Natriuretic Peptide-Potentiating Actions of Neutral Endopeptidase Inhibition in Rats with Experimental Heart Failure

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We developed a rat model of heart failure induced by myocardial infarction (MI) which preserves responsiveness to exogenously administered natriuretic peptide, and investigated the potentiating action of neutral endopeptidase (NEP) inhibition on the renal response to endogenous natriuretic peptide in MI rats, comparing with that in the established cardiac-failing model with arterio-venous fistula (AVF). The endogenous plasma concentration of α-rat atrial natriuretic peptide (α-rANP) in the MI rat was 6.4-fold higher than that in the normal rat, and intravenous infusion of phosphoramidon (165 nmol/min/kg), an NEP inhibitor, induced larger increases in circulating α-rANP levels and natriuresis in MI rats than in normal controls. The maximal natriuretic effect of phosphoramidon (165 nmol/min/kg) was equal to that of exogenously administered α-rANP (100 pmol/min/kg) in MI rats, whereas plasma α-rANP concentration under NEP inhibition was much lower than that after administration of α-rANP. The endogenous α-rANP levels in AVF rats were as high as those in MI rats. However, the natriuretic effect of phosphoramidon was less in AVF rats than in MI rats, which was consistent with the decreased natriuretic activity observed with administration of exogenous to α-rANP in the AVF rat. These results indicate that the natriuretic effect of NEP inhibition is dependent on elevated endogenous α-rANP levels in cardiac-failing rats, but cannot be accounted for simply in terms of the increase in circulating α-rANP levels. Endogenous natriuretic peptide-mediated natriuresis under NEP inhibition also appears to correlate with the responsiveness to the exogenously administered peptide.

KEY WORDS: arterio-venous fistula; atrial natriuretic peptide; heart failure; myocardial infarction; neutral endopeptidase; phosphoramidon.

INTRODUCTION

Clinical application of atrial natriuretic peptide (ANP)[5] is limited because of its structure (poor bioavailability following oral administration) and its short biological half-life (1). Endogenous circulating ANP is increased in several diseases for which ANP is considered of clinical benefit. An alternative to administering exogenous ANP is to enhance the activity of the endogenous circulating peptide by inhibiting its metabolism (2). The inactivation of circulating ANP is attributed to receptor-mediated internalization and enzymatic degradation. In our previous study (3), using the specific ligand for the non-guanylate cyclase-lined ANP receptor, so-called 'C' or clearance receptor, and neutral endopeptidase (EC 3.4.24.11, NEP) inhibitor, we demonstrated that the clearance receptor is involved in the clearance of the physiological levels of ANP, and that NEP plays a major role in the clearance of ANP in the higher plasma concentration range at which clearance receptors are thought to be saturated. The ability of NEP inhibition to increase the circulating levels and activity of coadministered ANP suggests that an NEP inhibitor (and/or its orally available prodrug) may be useful in augmenting the biological activity of elevated endogenous ANP levels in certain disease states (2,3,4).

Wilkins *et al.* (5) demonstrated that the endogenous α-rat ANP (α-rANP) concentration is increased in cardiac-failing rats with arterio-venous fistula (AVF), and that treatment with thiorphan, an NEP inhibitor, elicits a significant renal effect, which is considerably inhibited by co-treatment with monoclonal antibodies against ANP. However, because the renal response to exogenous ANP is blunted in AVF rats, analysis of the underlying mechanisms and influencing factors for the ANP-potentiating actions of NEP inhibition is complicated. In the present study, we developed a rat model of heart failure induced by myocardial infarction (MI) which preserves the responsiveness to exogenously administered natriuretic peptide, and investigated the potentiating action of an NEP inhibitor, phosphoramidon, on the renal response to endogenous natriuretic peptide in MI rats, comparing with that in the AVF rat. In addition, we compared the plasma α-rANP concentration-renal response relationship under NEP inhibition in cardiac-failing rats with that after exogenous α-rANP administration.

MATERIALS AND METHODS

Animals

Male Wistar rats, each weighing 300–350 g, were used. Prior to the experiments, the rats were housed in a temperature- and humidity-controlled room with free access to water and standard rat chow.

Materials

Sodium pentobarbital was purchased from Abbott (Chicago, IL). Cyanoacrylate glue was obtained from Sankyo Co. Ltd. (Tokyo, Japan). Phosphoramidon and α-rANP were purchased from Peptide Institute (Osaka, Japan). All other chemicals were the highest purity available.

Induction of MI

MI was induced by left coronary arterial ligation as described previously (6). Briefly, rats were anesthetized with 50 mg/kg sodium pentobarbital, and artificially ventilated

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[5] Abbreviations used: ANP, atrial natriuretic peptide; α-rANP, α-rat ANP; AVF, arterio-venous fistula; MI, myocardial infarction; NEP, neutral endopeptidase.

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with a respirator (Harvard Apparatus Model 683, MA). The chest was opened by a left thoracotomy at the fourth intercostal space, and a 6/0 braided silk suture attached to a 10 mm micro-pointed reverse cutting needle (Nescosture ER1006s, Nihon-shoji, Osaka, Japan) was introduced under the left coronary artery near its origin with which the artery was permanently occluded. The thoracic cavity was closed with separate silk thread stitches. Preliminary studies showed the endogenous plasma α-rANP concentration to be significantly increased by one day after induction of MI. In the present study, the experiment was performed three days after induction of MI.

Induction of AVF

AVF was induced as described previously (5,7). Briefly, the vena cava and abdominal aorta were exposed by opening the abdominal cavity via a midline incision in the anesthetized rat. The aorta was punctured at the union of the segment two thirds caudal to the renal artery and the one third cephalic to the aortic bifurcation with an 18-gauge disposable needle. The needle was advanced into the aorta, perforating its adjacent wall and penetrating the vena cava. A vascular clamp was placed across the aorta caudal to the left renal artery, then the needle was fully withdrawn. A drop of cyanoacrylate glue was used to seal the aortal puncture, and the clamp was removed after 1 min. The peritoneal cavity was closed with separate silk thread stitches. The experiment was performed three weeks after induction of AVF (5).

Experimental Protocol

Cardiac-failing and normal rats were anesthetized with 35 mg/kg sodium pentobarbital. Supplemental doses of anesthetics were administered as required. Body temperature was maintained with heating lamps. Systemic arterial blood pressure was recorded from the femoral artery by a capacitance transducer (Toyo Baldwin MPU-0.5-290-0-III, Tokyo, Japan). Arterial blood pressure and heart rate were monitored continuously with a polygraph (Nihon-dennki San-ei Model 366, Tokyo, Japan) and a recorder (Nihon-dennki San-ei Model 8K21-L, Tokyo, Japan). To maintain the urine flow rate, isotonic saline was infused via the femoral vein at a constant rate of 110 μl/min throughout the experiment including a 40 min stabilizing period (4). The 28-amino acid α-rANP was dissolved in phosphate-buffered saline (pH 7.4) containing 0.1% bovine serum albumin, and 30 pmol/min/kg and 100 pmol/min/kg were infused sequentially for 20 min each via the jugular vein (4). In a separate experiment, phosphoramidon was dissolved in isotonic saline, and administered via the femoral vein at a constant rate of 165 nmol/min/kg (3,4). For measurement of circulating α-rANP concentration, arterial blood samples (0.4 ml) were periodically withdrawn via a catheter in the femoral artery. A catheter was placed into the bladder for urine collection, and urine volume was deduced from the sample weight assuming a density of 1.0.

Assays

Plasma concentration of α-rANP was determined by specific radioimmunoassay as described previously (1). Urinary sodium concentration was determined using an ion meter (Horiba F-8AT, Kyoto, Japan) with an ion-specific electrode (Horiba Sera-100, Kyoto, Japan).

Data Analysis

Values are expressed as mean ± SE. Multiple comparison was performed using Scheffé-type test following Kruskal-Wallis analysis. A P value of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

In the present study, using two kinds of rats with experimentally-induced heart failure, we investigated the potentiating actions of an NEP inhibitor, phosphoramidon, on the renal response to endogenous natriuretic peptide, and compared the plasma α-rANP concentration-response relationship under NEP inhibition with that after administration of exogenous α-rANP. Table I summarizes the effects of MI- and AVF-induced heart failure on various characteristics of rats. The heart weight normalized by the body weight was significantly higher in MI and AVF than in normal control rats. The mean arterial blood pressure determined under pentobarbital anesthesia was significantly lower in AVF than in normal and MI rats, but the heart rate was similar in all three groups. Endogenous plasma α-rANP concentration was increased 6.4- and 7.1-fold by induction of MI and AVF, respectively.

We investigated the natriuretic effect of exogenously administered α-rANP (30 and 100 pmol/min/kg) in normal, MI, and AVF rats. Figure 1 shows the plasma α-rANP concentration before and during intravenous infusion of the peptide. The baseline α-rANP concentration in MI and AVF rats was higher than that in normal controls ($P < 0.05$), whereas there were no differences in plasma α-rANP concentrations during peptide infusion between the three groups. Figure 2 shows the time courses of urinary sodium excretion in nor-

Table I. Characteristics of MI and AVF Rats[a]

	Normal $n = 13$	MI $n = 12$	AVF $n = 13$
Heart weight (g/kg of body weight)	2.89 ± 0.11	3.60 ± 0.12*	3.79 ± 0.23*
Mean arterial blood pressure (mmHg)	118 ± 4	111 ± 5	103 ± 3*
Heart rate (beats/min)	366 ± 12	398 ± 9	380 ± 9
Plasma α-rANP concentration (pM)	15.4 ± 3.5	98.5 ± 22.4*	109 ± 26*

[a] The results are presented as mean ± SE for n animals.
* Significantly different from the normal group, $P < 0.05$.

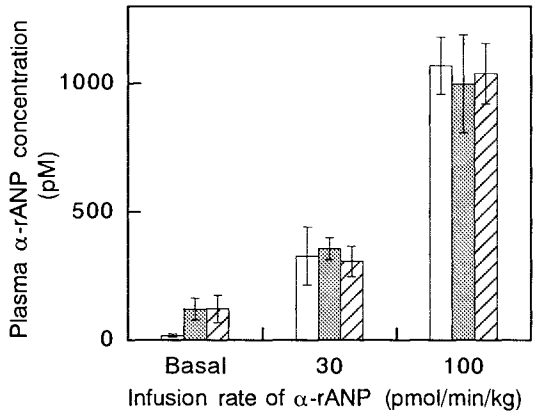

Fig. 1. Mean (±SE) plasma concentration of α-rANP following intravenous infusion of the peptide at 30 and 100 pmol/min/kg in normal (open columns), MI (dotted columns), and AVF (hatched columns) rats. Values are means of five or six rats.

mal, MI, and AVF rats. The normal and MI rats showed a similar curve in urinary sodium excretion in response to increasing doses of α-rANP. On the other hand, the natriuretic response to exogenous α-rANP was attenuated in AVF rats, despite maintaining similar plasma α-rANP levels during peptide infusion (Figure 1). The resistance in renal response to exogenous α-rANP in AVF rats was consistent with that observed previously (5), whereas natriuretic activity to exogenous α-rANP was shown to be preserved in MI rats.

We also investigated the effects of NEP inhibition on plasma α-rANP levels and renal responses to the endogenous peptide in normal, MI, and AVF rats, where the dosing rate of phosphoramidon (165 nmol/min/kg) was sufficient to completely inhibit NEP activity (3). Figure 3 shows plasma α-rANP concentrations following intravenous infusion of the drug to normal, MI, and AVF rats. Plasma α-rANP concentration was not affected by NEP inhibition in normal rats, while that in MI and AVF animals increased gradually with NEP inhibition, reaching levels 2 to 3-fold higher than baseline values at the end of the experiments. The circulating α-rANP concentrations in MI and AVF rats were significantly higher than that in normal rats throughout the experiments ($P < 0.05$). These findings confirmed our previous observations that a clearance receptor-mediated mechanism is largely involved in the clearance of α-rANP at physiological levels, and that NEP plays a significant role in the clearance of the peptide at pharmacological doses or at supraphysiological levels (3,4).

Figure 4 shows the time courses of urinary sodium excretion following intravenous infusion of phosphoramidon in normal, MI, and AVF rats. Urinary sodium excretion in normal rats was not affected for 60 min after the start of drug infusion, after which time it gradually increased. On the other hand, the natriuretic effect of phosphoramidon in MI rats was significantly higher than that in normal rats from 15 min to 120 min after administration ($P < 0.05$). The sodium excretion rate also increased after administration of phosphoramidon in AVF rats, although the natriuretic effect was less than that in MI rats. The data suggest that the natriuretic effect of NEP inhibition was distinctive for the cardiac-failing rats in which endogenous α-rANP levels were elevated (Figure 3 and 4). In addition, Figure 5 shows comparison of the plasma α-rANP concentration-renal response relationship under NEP inhibition with that after the exogenous α-rANP administration in cardiac-failing rats. The maximal natriuretic effect of phosphoramidon (165 nmol/min/kg) was equal to or greater than that of exogenously administered α-rANP (100 pmol/min/kg) in MI (Figure 5a) and AVF (Figure 5b) rats, whereas the circulating α-rANP concentrations under NEP inhibition were much lower than those during α-rANP infusion. The findings assert that the natriuretic response to phosphoramidon cannot be explained simply in terms of an increase in circulating α-rANP level (2,4,5).

Besides ANP, NEP is involved in the metabolism of several other peptides including kinins, enkephalins, and neurotensins. However, in conjunction with the present finding that renal responses to phosphoramidon are more pronounced where circulating ANP levels are elevated, accumulating evidence suggests that ANP is an important mediator of the effect of NEP inhibition (2). A plausible explanation for the higher natriuretic response than that expected from the circulating α-rANP levels under NEP inhibition is that metabolism of the peptide is inhibited by an NEP inhib-

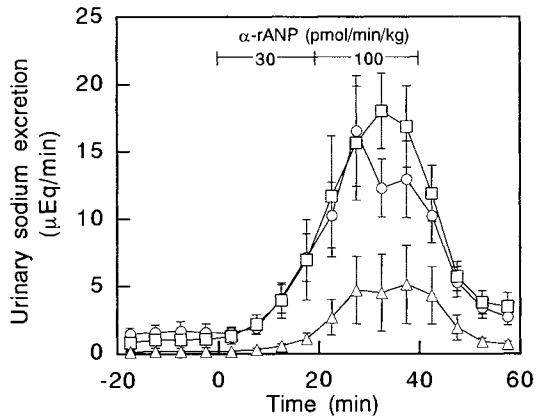

Fig. 2. Time course of mean (±SE) urinary sodium excretion following intravenous infusion of α-rANP at 30 and 100 pmol/min/kg in normal (circles), MI (squares), and AVF (triangle) rats. Values are means of five or six rats.

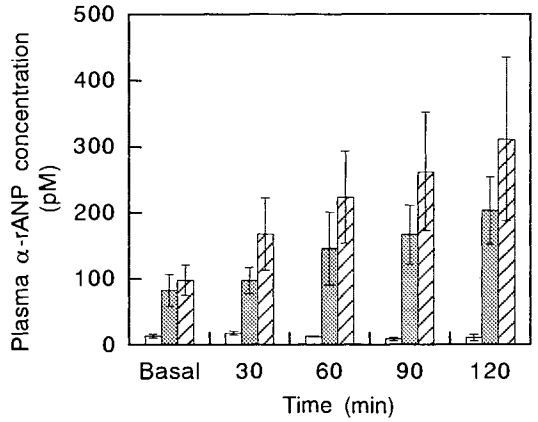

Fig. 3. Time course of mean (±SE) plasma concentration of α-rANP following intravenous infusion of phosphoramidon given at 165 nmol/min/kg in normal (open columns), MI (dotted columns), and AVF (hatched columns) rats. Values are means of seven rats.

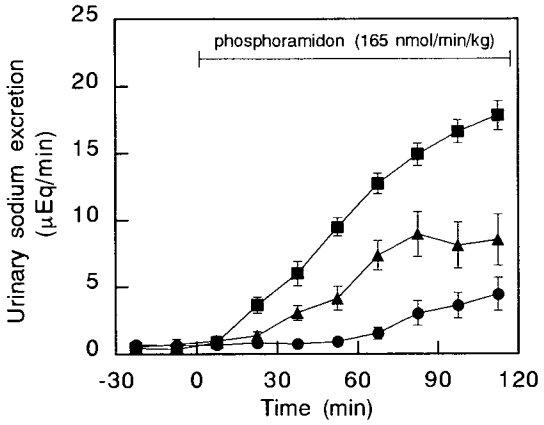

Fig. 4. Time course of mean (±SE) urinary sodium excretion following intravenous infusion of phosphoramidon at 165 nmol/min/kg in normal (circles), MI (squares), and AVF (triangles) rats. Values are means of seven rats.

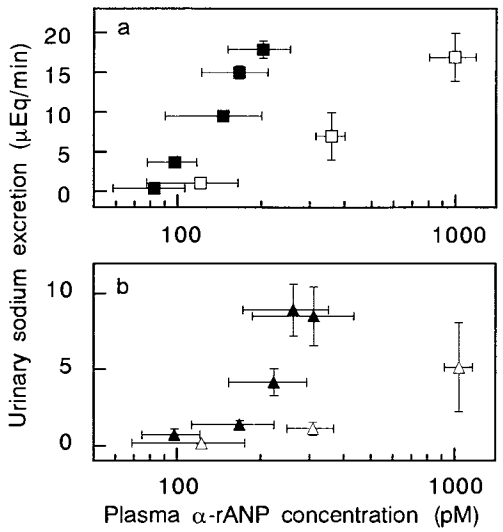

Fig. 5. Relationship between plasma α-rANP concentration and urinary sodium excretion in MI (a) and AVF (b) rats following intravenous infusion of α-rANP (open symbols) and phosphoramidon (filled symbols). Values are means of five to seven rats. Bars represent SE.

itor at a critical site where NEP plays a major role in the degradation of the natriuretic peptide. That is, the NEP inhibitor may protect filtrated α-rANP from degradation in the renal proximal tubule brush border, with consequent elevation of natriuretic peptide concentration in the nephron to the level required to elicit a renal response (2,5,9). This hypothesis was partly supported by the increase in immunoreactive ANP excreted into the urine observed with NEP inhibition in cardiac-failing and hypertensive rats (5,8), and by the increased urinary sodium excretion observed with intraluminal administration of ANP in the rat kidney (10).

The renal action of ANP is known to be influenced by factors other than the circulating peptide levels such as renal perfusion pressure. In our previous study (4), we analyzed the effects of the blood pressure-lowering activity of exogenously administered α-rANP on renal response, and demonstrated that decreased renal perfusion caused by excessive hypotension significantly attenuates the renal actions of the peptide. Therefore, we further explored the hypotensive effects of phosphoramidon in MI and AVF rats, comparing with that after exogenously administered α-rANP. Figure 6 shows plots of the blood pressure-lowering effects vs natriuretic effects of phosphoramidon in MI (a) and AVF (b) rats. Blood pressure was decreased transiently and slightly just after administration of phosphoramidon (165 nmol/min/kg) in MI rats. However, the hypotensive effect of the drug was less than that of exogenously administered α-rANP (30–100 pmol/min/kg). Thus, the lack of induction of excessive hypotension by phosphoramidon may be partly responsible for maintenance of the renal action to endogenous peptide in MI and AVF rats.

The present study also demonstrated that renal responsiveness to NEP inhibition is variable in heart failure, and that the natriuretic response to endogenous ANP under NEP inhibition correlates with the responsiveness to exogenously administered peptide in heart failure (Figure 2 and 4). That is, the natriuretic effect of phosphoramidon was less in AVF rats than in MI rats, whereas the endogenous α-rANP levels were equivalent in both cardiac-failing rats, consistent with the decreased natriuretic activity induced by exogenously administered α-rANP in AVF rats (Figure 5). Several studies have demonstrated an attenuated renal response to exogenous ANP in both experimental animals and human subjects with congestive heart failure, and multiple factors are thought to contribute to the blunted effect of ANP in congestive heart failure, including decreased renal perfusion pressure, decreased sodium delivery to the tubular site of action for ANP, enhanced degradation of ANP, receptor down-regulation, altered post-receptor signal transduction, and activation of antagonistic systems to ANP such as the sympathetic nerve system and renin-angiotensin-aldosterone system (11). In fact, decreased renal perfusion pressure may contribute to the renal response to ANP observed in AVF

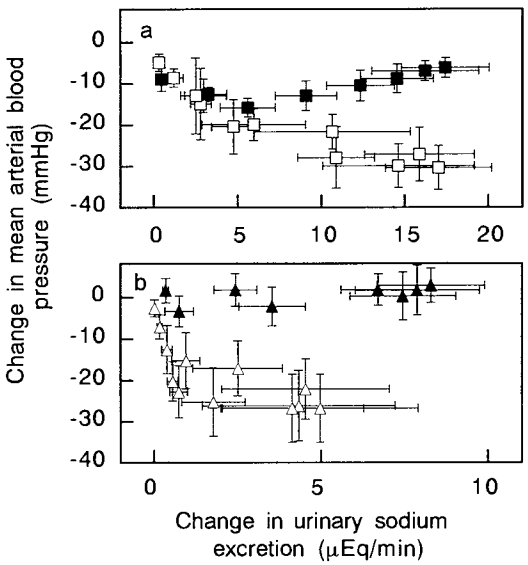

Fig. 6. Relationship between change in urinary sodium excretion and change in mean arterial blood pressure in MI (a) and AVF (b) rats following intravenous infusion of α-rANP (open symbols) and phosphoramidon (filled symbols). Values are means of five to seven rats. Bars represent SE.

rats, since the mean arterial blood pressure was decreased in AVF rats as compared to that in the normal and MI rats (Table I). In addition, Wilkins *et al.* (5) proposed that the renal tubule sites of action of filtered ANP under NEP inhibition are normally inaccessible to the peptide and are thus protected from down-regulation by high circulating ANP levels in AVF rats. However, Yechieli *et al.* (12) have recently demonstrated that the density of biologically active renal glomerular and papillary ANP receptors is significantly reduced in rats with AVF, and suggested that down-regulation of this receptor may contribute to renal hyporesponsiveness to ANP in congestive heart failure. These multiple factors may not be mutually exclusive as mechanisms of blunted renal response to ANP and NEP inhibitors in congestive heart failure, and remain to be evaluated (2,11).

In conclusion, we have used two kinds of rats with experimentally-induced heart failure and demonstrated that the natriuretic effect of NEP inhibition is dependent on elevated endogenous α-rANP levels and on responsiveness to the peptide in heart failure, but cannot be explained simply in terms of the increase in circulating α-rANP levels. These findings may provide new insights for the clinical application of NEP inhibitors.

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