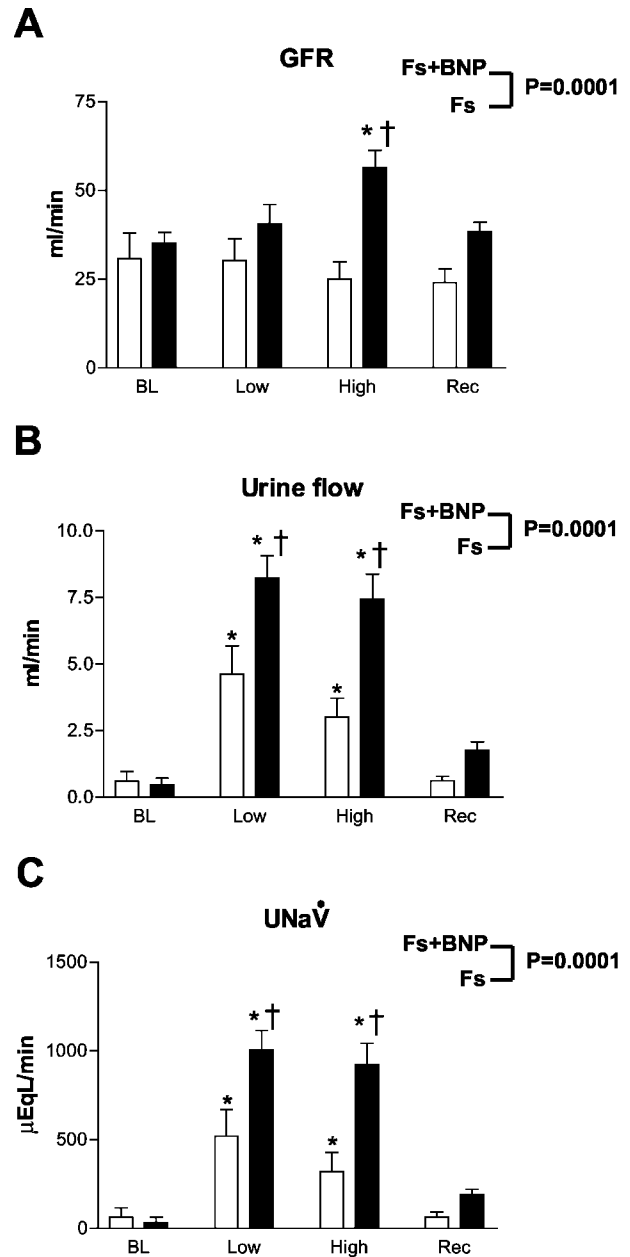


Figure 1. Open bar, Fs; closed bar, Fs+BNP. BL, baseline; Low, low-dose ($2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP plus Fs; High, high-dose ($10 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP plus Fs; Rec, recovery. A, GFR in Fs and Fs+BNP groups. * $P<0.005$ vs BL, 1-way ANOVA, Dunnett posttest. † $P<0.001$ vs Fs, 2-way ANOVA, Bonferroni posttest. $P<0.0001$ between Fs and Fs+BNP groups by 2-way ANOVA. B, Urine flow in Fs and Fs+BNP groups. * $P<0.0001$ vs BL, 1-way ANOVA, Dunnett posttest. † $P<0.001$ vs Fs, 2-way ANOVA, Bonferroni posttest. $P<0.0001$ between Fs and Fs+BNP groups by 2-way ANOVA. C, Urinary sodium excretion in Fs and Fs+BNP groups. * $P<0.0001$ vs BL, 1-way ANOVA, Dunnett posttest. † $P<0.001$ vs Fs, 2-way ANOVA, Bonferroni posttest. $P<0.0001$ between Fs and Fs+BNP groups by 2-way ANOVA.

pressure, RAP, pulmonary artery pressure, pulmonary capillary wedge pressure, CO, SVR, renal vascular resistance, and renal blood flow, there were some differential responses between these 2 groups in some hemodynamic parameters. Specifically, when high-dose BNP was coadministered with Fs, CO, RAP, and pulmonary capillary wedge pressure were

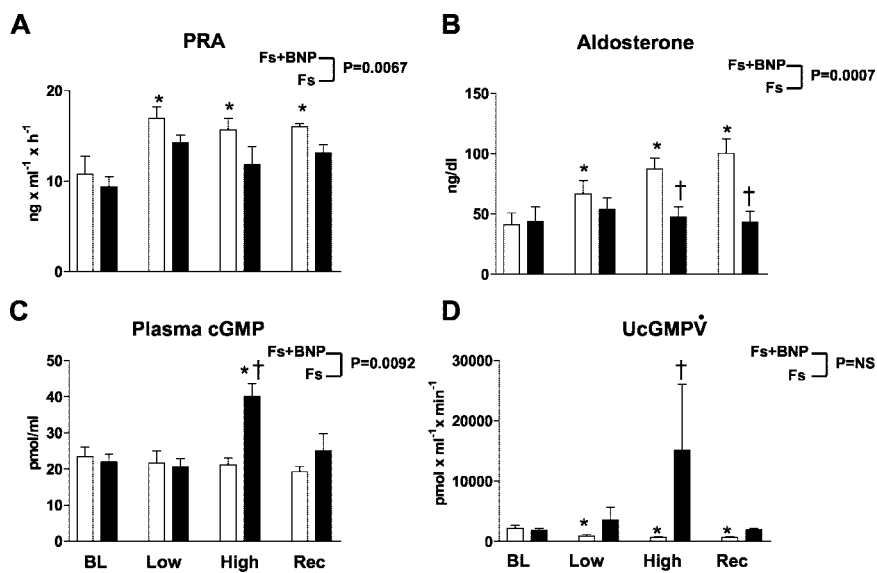


Figure 2. Open bar, Fs; closed bar, Fs+BNP. BL, baseline; Low, low-dose ($2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP plus Fs; High, high-dose ($10 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) BNP plus Fs; Rec, recovery. A, PRA in Fs and Fs+BNP group. $*P < 0.001$ vs BL, 1-way ANOVA, Dunnett posttest. $P = 0.0067$ between Fs and Fs+BNP groups by 2-way ANOVA. B, Plasma aldosterone in Fs and Fs+BNP groups. $*P < 0.001$ vs BL, 1-way ANOVA, Dunnett posttest. $\dagger P < 0.01$ vs Fs 2-way ANOVA, Bonferroni posttest. $P = 0.0007$ between Fs and Fs+BNP groups by 2-way ANOVA. C, Plasma $3',5'$ -cGMP in Fs and Fs+BNP groups. $*P < 0.001$ vs BL, 1-way ANOVA, Dunnett posttest. $\dagger P < 0.001$ vs Fs, 2-way ANOVA, Bonferroni posttest. $P = 0.0092$ between Fs and Fs+BNP groups by 2-way ANOVA. D, Urinary cGMP in Fs and Fs+BNP groups. $*P < 0.001$ vs BL, 1-way ANOVA, Dunnett posttest. $\dagger P < 0.05$ vs Fs, 2-way ANOVA, Bonferroni posttest. $P = \text{NS}$ between Fs and Fs+BNP groups by 2-way ANOVA.

reduced without increase in SVR. The lack of increase in SVR may be a result of differential modulation of angiotensin II, which was inhibited with BNP and the vasodilator properties of BNP. Interestingly, BNP did not suppress sympathetic system activation induced by Fs, as indicated by a similar increase in NE in both groups. This observation is similar to reports by Francis et al.⁴ Specifically, the authors demonstrated combined neurohormonal stimulation and concomitant hemodynamic deterioration with Fs in CHF patients.⁴ Goldsmith et al²⁴ further reported a partial suppression of sympathetic activity induced by Fs with ACE inhibitor (ACEI) in human CHF concomitant with a blunting of the pressor responses to acute Fs. It would be interesting in future studies to investigate whether coadministration of Fs and BNP together with an ACEI, an angiotensin receptor blocker (ARB), or a β -blocker could further suppress sympathetic system activation induced by Fs. In addition, the reduction in CO, RAP, and PWCP may be related to the intense diuretic actions observed with Fs+BNP and to the venous and arterial dilating actions of BNP. The reduction of CO could also be related to the activation of NE. BNP also increased renal blood flow at low and high doses because of vasodilatation of the preglomerular vessels.²⁵ With high-dose BNP, we observed an increase in GFR concomitant with an increase of plasma and urinary cGMP. The increase in GFR is consistent with known properties of the natriuretic peptides to increase ultrafiltration coefficient and inhibit tubuloglomerular feedback.²⁶ BNP also augmented Fs-mediated increases in urinary flow and sodium excretion. The mechanism of this enhanced diuresis and natriuresis may be multifactorial. The concomitant increase of GFR may have resulted in greater delivery of water and sodium to

Henle's loop, resulting in greater increases in water and sodium excretion. However, it should be noted that GFR increased only with high-dose BNP, whereas the greater diuretic and natriuretic responses were observed even with low-dose BNP. Therefore, other mechanisms should be taken into consideration. First, BNP decreases proximal and distal reabsorption of water and sodium, thus further decreasing water and sodium resorption secondary to Fs.¹⁴ Another mechanism may be the lack of activation of renin and aldosterone. Suppression of the RAAS may have prevented the antinatriuretic actions of angiotensin II on the proximal tubule and of aldosterone on the distal tubule.^{27,28} It should be noted that our findings are not completely consistent with those of previous clinical studies. In particular, improvements in GFR with BNP have not been observed in some CHF patients.^{18,29} In the Prospective Randomized ECTopy Evaluation on DobutaminE or Nesiritide Therapy (PRECEDENT) trial, the incidence of elevations in serum creatinine above baseline through day 14 was higher in the BNP group than with standard therapy. In the VMAC trial, through day 30, the incidence of elevations in creatinine to $>0.5 \text{ mg/dL}$ above baseline was 28% and 21% in the BNP and nitroglycerin groups, respectively.¹⁹ The findings of these trials and of the present investigation warrant further studies of the renal action of BNP in human CHF using a wide range of doses.

We previously reported that enhancement of the natriuretic peptide system via vasopeptidase inhibition improves the cardiorenal hemodynamic actions of Fs in experimental CHF.³⁰ The present study aimed to address whether exogenous BNP could acutely potentiate the diuretic actions of Fs and maintain GFR without activation of aldosterone in severe experimental CHF. This setting has clinical relevance, because studies have shown that over time, patients with advanced CHF become resistant to loop diuretics. This is due in part because of decreased renal perfusion and increased sodium reabsorption at the diuretic-insensitive sites in the nephron and of the concomitant increase in sodium-retaining hormones such as angiotensin II and aldosterone. Indeed, activation of the RAAS and of the sympathetic system may contribute to the reduction in GFR secondary to both acutely

TABLE 2. Plasma Norepinephrine Concentration

NE, pg/mL	Baseline	Low Dose	High Dose	Recovery
Fs	417.1 \pm 22	503.7 \pm 13*	508.8 \pm 13*	460.2 \pm 33
Fs+BNP	414.8 \pm 25	509.2 \pm 25*	506.6 \pm 22*	404.4 \pm 13

Values are expressed as mean \pm SEM.

* $P < 0.05$ vs Baseline.

and chronically administered loop diuretics.^{3,31} Hillege et al⁶ reported that decreased GFR is a major predictor of morbidity and mortality in patients with CHF. Furthermore, activation of aldosterone may have deleterious actions on cardiac remodeling and fibrosis, thus accelerating progression of CHF and prompting use of aldosterone antagonists. The use of spironolactone has been encouraged by the results of the Randomized Spironolactone Evaluation Study (RALES).³² However, more recently, Bozkurt et al³³ reported that complications with the use of spironolactone in patients with CHF are greater in clinical practice than reported in the RALES, implying the need for longer-term studies. We recently reported that concomitant use of ARBs might prevent detrimental renal actions of acute diuretics in human CHF.³⁴ Furthermore, our present study supports the use of BNP to reduce resistance to loop diuretics and oppose their potential side effects, especially in the setting of acute CHF. Thus, BNP may represent a unique therapeutic strategy aimed to both reduce the renal resistance and to minimize the side effects of loop diuretics in CHF. However, it should be mentioned that at present, the use of BNP has been approved only for acute CHF, whereas ACEIs, ARBs, and aldosterone antagonists have been effective in reducing cardiovascular events in CHF patients and are currently available for chronic treatment.

There are limitations to the present study. First, the study was performed in an experimental model of CHF; thus, further studies in humans are required. Furthermore, it should be also noted that high doses ($10 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) of BNP used in the present study were equivalent to the high doses ($0.03 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) recommended for the treatment of human CHF. However, we did not precede continuous infusion of BNP with a high dose ($2 \mu\text{g}/\text{kg}$ bolus) of BNP; thus, the overall dose of BNP used in the present study was lower than the overall dose of BNP often used in the treatment of acutely decompensated human CHF. We chose this dose on the basis of preliminary studies indicating that these are the most effective doses to ensure complete pharmacological action of BNP in dogs (data not shown). A further limitation is that mean arterial pressure in our model was greater than that often observed in patients with overt CHF. Further studies are also warranted to address the modulating actions of coadministration of Fs and BNP with ACEIs, ARBs, or β -blockers, which are often concomitant therapy in patients with CHF. Indeed, the concomitant use of an ACEI or ARB and Fs may blunt the activation of the RAAS, as observed after Fs alone in the present study. Although we did not observe any rebound activation of RAAS 60 minutes after discontinuation of BNP, a later activation is possible. Thus, more prolonged studies are warranted.

In conclusion, our study represents a highly controlled report of combined Fs and BNP in a model of experimental CHF. This study provides important new insights into interactions between exogenous BNP and loop diuretics in this canine model of CHF. Specifically, our studies demonstrate that coadministration of BNP and furosemide unloads the heart and inhibits activation of aldosterone while maximizing

natriuresis and diuresis and preserving renal function in this model of experimental CHF.

Acknowledgments

This study was supported by grants HL-36634 and HL-07111 from the National Institutes of Health, the Miami Heart Research Institute, the Mayo Foundation, an Unrestricted Grant for Cardiovascular Research by Bristol-Myers Squibb Co, and the Marriott Foundation. The authors gratefully acknowledge the assistance of Linda Combs, Cindy Reed, Gail Harty, Sharon Sandberg, and Brenda Huntley.

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