Cardiorenal Actions of Neutral Endopeptidase Inhibition in Experimental Congestive Heart Failure

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The present studies were designed to determine the action of neutral endopeptidase inhibition (NEP-I), an inhibitor of the degradation of atrial natriuretic factor (ANF), in congestive heart failure (CHF). Studies were conducted in two groups of anesthetized dogs with CHF induced by 8 days of rapid right ventricular pacing. Group 1 (n=5) received a specific NEP-I (SQ 28,603) at two doses administered sequentially—30 mg/kg followed by a 60 mg/kg i.v. bolus. Group 2 (n=5) received intravenous infusion of exogenous ANF (100 ng/kg/min) to achieve increases in plasma ANF concentration as observed in group 1. NEP-I resulted in a diuresis and natriuresis (p<0.05) with increases in the fractional excretion of sodium and fractional excretion of lithium, the latter a marker for proximal tubule sodium delivery. Such tubular actions occurred in the absence of increases in glomerular filtration rate or renal blood flow but were associated with significant increases in urinary ANF and urinary cyclic GMP. Plasma ANF increased after the 30 mg/kg NEP-I dose. In contrast, in group 2 with exogenous ANF and despite a marked increase in plasma ANF, no natriuresis was observed. Arterial pressure did not change in either group. These studies demonstrate for the first time in CHF that NEP-I may potentiate the natriuretic action of endogenous ANF by a mechanism that is independent of systemic or renal hemodynamics and does not parallel increases in plasma ANF. These studies support an important therapeutic role for NEP-I in CHF. (Circulation 1990;82:196–201)

Atrial natriuretic factor (ANF) is a peptide hormone of cardiac origin with vasorelaxant properties that may increase sodium and water excretion and inhibit the renin-angiotensin-aldosterone system.1–3 Based on such unique cardiorenal actions, a role has been advanced for ANF in the treatment of congestive heart failure (CHF).4 In support of such a therapeutic role are studies by Saito et al5 and Molina et al,6 which have demonstrated that high-dose ANF may decrease peripheral vascular resistance and increase cardiac output. Despite such vasorelaxant responses, CHF is characterized by markedly blunted diuretic and natriuretic responses to exogenous ANF despite increases in glomerular filtration rate (GFR).7,8

Recent studies by Berg et al9 have demonstrated that urinary ANF is rapidly metabolized by nonsaturable degradative neutral endopeptidases (NEP) within the nephron that are concentrated mostly within brush border vesicles of the proximal tubule.10–11 Shima et al12 have also reported NEP activity within the glomerulus, although at lower concentrations. Recent preliminary studies have reported that inhibition of neutral endopeptidases

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(NEP-I) may be natriuretic if plasma ANF is increased either endogenously with intravascular volume expansion or by exogenous ANF infusion.13,14 A role for NEP-I in CHF as a unique natriuretic potentiator of elevated endogenous ANF is supported by these two previous reports.

To date, the action of NEP-I on cardiorenal function in severe CHF has not been reported. In preliminary studies, NEP-I in the dog with mild CHF resulted in a natriuresis with an increase in endogenous ANF.15 NEP-I administration in humans with
mild left ventricular dysfunction also resulted in a natriuresis with elevation in plasma ANF. Despite the known attenuated renal natriuretic response to increases in plasma ANF in CHF, the investigators of these recent preliminary reports speculated that the natriuretic response to NEP-I in CHF is mediated by an increase in circulating atrial peptide produced by an inhibition of its degradation. The renal mechanisms of this natriuretic response were not investigated.

Based on the markedly high concentration of NEP within the brush border vesicles of proximal tubules of the kidney, the present study tested the hypothesis that NEP-I in CHF may mediate a natriuretic action by renal tubular mechanism that is not dependent on increases in renal hemodynamics or parallel changes in circulating ANF. The present study was therefore designed to determine the cardioenal response to a specific NEP-I (SQ 28,603) in experimental CHF in the dog induced by rapid ventricular pacing.

Methods

Experiments were performed in two groups of mongrel dogs weighing 16–24 kg with experimental CHF induced by 8 days of rapid right ventricular pacing. This model is characterized by changes in cardiac hemodynamics, circulating hormone adjustments, and marked sodium retention as observed in humans with chronic CHF. These animal studies conformed to the guiding principles of the American Physiological Society.

Two weeks before the acute experiment, programmable pacemakers (Medtronic 5320, Minneapolis, Minnesota) were implanted under pentobarbital anesthesia (30 mg/kg i.v.). The heart was exposed through a left thoracotomy incision at the fourth intercostal space. The pericardium was opened, and a screw-in epicardial pacemaker lead was implanted into the right ventricular myocardium. The pacemaker lead was then connected to the pacemaker generator, which was implanted subcutaneously within the chest. A chest tube was placed in the left thorax for drainage, and the thoracotomy incision closed. The chest tube was removed after adequate drainage. The dogs were then allowed to recover. Three to 5 days after implantation, the pacemaker was programmed at 250 beats/min. Dogs were then paced for 8 days, which resulted in the clinical signs of CHF.17,18

The dogs were fasted overnight before the acute experiment and given an oral dose of lithium (300 mg). Before the acute experiment, the dogs were anesthetized with fentanyl (0.005–0.01 mg/kg) and sodium pentobarbital (5–10 mg/kg) given intravenously. The anesthetics were slowly titrated with supplemental doses given as needed. The dogs were then intubated and artificially ventilated (Harvard Respirator, Harvard Apparatus, Millis, Massachusetts) with supplemental oxygen at 4 l/min.

The right external jugular vein was exposed and a flow-directed balloon-tipped thermolodution catheter (model 93-121A, 7F, American Edwards Laboratories, Santa Ana, California) was advanced into the pulmonary artery. Right atrial pressure was measured from the atrial port of the Swan-Ganz catheter. Both femoral veins were cannulated with polyethylene catheters for intravenous infusions of insulin and ANF or NEP-I. The right femoral artery was cannulated for measurement of arterial pressure and sampling of arterial blood.

A flank incision was made, and the left kidney was exposed. The ureter was cannulated for timed urine collections. An electromagnetic flow probe was placed on the left renal artery and connected to a flowmeter (model FM 5010, Carolina Medical Electronics, King, North Carolina). Blood flows and arterial pressure were recorded on a Gould Model 2200 strip recorder (Gould Electronics, Minneapolis, Minnesota).

Experimental Protocol

After completion of the surgical preparations, insulin and normal saline infusions at 1 ml/min were initiated. The amount of insulin infused was calculated to achieve a plasma concentration of 50 mg/dl. The dogs were allowed to stabilize for 60 minutes without intervention. At the end of the equilibration period, two consecutive 15-minute baseline clearances were obtained. The remainder of the protocol differed among the two groups. In group 1 (n=5) after the two baseline clearances, a 30 mg/kg i.v. bolus of a specific NEP-I (SQ 28,603, Squibb Institute for Medical Research, Princeton, New Jersey), with a structure similar to that recently reported by Delaney et al,19 was given over 5 minutes, and a 15-minute lead-in period was observed. Three 15-minute clearances were then obtained after which NEP-I 60 mg/kg by i.v. bolus was administered. Three 15-minute experimental clearances were then obtained. In group 2 (n=5), after two baseline 15-minute clearances, ANF (α-hANF, Peninsula, Belmont, California) was infused intravenously at 100 mg/kg/min, a dose estimated to achieve similar peak plasma concentrations for ANF as observed during NEP-I administration. After a 15-minute lead in infusion, three experimental 15-minute clearances were obtained.

Each clearance period consisted of a 15-minute urine collection, measurement of hemodynamic parameters, and withdrawal of 20 ml of arterial blood for hormonal analysis and electrolyte determination. Extracted arterial plasma levels of ANF and urinary ANF levels were measured by radioimmunoassay to α-hANF as previously described.20 Urine for cyclic GMP (cGMP) levels was collected on ice, and cGMP was measured by radioimmunoassay using the method of Steiner et al.21

The hemodynamic data collected included mean arterial pressure (MAP), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP) (to assess left atrial pressure), cardiac output (CO), and renal blood flow (RBF). CO was measured by ther-
modulation using American Edwards Cardiac Output model 9510-A computer (American Edwards Laboratories, Irvine, California). For each clearance, CO was determined in triplicate and averaged.

Plasma and urinary sodium concentrations were quantified by use of ion-selective electrodes using a Beckman E2A analyzer (Beckman Instruments, Brea, California). GFR was determined by the clearance of inulin. Plasma and urine inulin concentrations were measured by the anthrone method.22

Whole kidney proximal tubule reabsorption of sodium was determined by the lithium-clearance technique. While lithium is exclusively reabsorbed by the proximal tubule and is thus a reliable index of proximal tubule handling of sodium in normal physiological states, distal reabsorption may occur in pathophysiological states such as marked sodium depletion. Plasma and urine lithium levels were determined by flame-emission spectrophotometry (model 357, Instruments Lab, Los Angeles, California).

Data Analysis

Data from the two baseline clearances were combined, and the means were calculated. Data from each experimental clearance obtained in group 1 with NEP-I 30 mg/kg and 60 mg/kg were combined, as were data from group 2 during the ANF infusion.

| Table 1. Systemic Hemodynamic Response to NEP-I or ANF Infusion in Congestive Heart Failure |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| MAP (mm Hg)                                  | CO (l/min)                                    | SVR (dyne · sec · cm⁻²)                       | RAP (mm Hg)                                  | LAP (mm Hg)                                  |
| NEP-I (group 1; n=5)                         |                                               |                                               |                                               |                                               |
| Baseline                                     | 97.1±2.4                                      | 1.78±0.19                                     | 4,373±612                                    | 9.4±0.5                                       | 21.6±2.2                                      |
| NEP-I 30 mg/kg                               | 101.3±4.2                                     | 1.52±0.16                                     | 5,237±721*                                   | 8.1±0.5*                                      | 20.9±2.3                                      |
| NEP-I 60 mg/kg                               | 103.6±4.1                                     | 1.48±0.14                                     | 5,416±663*                                   | 7.8±1.4                                       | 24.4±1.7                                      |
| ANF (100 ng/kg/min) (group 2; n=5)           |                                               |                                               |                                               |                                               |
| Baseline                                     | 98.6±2.5                                      | 1.85±0.29                                     | 4,224±513                                    | 4.8±1.4                                       | 19.6±2.1                                      |
| ANF                                          | 97.0±3.2                                      | 1.71±0.23                                     | 4,616±572*                                   | 5.0±1.9                                       | 19.2±2.6                                      |

Values are given as mean±SEM. Statistical analysis of data was completed by analysis of variance (ANOVA) for repeated measures. Statistical significance was defined as p of less than 0.05.

Results

Tables 1 and 2 and Figures 1 and 2 summarize the cardiovascular, renal, and endocrine data in the two groups of dogs with CHF. The systemic hemodynamic response to NEP-I in dogs with severe CHF was characterized by no significant changes in MAP or CO and an increase in the systemic vascular resistance. RAP decreased in response to 30 mg/kg NEP-I, but LAP did not change.

NEP-I with SQ 28,603 in group 1 resulted in a diuresis and natriuresis with increases in urinary flow, urinary sodium excretion, and fractional excretion of sodium (FE₂Na). In addition, the fractional excretion of lithium (FE₁Li) increased. This observed diuresis and natriuresis was independent of changes in either the glomerular filtration rate or the renal blood flow.

In group 1, with NEP-I, plasma ANF increased significantly with 30 mg/kg NEP-I in the presence of a decrease in RAP with no effect of NEP-I on RAP or plasma ANF at 60 mg/kg. Further, in association with a decrease in RAPs, urinary ANF tended to

| Table 2. Renal Hemodynamic and Excretory Response to NEP-I or ANF Infusion in Congestive Heart Failure |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| GFR (ml/min)                                  | RBF (ml/min)                                  | U₁₉, V (μg/min)                               | FE₂Na (%)                                    |
| NEP-I (group 1; n=5)                         |                                               |                                               |                                               |
| Baseline                                     | 36±6                                          | 168±22                                       | 14.87±7.81                                   | 0.44±0.3                                     | 28.4±7.1                                     | 0.23±0.11                                    | 372±81                                       |
| NEP-I 30 mg/kg                               | 40±7                                          | 168±26                                       | 57.18±14.54*                                 | 1.18±0.32*                                   | 46.3±11.8*                                   | 0.68±0.21*                                   | 518±73*                                      |
| NEP-I 60 mg/kg                               | 38±5                                          | 190±35                                       | 125.6±29.77*                                 | 2.23±0.28*                                   | 49.5±5.7*                                    | 1.07±0.43*                                   | 489±76                                       |
| ANF (100 ng/kg/min) (group 2; n=5)           |                                               |                                               |                                               |
| Baseline                                     | 27±3                                          | 130±18                                       | 14.37±9.76                                   | 0.43±0.17                                    | 24.5±6.0                                     | 0.15±0.05                                    | 372±76                                       |
| ANF                                          | 36±3*                                         | 126±18                                       | 50.63±25.8                                   | 0.91±0.47                                    | 31.5±5.0                                     | 0.37±0.11                                    | 638±41*                                      |

Values are given as mean±SEM. Statistical analysis of data was completed by analysis of variance (ANOVA) for repeated measures. Statistical significance was defined as p of less than 0.05.

NEP-I, neutral endopeptidase inhibitor; ANF, atrial natriuretic factor; GFR, glomerular filtration rate; RBF, renal blood flow; U₁₉, V, urinary sodium excretion; FE₂Na, fractional excretion of sodium; FE₁Li, fractional excretion of lithium; V, urine flow.
increase with 30 mg/kg NEP-I and significantly increased with 60 mg/kg NEP-I. The urinary clearance of ANF increased with 30 mg/kg NEP-I and remained significantly elevated with 60 mg/kg NEP-I. In association with the increase in urinary ANF, urinary cGMP increased with both 30 mg/kg NEP-I and 60 mg/kg NEP-I.

With ANF infusion in group 2, in dogs with a similar degree of CHF as in group 1, MAP and CO did not change in association with a significant increase in systemic vascular resistance. RAPs and LAPs also did not change. ANF infusion in group 2 resulted in no significant increase in absolute excretion of sodium or lithium or \( \text{FE}_{\text{\(\text{Na}\)}} \) or \( \text{FE}_{\text{Li}} \) despite an increase in the GFR. RBF did not change. This blunted natriuresis with the exogenous infusion of \( \alpha \)-hANF was observed despite a significant increase in circulating concentrations of ANF.

**Figure 2.** Bar graphs of peak changes in plasma atrial natriuretic factor (ANF), urinary sodium excretion (\( \text{UNaV} \)) and fractional excretion of sodium (\( \text{FE}_{\text{\(\text{Na}\)}} \)) in congestive heart failure with neutral endopeptidase inhibitor (NEP-I) 30 mg/kg following by NEP-I (60 mg/kg) or with exogenous ANF (100 mg/kg/min). *p<0.05 NEP-I group versus ANF group.

**Discussion**

The present study reports for the first time the renal and cardiovascular actions of NEP-I in experimental CHF. In CHF with elevated endogenous ANF, NEP-I produced a significant natriuresis and diuresis that was not dependent on increases in GFR or RBF and did not parallel the increases in plasma ANF. Further, administration of exogenous ANF in a separate CHF group to mimic plasma levels achieved with NEP-I failed to enhance sodium and water excretion. Arterial pressure did not change with either NEP-I or exogenous ANF. Thus, these studies support a renal tubular action for NEP-I in CHF.

The current studies importantly extend previous published studies of the in vivo action of NEP-I. In studies by Koepke et al., NEP-I with thiorphan in the normal rat did not result in changes in plasma ANF, sodium excretion, or arterial pressure. Only in the presence of coadministration of a blocker of the ANF clearance receptor with an increase in plasma ANF was NEP-I associated with an increase in sodium excretion. No decrease in arterial pressure was observed. No assessment of renal hemodynamic or tubular function was performed. In preliminary studies by Samuels et al., NEP-I also was natriuretic only in the presence of increased plasma ANF produced by volume expansion. The present studies now demonstrate that NEP-I in CHF with chronically increased endogenous circulating ANF is natriuretic.

The most significant action of NEP-I in the present study in experimental CHF was to increase sodium excretion in the absence of changes in renal hemodynamics, although small but significant immeasurable changes cannot be excluded. Nevertheless, neither GFR nor RBF increased in response to NEP-I. Thus, the mechanism of the increase in urinary sodium excretion, not investigated in previous investigations, is via inhibition of tubular reabsorption of sodium.

The renal tubular mechanism(s) of action of NEP-I remain unclear but is suggested by the localization of markedly high concentrations of NEP within proximal tubule brush border vesicles of the kidney. Studies have recently reported that the metabolic
degradation of ANF by proximal tubule vesicles may be nonsaturable, thus serving to markedly limit, beyond the proximal tubule, the availability of ANF. The present study demonstrates that NEP-I resulted in an increase in sodium excretion secondary to a decreased tubular reabsorption of sodium associated with an increase in urinary ANF and urinary cGMP. In the absence of a significant increase in GFR, the principal renal mechanism of action of NEP-I in the current study in CHF may therefore be to decrease the degradation of ANF within proximal tubule brush border potentiating the renal action of ANF within the nephron at or beyond the proximal tubule, supported by the observation that NEP-I decreased proximal tubule reabsorption as determined by an increase in the FE\(_{\text{Na}}\). Such an action was not observed with exogenous ANF, suggesting that part of the attenuated natriuretic response to ANF in chronic CHF may be localized to the proximal tubule in contrast to an intact response reported in acute CHF. Alternatively, as NEP-I may inhibit the degradation of other peptides, NEP-I natriuresis could also involve modulation of sodium excretion by potentiating other peptide hormones. Moreover, modulation of other intrarenal hormonal systems, such as the intrarenal renin-angiotensin system\(^\text{27}\) or intrarenal prostaglandins, cannot be excluded.

Wong et al\(^\text{28}\) have demonstrated that an increase in urinary cGMP is a marker for the renal biological action of ANF. Thus, the increase in urinary cGMP in the current study with NEP-I further supports an action of NEP-I to potentiate the renal tubular action of endogenous ANF.\(^\text{28}\) The present study supports the concept of Kenny and Stephenson\(^\text{29}\) that NEP-I may permit, by inhibiting the degradation of ANF in the proximal tubule, pharmacological rather than physiological concentrations of ANF to reach more distal segments of the nephron. These high intraluminal concentrations could also act on contraluminal receptors. The increase in FE\(_{\text{Na}}\), a marker for reabsorption of sodium within the proximal tubule, indicates that the natriuretic action of NEP-I includes a decrease in proximal tubule reabsorption, perhaps by inhibiting intrarenal angiotensin II concentrations in the pathophysiological state of CHF associated with activation of the renin-angiotensin-aldosterone system\(^\text{8,30}\) with a link to the cGMP second-messenger system as suggested in recent in vitro studies by Garvin.\(^\text{31}\)

The current study also importantly extends our understanding of the renal hyporesponsiveness to elevated concentrations of endogenous ANF in CHF. Previous studies have suggested that the attenuated natriuretic response to elevated plasma ANF in CHF may be related to ANF receptor down-regulation\(^\text{32}\) and the opposing actions of counter-regulatory systems such as renal nerves\(^\text{33}\) and the intrarenal renin-angiotensin system\(^\text{27}\) as well as decreased renal perfusion pressure.\(^\text{34}\) The increase in sodium excretion in the present study in response to NEP-I, not observed with exogenous ANF, suggests that enhanced renal degradation of endogenous ANF in CHF may also contribute to the attenuated renal natriuretic response in CHF.

NEP-I increased circulating concentrations of ANF in our model of experimental CHF. Thus, inhibition of NEP may also serve as an important physiological and therapeutic tool to increase plasma ANF independent of exogenous peptide infusions. While a limited cardioenlargement response to exogenous ANF was observed in the present study as compared with the renal action of NEP-I, more long-term administration of NEP-I to chronically elevate ANF in CHF may result in more long-term alterations in cardioenlargement function as has been documented with chronic infusions of exogenous ANF in both animals and humans.\(^\text{35,36}\)

In the present study, NEP-I did not decrease arterial pressure—nor did arterial pressure decrease with exogenous ANF infusion. This attenuated vasoactive action on arterial pressure has been previously documented in both animals and humans with CHF.\(^\text{8,37}\) Thus, NEP-I in CHF has a selective renal action independent of changes in arterial pressure.

In conclusion, the present study demonstrates that NEP-I in CHF acts to decrease tubular reabsorption of sodium in this sodium-retaining state. Such an action may be secondary to inhibition of the renal degradation of endogenous ANF. This action appears independent of changes in systemic or renal hemodynamics and did not parallel changes in plasma ANF. Thus, NEP-I may serve as a novel therapeutic tool to enhance the renal natriuretic action of elevated endogenous ANF in CHF.

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