Clearance receptors and endopeptidase: equal role in natriuretic peptide metabolism in heart failure

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Radeemaker, Miriam Tessa, Christopher John Charles, Teddy Kosoglou, Andrew A. Protter, Eric Arnold Espiner, Michael Gary Nicholls, and Arthur Mark Richards. Clearance receptors and endopeptidase: equal role in natriuretic peptide metabolism in heart failure. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2372–H2379, 1997.—The effects of separate and combined endopeptidase inhibition (by SCH-32615) and natriuretic peptide receptor C blockade [by C-ANP-(4—23)] on the clearance and bioactivity of atrial (ANP) and brain (BNP) natriuretic peptides was investigated in eight sheep with heart failure. SCH-32615 and C-ANP-(4—23) administered separately induced significant and proportionate dose-dependent rises in plasma ANP, BNP, and guanosine 3′,5′-cyclic monophosphate (cGMP) levels. Associated with these changes were reductions in arterial pressure, left atrial pressure, and peripheral resistance and increases in cardiac output, urine volume, sodium excretion, and creatinine clearance. SCH-32615 induced greater diuresis and natriuresis than C-ANP-(4—23). Combined administration of SCH-32615 and C-ANP-(4—23) induced greater than additive rises in plasma ANP, BNP, and cGMP concentrations, with enhanced hemodynamic effects, diuresis, and natriuresis and reduced plasma aldosterone levels. In conclusion, we find that the enzymatic and receptor clearance pathways contribute equally to the metabolism of endogenous ANP and BNP in sheep with heart failure. Combined inhibition of both degradative pathways was associated with enhanced hormonal, hemodynamic, and renal effects and may have greater potential therapeutic value than either agent separately.

guanosine 3′,5′-cyclic monophosphate; ventricular pacing; hemodynamics; natriuresis

ATRIAL NATRIURETIC PEPTIDE (ANP) and brain natriuretic peptide (BNP) are circulating hormones of primarily cardiac origin with diuretic, natriuretic, and hypotensive actions (9). Both natriuretic peptides are intimately involved in pressure and body fluid homeostasis, and their circulating levels are significantly raised in congestive heart failure (CHF) (17), a disease characterized by cardiac and volume overload. Despite already elevated levels, administration of both ANP and BNP in CHF results in beneficial cardiovascular and renal effects (22). Although the rate of synthesis and release of the natriuretic peptides is a major regulator of plasma concentrations, the rate of removal of the peptides from the circulation is also important. The metabolism of both ANP and BNP involves two main pathways: enzymatic degradation by neutral endopeptidase (NEP) 24.11 (10, 19) and receptor-mediated endocytosis via the natriuretic peptide receptor C (NPR-C) or clearance receptor (19). NEP 24.11 is widely distributed throughout the body and is particularly concentrated at the brush border membranes in the proximal tubule of the kidney (25). The NPR-C make up the majority of the natriuretic peptide receptors and are located in several tissues including vascular endothelium and smooth muscle, heart, adrenal gland, and kidney (9, 16).

Numerous studies have investigated the effects of blockade of both these degradative pathways on the clearance and bioactivity of ANP through the administration of NEP inhibitors and NPR-C ligands, either separately or in combination. In normal animals, co-administration of NEP and NPR-C produces greater increases in plasma ANP concentrations, urine volume, and sodium excretion and falls in blood pressure than are achieved by either agent alone (7, 16, 31). In the setting of heart failure, in which plasma levels of the natriuretic peptides are raised, separate NEP inhibition (18, 23) and NPR-C blockade (21) have been shown to induce significant rises in plasma ANP in association with vasodilation and natriuresis and diuresis. Results from comparative studies (8, 12) suggest that, although both pathways contribute, the NPR-C may play a dominant role in ANP metabolism at physiological plasma concentrations at which occupancy of the receptor is thought to be <5% (19). However, it has been hypothesized that in states of chronically elevated endogenous ANP, such as occurs in CHF, the clearance receptor may play a lesser role than that of NPR in the metabolism of the peptide because of increased receptor occupancy (8, 12). Furthermore, there is some evidence that ANP pretreatment and/or raised plasma levels may promote downregulation of NPR-C (15) and decrease internalization of the receptor-ligand complex. Changes in NEP expression or activity may also occur. Increased natriuretic peptide response to NEP inhibition in heart failure has prompted speculation that the enzyme may be induced in the setting of increased hormone secretion (6, 30). On the other hand, in rats with decompensated heart failure, pulmonary NEP mRNA is reduced in correlation with the severity of the disorder, whereas renal NEP mRNA, protein levels, and activity are unchanged compared with normal rats (1).

To our knowledge, no studies have previously investigated the quantitative contributions of the enzymatic and clearance-receptor pathways in the metabolism of endogenous natriuretic peptides (ANP and BNP) in the...
setting of CHF. Accordingly, we administered incremental doses of an NEP inhibitor and an NPR-C ligand, both separately and in combination, to sheep with pacing-induced heart failure. We also examined the concomitant hormonal, hemodynamic, and renal effects.

METHODS

Surgical preparation. Eight Coopworth ewes (body wt 40–50 kg) were instrumented as previously described (10) via a left lateral thoracotomy. Under general anesthesia [induced by thiopentone sodium (17 mg/kg) and maintained with halothane and nitrous oxide], two polyvinyl chloride catheters were inserted in the left atrium for blood sampling and left atrial pressure (LAP) determination, a Konigsberg (P6.0) high-fidelity pressure-tip transducer was inserted in the aorta for measurement of mean arterial pressure (MAP), an electromagnetic flow probe was placed around the ascending aorta to measure cardiac output (CO), a 7-Fr Swan-Ganz catheter was inserted in the pulmonary artery for infusions, and a 7-Fr His-bundle electrode was stitched subepicardially to the wall of the left ventricle for subsequent left ventricular pacing using an external pacemaker made in our department. All leads were externalized through individual incisions in the neck. An indwelling bladder catheter was inserted per urethra for subsequent urine collections. The animals received meperidine (pethidine; 50 mg im) postoperatively and were allowed to recover for at least 14 days before the study protocol commenced. During the experiments, the animals were held in metabolic cages, had free access to water, and ate a diet of chaff and sheep pellets (containing 40 mmol/day sodium and 200 mmol/day potassium) supplemented with a further 40 mmol of sodium administered orally each morning as NaCl tablets using an applicator.

Study protocol. Heart failure was induced by rapid left ventricular pacing at 225 beats/min for 7 days (10). On days 8, 10, 12, and 14 of pacing, the sheep received in balanced random order vehicle, the NEP inhibitor SCH-32615 alone, the NPR-C ligand C-ANP-(4—23) alone, and the combination of both compounds. SCH-32615 was given as intravenous boluses in three incremental doses (1, 5, and 25 mg/kg at 90-min intervals) accompanied by a vehicle infusion. C-ANP-(4—23) was administered as a continuous intravenous infusion in three incremental doses (20, 100, and 500 pmol·kg⁻¹·min⁻¹ for 90 min each) accompanied by boluses of vehicle. Vehicle controls consisted of 10 ml of 0.9% saline for the boluses and 60 ml of Haemaccel (Behring Institut, Behringwerke, Marburg, Germany) for the infusions. All treatments were administered via the pulmonary artery catheter commencing at 1000.

C-ANP-(4—23), an analog of ANP (in which 5 amino acids in the COOH-terminal, 3 in the NH₂-terminal, and 5 in the ring are deleted), was synthesized by solid-phase peptide synthesis at Scios Nova (Palo Alto, CA) and was purified by high-performance liquid chromatography. SCH-32615 [N-[L-(1-carboxy-2-phenyl)ethyl]-L-phenylalanine]-alanine, the parenteral form of the prodrug SCH-34826, is a potent, competitive inhibitor of NEP 24.11 with an inhibitory constant of 19 nM (28). It is inactive against other metalloenzymes including angiotensin-converting enzyme.

Hemodynamic recordings [MAP, LAP, CO, and calculated total peripheral resistance (CTPR = MAP/CO)] were performed at 15-min intervals in the hour before treatment (baseline) and at 15, 30, 45, 60, and 90 min during each incremental dose. A further two recordings were made at 15 and 30 min after cessation of the highest dose. All measurements were made with the sheep standing quietly in the metabolic cage. The left atrial catheter was connected to a Statham P50 strain-gauge transducer positioned at the level of the atria and linked to a hemodynamic monitor (M17294; Mennen-Greatbatch, Rehevot, Israel) for pressure determination relative to atmospheric pressure. The Konigsberg pressure transducer was connected to a preamplifier before display by the monitor. Hemodynamic measurements were determined by on-line computer-assisted analysis using methods previously described (11).

Blood samples were drawn from the left atrium for the assay of C-ANP-(4—23), ANP, BNP, and guanosine 3',5'-cyclic monophosphate (cGMP) at 30 min and immediately before treatment (baseline), at 30, 60, and 90 min during each dose, and at 30 min after cessation of the highest dose. Additional blood samples were drawn at each change in dose for measurement of plasma renin activity (PRA), aldosterone, and cortisol concentrations (7, 10). An index of NEP activity was measured at 30 and 90 min of each dose and 30 min after completion of the highest dose. To assess the effects of SCH-32615 circulating in the serum, we added exogenous NEP activity to each sample and monitored for inhibition of this activity by the SCH-32615 present in the sample. Ten microliters of ovine kidney microvillar NEP preparation containing −2.35 nmol·ml⁻¹·min⁻¹ activity was added to 20 μl to this tube and an identical control tube, 50 μl of substrate solution were added to yield a final concentration of 0.6 mmol/l. Phosphoromycin (final concn 10 μM; Sigma Chemical, St. Louis, MO) was added to the control tube. Both tubes were incubated at 37°C for 30 min, at which time phosphoromycin was added to the sample tube. The tubes were then incubated for 40 min with excess aminopeptidase M (Boehringer Mannheim) to release free 7-amino-4-methylcoumarin (7-AMC). The 7-AMC released was measured fluorometrically after dilution with 3 ml of buffer, and NEP activity was calculated from the difference between the sample and control tubes.

All blood was taken into tubes on ice, centrifuged at 4°C, and stored at −80°C. All samples from each animal were measured in the same assay to avoid interassay variability. Hematocrit was measured with every blood sample taken, whereas plasma sodium, potassium, and creatinine samples were taken at the change of each dose. Urine volume and samples for the measurement of sodium, potassium, and creatinine excretion were collected in the 90 min before treatment (baseline) and over the 90-min period of each dose. The protocol was approved by the Animal Ethics Committee of the Christchurch School of Medicine.

Statistics. Results are expressed as means ± SE. Baseline hemodynamic and hormone values represent the means of four and two measurements, respectively, made within the hour immediately before infusion. Statistical analysis was performed by repeated-measures analysis of variance (ANOVA) using the BMDP P2V package. Baseline data from the vehicle-, SCH-32615-, C-ANP-(4—23)-, and combined-treatment days were compared. Treatment and time differences among all four study days were determined using a two-way ANOVA. Overall treatment-time interactions from ANOVA are quoted in the text unless otherwise stated. Incremental changes in plasma ANP, BNP, and cGMP concentrations during combined treatment were tested for synergism by comparing the changes from baseline at each time point during combined treatment (Δ) with the sum of those during each treatment separately (Δ + Δ) in a two-way ANOVA. Significance was assumed when P < 0.05.
RESULTS

There were no significant intergroup differences in pretreatment baseline data for any hormonal, hemodynamic, or metabolic variable. After 7 days of rapid ventricular pacing, all sheep exhibited the hemodynamic and hormonal hallmarks of established heart failure (10). As observed in previous studies (10), MAP, CO, and CTPR were reduced, whereas LAP and plasma ANP, BNP, cGMP, PRA, and aldosterone levels were elevated.

As shown in Fig. 1, infusion of incremental doses of C-ANP-(4—23) alone resulted in dose-dependent increases in plasma C-ANP-(4—23) concentrations (treatment-time interaction, ANOVA, \( P < 0.001 \)). When C-ANP-(4—23) was administered in combination with SCH-32615, plasma levels were further increased by 22 [not significant (NS)], 38 (\( P < 0.001 \)), and 62% (\( P < 0.001 \)) above those achieved during the low, medium, and high doses, respectively. The index of plasma NEP activity was reduced to a similar extent after administration of SCH-32615 alone and in combination with C-ANP-(4—23) (Fig. 1); at 30 min after each incremental dose, NEP activity was reduced by 63, 88, and 97%, respectively (\( P < 0.001 \)). After cessation of treatments, plasma levels of C-ANP-(4—23) fell promptly whereas the index NEP activity was largely unchanged (Fig. 1).

Compared with vehicle control data, incremental infusions of C-ANP-(4—23) alone induced significant and proportionate dose-dependent increases in plasma ANP (1.1-, 1.5-, and 1.8-fold during low, medium, and high doses, respectively; \( P < 0.001 \)) and BNP (1.2-, 1.5-, and 1.7-fold; \( P < 0.001 \)) (Fig. 2). Identical fivefold increases in dose of SCH-32615 alone elicited similar proportionate dose-dependent rises in plasma ANP (1.2-, 1.5-, and 1.9-fold; \( P < 0.001 \)) and BNP (1.2-, 1.5-, and 1.9-fold; \( P < 0.001 \)) (Fig. 2). There were no significant treatment differences between the responses to each agent. When C-ANP-(4—23) and SCH-32615 were administered together, augmented but still proportionate dose-dependent increases in plasma concentrations of both natriuretic peptides were observed (ANP: 1.3-, 2.2-, and 3.8-fold, \( P < 0.001 \); BNP: 1.5-, 2.2-, and 3.7-fold, \( P < 0.001 \)). These increases were significantly greater than the additive increments induced by either treatment alone during the middle (\( P < 0.01 \)) and high (\( P < 0.001 \)) combined doses for BNP [high dose: C-ANP-(4—23) (37 pmol/l) + SCH-32615 (49 pmol/l) = 86 pmol/l; combined = 148 pmol/l] and during the high combined dose for ANP [C-ANP-(4—23) (137 pmol/l) +
SCH-32615 (162 pmol/l) = 299 pmol/l; combined = 472 pmol/l, P < 0.001. As observed with the natriuretic peptides, C-ANP-(4–23) and SCH-32615 given separately resulted in similar dose-dependent rises in plasma cGMP (both P < 0.001) and more than additive increases during combined administration at the high dose [C-ANP-(4–23) (31 nmol/l) + SCH-32615 (31 nmol/l) = 62 nmol/l; combined = 86 nmol/l, P < 0.01] (Fig. 2). Plasma ANP, BNP, and cGMP levels fell promptly and similarly after cessation of both C-ANP-(4–23) alone and the combined treatment, whereas plasma concentrations were virtually unchanged at 2 h after the last bolus of SCH-32615.

The comparable rises in plasma cGMP levels during SCH-32615 and C-ANP-(4–23) alone were associated with significant and similar dose-dependent falls in MAP [high dose: C-ANP-(4–23) 6.3 mmHg, SCH-32615 6.6 mmHg; both P < 0.001], LAP [C-ANP-(4–23) 4.1 mmHg, SCH-32615 4.7 mmHg; both P < 0.001], and CTPR [C-ANP-(4–23) 9.9 mmHg·l⁻¹·min, SCH-32615 9.9 mmHg·l⁻¹·min; both P < 0.001] and increases in CO [C-ANP-(4–23) 0.36 l/min, SCH-32615 0.37 l/min; both P < 0.001] compared with vehicle control data (Fig. 3). The slightly greater reduction in LAP observed during SCH-32615 compared with C-ANP-(4–23) approached statistical significance (1.0 > P > 0.05). The combined administration of C-ANP-(4–23) and SCH-32615 resulted in significantly enhanced falls in MAP (9 mmHg, P < 0.05 vs. either compound alone), LAP (6.8 mmHg, P < 0.001 vs. either alone) and CTPR (13.7 mmHg·l⁻¹·min, P < 0.001 vs. either alone) and rise in CO (0.66 l/min, P < 0.001 vs. either alone) compared with either C-ANP-(4–23) or SCH-32615 alone. Hematocrit was increased relative to control data by all treatments (all P < 0.01; Table 1).

Compared with vehicle control data, all treatments significantly and dose dependently increased urine volume [C-ANP-(4–23) 3.2-fold, SCH-32615 4.8-fold, combined 9.2-fold; all P < 0.01], urine sodium [C-ANP-(4–23) 5.8-fold, SCH-32615 22.7-fold, combined 39.2-fold; all P < 0.01], potassium [C-ANP-(4–23), P < 0.05; SCH-32615 and combined, P < 0.01], and creatinine excretion (all P < 0.01) (Fig. 4), and creatinine clearance [C-ANP-(4–23), P < 0.05; SCH-32615 and combined, P < 0.01] (Table 1). Urine sodium excretion was significantly greater during SCH-32615 compared with C-ANP-(4–23) (P < 0.05), whereas combined administration of SCH-32615 and C-ANP-(4–23) increased both urine output (P < 0.05 vs. both) and sodium excretion (P < 0.05 vs. both) to a greater extent than either compound separately.

PRA was not significantly altered by any treatment compared with control levels, whereas plasma aldosterone concentrations were significantly reduced only during the combined administration of C-ANP-(4–23) and SCH-32615 (P < 0.05; Fig. 5). Plasma cortisol (Fig. 5) and plasma sodium, potassium, and creatinine levels were not affected by any of the treatments (data not shown).

DISCUSSION

The present vehicle-controlled study examines for the first time the dose-dependent biological actions of an NEP inhibitor and an NPR-C ligand, separately and in combination, in heart failure. We found that incremental doses of SCH-32615 and C-ANP-(4–23) administered separately induced significant and proportionate dose-related rises in plasma ANP, BNP, and cGMP levels. These changes were associated with remarkably similar reductions in MAP, LAP, and CTPR and increases in CO and relative hemoconcentration. Both compounds increased urine volume, urine sodium, potassium, and creatinine excretion, and creatinine clearance. The diuretic and natriuretic responses during NEP inhibition were significantly greater than during NPR-C blockade. Combined administration of SCH-32615 and C-ANP-(4–23) resulted in greater than...
Table 1. Effects of vehicle, C-ANP-(4—23), SCH-32615, and combined treatment in sheep with heart failure

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Dose 1</th>
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<th>Dose 3</th>
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<td>Hematocrit, %</td>
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<tr>
<td>Vehicle</td>
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<td>21.9±1.1</td>
<td>21.7±1.2†</td>
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<td>SCH-32615</td>
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<td>21.7±1.2</td>
<td>21.3±1.2</td>
<td>21.1±1.2‡</td>
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<tr>
<td>Combined</td>
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<td>21.8±1.2</td>
<td>21.6±1.3‡</td>
<td>21.3±1.3‡</td>
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<td>78±6</td>
<td>92±10*</td>
<td>94±11‡</td>
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Values are means ± SE. Hematocrit and creatinine clearance responses before (baseline) and during incremental doses of vehicle, C-ANP-(4—23) alone (20, 100, and 500 pmol·kg⁻¹·min⁻¹ = doses 1, 2, and 3, respectively), SCH-32615 alone (1, 5, and 25 mg/kg = doses 1, 2, and 3, respectively), and combination of both compounds in 8 sheep with pacing-induced heart failure. Significant differences between vehicle control and treatments: *P < 0.05, †P < 0.01, ‡P < 0.001.

Although data from a number of studies in normal animals have suggested that NPR-C blockade has a greater effect on ANP clearance than do NEP inhibitors (8, 12), others have demonstrated that the enzymatic and receptor clearance pathways contribute equally to the degradation of ANP (7, 16) and BNP (7) at physiological plasma concentrations. However, it has been suggested that in states of chronically elevated endogenous ANP, such as occurs in CHF, the clearance receptor may play a lesser role than NEP in the metabolism of the peptide because of increased receptor occupancy (8, 12). There is also some evidence that ANP pretreatment and/or raised plasma ANP levels promote downregulation of NPR-C (15). Schiffrin (24) observed reduced NPR-C in platelets of patients with severe CHF, although other studies have reported that receptor downregulation may vary regionally (21) or be nonexistent (4) in heart failure. In the present study, we directly additive (but still proportionate) increments in plasma ANP, BNP, and cGMP concentrations with augmented hemodynamic effects, a reduction in plasma aldosterone levels, and enhanced diuresis and natriuresis compared with either agent alone.

Numerous studies in experimental (23, 33) and human (18) heart failure have demonstrated increased endogenous ANP levels after NEP inhibition. A limited number have also reported rises in plasma BNP levels (see Ref. 23). In the present study, NEP inhibition induced significant and proportionately similar dose-dependent increases in plasma ANP and BNP concentrations. These results are in agreement with previous work in sheep with heart failure (23) and with in vitro data (14) showing that the enzyme has a similar affinity for both ANP and porcine (and ovine (2)) BNP. Although many studies have examined the effects of NPR-C blockade on the clearance of the natriuretic peptides in normal animals (7, 8, 16, 31), information regarding the contribution of the NPR-C to the metabolism and clearance of endogenous ANP in the setting of heart failure is sparse (4, 21) or, in the case of BNP, nonexistent. In normal sheep, Charles et al. (7) reported proportionate increases in both plasma ANP and BNP concentrations after infusion of the NPR-C ligand C-ANP-(4—23). In dogs with heart failure, clearance-receptor blockade has also been shown to significantly increase endogenous ANP (4, 21). In the present study, infusion of C-ANP-(4—23) in sheep with pacing-induced heart failure induced proportionately similar increases in both plasma ANP and BNP levels. This observation is consistent with the results in normal sheep (7) and with in vitro studies showing that the NPR-C binds the ovine forms of ANP and BNP with similar affinity (32). It should be noted, however, that the similar responses of plasma ANP and BNP observed during both NPR-C blockade and NEP inhibition in sheep may not apply to humans and other species, in which the affinity of the receptor and the enzyme for species-specific forms of BNP are likely to differ (9, 14, 20).

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Numerous studies in experimental (23, 33) and human (18) heart failure have demonstrated increased endogenous ANP levels after NEP inhibition. A limited number have also reported rises in plasma BNP levels (see Ref. 23). In the present study, NEP inhibition induced significant and proportionately similar dose-dependent increases in plasma ANP and BNP concentrations. These results are in agreement with previous work in sheep with heart failure (23) and with in vitro data (14) showing that the enzyme has a similar affinity for both ANP and porcine (and ovine (2)) BNP. Although many studies have examined the effects of NPR-C blockade on the clearance of the natriuretic peptides in normal animals (7, 8, 16, 31), information regarding the contribution of the NPR-C to the metabolism and clearance of endogenous ANP in the setting of heart failure is sparse (4, 21) or, in the case of BNP, nonexistent. In normal sheep, Charles et al. (7) reported proportionate increases in both plasma ANP and BNP concentrations after infusion of the NPR-C ligand C-ANP-(4—23). In dogs with heart failure, clearance-receptor blockade has also been shown to significantly increase endogenous ANP (4, 21). In the present study, infusion of C-ANP-(4—23) in sheep with pacing-induced heart failure induced proportionately similar increases in both plasma ANP and BNP levels. This observation is consistent with the results in normal sheep (7) and with in vitro studies showing that the NPR-C binds the ovine forms of ANP and BNP with similar affinity (32). It should be noted, however, that the similar responses of plasma ANP and BNP observed during both NPR-C blockade and NEP inhibition in sheep may not apply to humans and other species, in which the affinity of the receptor and the enzyme for species-specific forms of BNP are likely to differ (9, 14, 20).
compared the effects of inhibition of both metabolic pathways for the first time in the setting of heart failure and found the pattern of plasma ANP and BNP responses during clearance-receptor blockade by C-ANP-(4—23) to be comparable to that seen during NEP inhibition. Furthermore, the maximum natriuretic peptide increments observed in these sheep with heart failure after either inhibitor (1.7- to 1.9-fold for both ANP and BNP) were similar to those observed previously in normal sheep under an identical treatment protocol (7). This suggests that the contributions of NPR-C and NEP 24.11 to ANP and BNP degradation do not alter significantly in the presence of severe cardiac decompensation. These findings indicate not only that the NPR-C has a significant role in the metabolism of the peptides in heart failure (despite higher receptor occupancy and possible downregulation) but also that its contribution is equivalent to that of the NEP enzyme, at least in sheep with pacing-induced CHF and for the dose range of the inhibitors used. We observed no evidence of clearance-receptor saturation in the present study, because each incremental C-ANP-(4—23) dose (both alone and in combination with SCH-32615) produced a similar, if not greater, increase in natriuretic peptide concentrations.

The effects of blocking both degradative pathways simultaneously in normal animals have previously been investigated by several groups. These studies report additive (8, 16) as well as synergistic (7, 31) increments in plasma ANP and BNP levels associated with enhanced biological effects. Results from the present study show for the first time in heart failure that combined inhibition produces more than additive increments in plasma natriuretic peptide concentrations compared with either SCH-32615 or C-ANP-(4—23) alone. These synergistic responses may be caused (in part) by the protection of the NPR-C ligand C-ANP-(4—23) from NEP-mediated degradation, because C-ANP-(4—23) has been shown to be an effective substrate for the NEP enzyme (14). Indeed, we found plasma levels of C-ANP-(4—23) to increase 1.6-fold when SCH-32615 was coadministered. It is unlikely that C-ANP-(4—23) itself was a competitive inhibitor of the NEP enzyme (14), because NEP activity was reduced in an identical fashion during SCH-32615 alone and in combination with the NPR-C ligand. Furthermore, we have shown that much higher concentrations of C-ANP-(4—23) than achieved in our current study fail to affect the hydrolysis of ANP by NEP 24.11 in vitro (7). These results indicate that augmentation of the natriuretic peptides occurred via increased blockade of the NPR-C rather than via inhibition of the enzyme [which is consistent with the similar pattern of natriuretic peptide reductions after cessation of C-ANP-(4—23) alone and in combination with SCH-32615].

The natriuretic peptide responses to each treatment were associated with a similar pattern of increments in the second messenger cGMP. The comparable increases in plasma cGMP levels during SCH-32615 and C-ANP-(4—23) alone were reflected in remarkably similar hemodynamic responses to each agent, including significant dose-dependent falls in MAP, LAP, and CTPR and increases in CO. The tendency for LAP to decline further during SCH-32615 compared with C-ANP-(4—23) may be caused by the significantly greater diuresis induced by this compound. Similar hemodynamic responses after NEP inhibition have been observed previously in heart failure (18, 23), whereas the effects of C-ANP-(4—23) are consistent with those reported during exogenous infusions of both ANP and BNP (22). For the first time in heart failure, we document enhanced hemodynamic responses to combined inhibition of the NEP enzyme and clearance receptor. Despite the synergism observed in natriuretic peptide and cGMP levels during combined treatment, the concomitant hemodynamic responses were less than additive, possibly at least in part because of increased activation of counterregulatory mechanisms. Our findings are in agreement with studies in normal (7, 16) and hypertensive (29) animals that have also demonstrated augmented hemodynamic effects during combined inhibition. These results show that blocking both natriuretic peptide degradative pathways is more effective than inhibiting either route separately and suggest that such a combination might therefore be a useful therapeutic tool for patients with cardiac dysfunction.

The administration of SCH-32615 and C-ANP-(4—23) alone induced significant increases in urine volume,
sodium, potassium, and creatinine excretion in these sheep with heart failure. However, despite near-identical increments in plasma natriuretic peptide levels and remarkably similar hemodynamic responses, the diuretic and natriuretic responses to NEP inhibition were comparatively greater than those to NPR-C blockade. These results are consistent with those of Cavero et al. (6), who found that NEP inhibition produced a greater natriuresis than infused ANP in dogs with experimental heart failure. It is thought that inhibition of NEP 24.11, in addition to elevating plasma natriuretic peptide concentrations (as does NPR-C blockade), also protects the peptides from degradation within the kidney. This view is supported by results from Seymour et al. (26), who observed an increase in both plasma and urinary ANP levels, in association with a significant natriuresis, after NEP inhibition in dogs with pacing-induced CHF. By inhibiting endopeptidase within the glomerulus (27) and proximal tubules (particularly at the brush border membranes where the enzyme is most concentrated; Ref. 25), NEP inhibitors may increase the local concentration of natriuretic peptides at a number of intrarenal sites to enhance natriuresis. The increase in urine sodium excretion induced by each agent may also be mediated by glomerular mechanisms, because it was associated with an increase in glomerular filtration rate (as evidenced by the rise in endogenous creatinine clearance). It is noteworthy that, despite the significantly greater fall in arterial (and hence renal perfusion) pressure, the combination of SCH-32615 and C-ANP-(4—23) elicited at least an additive diuretic and natriuretic response compared with the two agents separately. These enhanced renal effects most likely resulted from the direct natriuretic actions of significantly greater ANP and BNP concentrations and their protection within the kidney, but a minor contribution from the significant reduction in plasma aldosterone levels, seen only during combined blockade, cannot be ruled out. Interestingly, urine potassium excretion was not increased during combined treatment compared with either compound separately, despite the increased natriuresis and diuresis, which would be consistent with the lower levels of plasma aldosterone during combined treatment. Previous studies in normal (7, 31) and hypertensive (29) animals have also demonstrated enhanced renal effects during dual inhibition of the enzymatic and receptor metabolic pathways. Relative inhibition of renin secretion was evident during all treatments in view of the failure of PRA to rise significantly after the sizable falls in arterial pressure.

There is some evidence that the NPR-C may serve some function in addition to clearance of the natriuretic peptides from the circulation. In rat platelets, devoid of particulate guanylate cyclase, ANP inhibited adenylyl cyclase activity as well as reducing adenosine 3',5'-cyclic monophosphate (cAMP) concentrations (3). Hu et al. (13) reported that ANP as well as C-ANP-(4—23) and nanopiperezine ANP-(11—15)-NH2, agents selective for the NPR-C, inhibited the translation of endothelin message and endothelin secretion from cultured bovine aortic endothelial cells. This effect was reversed by 8-bromoadenosine 3',5'-cyclic monophosphate and amiloride, compounds that prevent the inhibition of adenyl cyclase by ANP, but was unchanged by an inhibitor of ANP-induced cGMP generation. Others have shown that ANP inhibits vascular smooth muscle cell proliferation through the NPR-C (5). These data indicate that the NPR-C may be capable of eliciting physiological actions, possibly through their interaction with the cAMP signal transduction mechanism, and suggest that NPR-C blockade by an agonist may have additional therapeutic value to that of raising endogenous natriuretic peptide levels.

In summary, NEP 24.11 inhibition and NPR-C blockade contribute similarly to the clearance of endogenous ANP and BNP in sheep with heart failure. Both natriuretic peptides need to be taken into account when interpreting the actions of NEP inhibitors and NPR-C ligands, which are likely to be species specific (for BNP). We found the functional activity of the clearance-receptor pathway to be preserved in heart failure and its degradative role to be equal to that of the NEP enzyme for the dose range of inhibitors used in the current study. The enhanced hemodynamic, renal, and hormonal responses evident during combined inhibition indicate that preventing the elimination of endogenous ANP and BNP through inhibition of both metabolic pathways is of potentially greater therapeutic value than administering either agent separately.

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