Neutral endopeptidase inhibition: augmented atrial and brain natriuretic peptide, haemodynamic and natriuretic responses in ovine heart failure

Miriam Tessa RADEMAKER, Christopher John CHARLES, Eric Arnold ESPINER, Michael Gary NICHOLLS, Arthur Mark RICHARDS and Teddy KOSOGLOU* 

Department of Medicine, The Christchurch School of Medicine, Christchurch, New Zealand, and *Schering-Plough Research Institute, Kenilworth, New Jersey, U.S.A.

(Received 15 March/21 May 1996; accepted 28 May 1996)

1. Atrial and brain natriuretic peptide are both circulating hormones subject to degradation by neutral endopeptidase 24.11. Whereas endogenous levels of atrial natriuretic peptide are increased by neutral endopeptidase inhibition in most pathophysiological states, the effect on brain natriuretic peptide and the influence of cardiac status is less clear. To further evaluate the role of neutral endopeptidase 24.11, we directly compared the responses of atrial and brain natriuretic peptide, together with the effects on other vasoactive hormones, haemodynamics and renal indices, to a neutral endopeptidase inhibitor, SCH32615, and a vehicle control in eight conscious sheep before and during pacing-induced heart failure.

2. In normal animals, SCH32615 significantly increased concentrations of plasma atrial natriuretic peptide (22 ± 5 pmol/l compared with 14 ± 2 pmol/l in control, 1.6-fold increase) and brain natriuretic peptide (6.5 ± 1.2 pmol/l compared with 4.1 ± 0.7 pmol/l in control, 1.6-fold increase), whereas in heart failure, plasma levels of atrial natriuretic peptide (306 ± 38 pmol/l compared with 187 ± 25 pmol/l in control, 1.6-fold increase) and brain natriuretic peptide (93 ± 11 pmol/l compared with 55 ± 9 pmol/l in control, 1.7-fold increase) were elevated to a significantly greater absolute, but proportionately similar, extent. In both normal and heart-failed animals, SCH32615 induced reductions in mean arterial pressure and left atrial pressure and increases in haematocrit, plasma cGMP and endogenous creatinine clearance. However, only in heart failure did neutral endopeptidase inhibition induce a significant and marked natriuresis (>10-fold increase) and diuresis (4-fold increase), together with suppression of renin activity and haemodynamic effects including decreased peripheral resistance and raised cardiac output.

3. In conclusion, neutral endopeptidase inhibition increases plasma concentrations of atrial and brain natriuretic peptide to a proportionately similar extent in both normal and heart-failed sheep. The striking natriuresis and diuresis and additional haemodynamic effects demonstrated in sheep with heart failure, where natriuretic peptide levels are elevated compared with normal sheep, supports the concept that neutral endopeptidase inhibition augments endogenous atrial and brain natriuretic peptide.

INTRODUCTION

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are both circulating hormones of primarily cardiac origin and are intimately involved in the integrated control of cardiovascular and volume homeostasis [1]. Both natriuretic peptides are significantly raised in congestive heart failure [2], a disease characterized by cardiac and volume overload. Despite already elevated levels, administration of ANP [3, 4] or BNP [5] to patients with congestive heart failure results in beneficial cardiovascular and renal effects. However, the peptidic nature and relatively short half-life of ANP and BNP in plasma severely limits their clinical application. An alternative approach is to enhance levels of the endogenous peptides through inhibition of neutral endopeptidase (NEP) 24.11, a key factor in the enzymic degradation of both ANP and BNP [1, 6]. Several groups have demonstrated that NEP cleaves the Cys105–Phe106 bond of ANP, thereby disrupting the 17-amino-acid ring structure and producing a biologically inactive metabolite [1]. Unlike ANP, the amino acid sequence of BNP differs considerably among species and is cleaved at several different sites and different rates in a species-specific manner [6].

Previous work in both normal [7, 8] and congestive heart failure states [9–11] has demonstrated that endogenous ANP levels are increased by NEP inhibition and are associated with significant haemodynamic and renal effects in heart failure (HF).
In deoxycorticosterone acetate salt-loaded rats these responses were inhibited by pretreatment with ANP-immune serum [12], which suggests that the beneficial effects of NEP inhibition in rats were probably mediated by change in endogenous ANP rather than BNP. On the other hand, administration of NEP inhibitors in several species, including humans [13] and sheep [14], is reported to increase plasma levels of BNP. These findings raise the possibility that the actions of NEP inhibition in some settings may also be at least partly BNP-dependent. To our knowledge, no previous study has systematically compared the effects of endopeptidase inhibition on endogenous levels of natriuretic peptides under both physiological and pathophysiological conditions. Accordingly, we directly compared the responses of both ANP and BNP to NEP inhibition in conscious sheep before and during congestive heart failure produced by rapid left ventricular pacing and examined the concomitant haemodynamic, renal and hormonal effects.

**METHODS**

**Surgical preparation**

Eight coopworth ewes (body weight 42–56 kg) were instrumented as previously described [15] via a left lateral thoracotomy. Under general anaesthesia (induced by thiopentone (17 mg/kg) and maintained with halothane and nitrous oxide), two polyvinyl chloride catheters were inserted in the left atrium for blood sampling and pressure (LAP) determination; a Konigsberg (P 6.0) 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 French Swan-Ganz catheter was inserted in the pulmonary artery for infusions and a 7 French 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 pethidine post-operatively (50 mg intramuscularly) and were allowed to recover for at least 14 days before commencing the study protocol. 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 approximately 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**

The sheep received SCH32615 (5 mg/kg bolus followed by a 3 h intravenous infusion at 1 mg h⁻¹ kg⁻¹) and vehicle (0.9% saline) in random order before (study days 1 and 3) and during (study days 11 and 13) HF induced by rapid left ventricular pacing (225 beats/min for 7 days) [15]. All infusions were administered in a total volume of 60 ml via the pulmonary artery catheter commencing at 1000 h. Haemodynamic recordings (MAP, LAP and CO) were performed at 15 min intervals from 1 h before infusion (baseline) to 1 h after infusion, after which half-hourly measurements were made for a further hour. 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 haemodynamic monitor (M17294; Mennen-Greatbatch Ltd, Rehevot, Israel) for pressure determination relative to atmospheric pressure. The Konigsberg pressure transducer was connected to a pre-amplifier before display by the monitor. Haemodynamic measurements were determined by on-line computer assisted analysis using methods described previously [16].

Blood was drawn from the left atrium 30 min and immediately before infusion (baseline), at 30, 60, 120 and 180 min during infusion and at 30, 60 and 120 min after infusion. The blood was taken into tubes on ice, centrifuged at 4°C and stored at −80°C before assay of ANP [17], BNP ([18], cGMP [17], plasma renin activity, aldosterone and cortisol [15]. All samples from each animal were measured in the same assay to avoid inter-assay variability. Partial cloning of the ovine BNP gene in our laboratory indicates that ovine BNP is identical to the C-terminal (26-residue) portion of porcine pro-BNP [19]. Similarly, the ovine ANP amino acid sequence is identical to human and porcine forms [19]. Haematocrit concentrations were measured with every blood sample taken. Blood samples for analysis of plasma sodium, potassium and creatinine concentrations were taken immediately before infusion (baseline), at the end of infusion, and 2 h after infusion. Urine volume and samples for the measurement of sodium, potassium and creatinine excretion were collected hourly from the hour before infusion (baseline) to 2 h post-infusion.

The protocol was approved by the Animal Ethics Committee of the Christchurch School of Medicine.

**Statistics**

Results are expressed as mean±SEM. Baseline haemodynamic and hormone values represent the mean of the four and two pre-infusion measurements, respectively. Significant differences between the effects of vehicle and SCH 32615 in both the non-paced and paced phases were determined by analysis of variance or covariance using time as a repeated measure. The response of each variable to SCH32615 in the non-paced and paced states was compared by delta analysis. The response of ANP and BNP to SCH32615 was directly compared by
delta percentage analysis. Significance was assumed when \( P < 0.05 \). Where significant differences were identified, \textit{a priori} Fisher's protected least-square difference tests were used to identify time points significantly different from control.

**RESULTS**

After 7 days of rapid ventricular pacing, all sheep exhibited the haemodynamic and hormonal hallmarks of established HF [15]. As observed in previous studies [15], MAP (\( P < 0.001 \)) and CO (\( P < 0.001 \)) were reduced, whereas LAP (\( P < 0.001 \)), calculated total peripheral resistance (\( \text{CTPR} = \frac{\text{MAP}}{\text{CO}} \)) (\( P < 0.001 \)), plasma ANP (\( P < 0.001 \)), BNP (\( P < 0.001 \)) and cGMP (\( P < 0.01 \)) and plasma renin activity (\( P < 0.05 \)) levels were elevated.

In normal animals, SCH32615 increased levels of plasma ANP (\( P < 0.05 \)) [22 ± 5 pmol/l at 3 h compared with 14 ± 2 pmol/l in time-matched control (1.6-fold increase)] and BNP (\( P < 0.05 \)) [6.5 ± 1.2 pmol/l compared with 4.1 ± 0.7 pmol/l in control (1.6-fold increase)] in association with a rise in plasma cGMP (\( P < 0.05 \)) [39 ± 4 nmol/l compared with 13 ± 3 nmol/l in control (3-fold increase)]. In HF, SCH32615 increased plasma ANP (\( P < 0.001 \)) [306 ± 38 pmol/l compared with 187 ± 25 pmol/l in control (1.6-fold increase)], BNP (\( P < 0.01 \)) [93 ± 11 pmol/l compared with 55 ± 9 pmol/l in control (1.7-fold increase)] and cGMP (\( P < 0.001 \)) [78 ± 19 nmol/l compared with 38 ± 4 nmol/l in control (2.1-fold increase)] (Fig. 1) to a significantly greater extent than was observed during the normal phase (all \( P < 0.01 \)). Although NEP inhibition produced a greater absolute increase in plasma ANP than BNP in each state (\( P < 0.05 \), normal sheep; \( P < 0.01 \), HF sheep), and the rises in HF were greater than those in normal sheep, the percentage increases of both natriuretic peptides were similar in both normal and HF animals. In contrast, the percentage increase in cGMP was less in the HF state. Natriuretic peptide levels were still significantly elevated at 2 h post-infusion in both states.

![Graph showing plasma ANP, BNP, and cGMP response during 3h infusions of vehicle (○) and SCH 32615 (5mg/kg bolus and 3mg/kg per 3h infusion) (●) in eight sheep before (non-paced) and during pacing-induced heart failure (paced). Baseline data points represent the mean of two samples taken in the hour preceding treatment. Significant differences between control and SCH 32615 data are shown by: *P<0.05, **P<0.01, ***P<0.001. Values are mean ± SEM.](attachment://graph.png)
In both states, SCH32615 significantly reduced MAP (both $P<0.01$) (at 3h: $85 \pm 4$ mmHg in normal compared with $90 \pm 3$ mmHg in control; $68 \pm 3$ mmHg in HF compared with $74 \pm 2$ mmHg in control) and LAP (both $P<0.01$) (1.5 $\pm$ 0.4 mmHg in normal compared with $3.0 \pm 0.3$ mmHg in control; $18 \pm 1$ mmHg in HF compared with $22 \pm 1$ mmHg in control) and increased haematocrit relative to control (both $P<0.001$) (Fig. 2). In the normal animals, heart rate tended to increase (at 1h: $87 \pm 12$ beats/min compared with $71 \pm 4$ beats/min in control), but these changes did not reach statistical significance (Table 1). CO was increased ($P<0.01$) (at 75 min: $1.74 \pm 0.23$ l/min compared with $1.53 \pm 0.15$ l/min in control; Fig. 2) and CTPR was reduced ($P<0.01$) (Table 1) in HF animals only. Changes in MAP, and especially LAP and haematocrit were sustained well after completion of SCH32615 infusion, whereas the rise in CO in the HF state was not sustained beyond the infusion period. The LAP response was significantly greater during the HF than in the normal phase ($P<0.001$).

In both normal and HF animals, SCH32615 increased urine creatinine excretion (both $P<0.01$) (at 2h: $0.42 \pm 0.02$ mmol/h in normal compared with $0.35 \pm 0.03$ mmol/h in control; at 1h: $0.43 \pm 0.03$ mmol/h in HF compared with $0.32 \pm 0.04$ mmol/h in control, Fig. 3) and creatinine clearance (both $P<0.01$) (Table 1). However, only in HF were significant increases in urine volume ($P<0.01$) (at 3h: $303 \pm 100$ ml/h compared with $74 \pm 17$ ml/h in control), urine sodium ($P<0.01$) (at 2h: $24 \pm 8.9$ mmol/h compared with $1.8 \pm 0.7$ mmol/h in control) and potassium excretion observed.
appears to be some inconsistency in the ability of NEP inhibition to increase plasma concentrations of ANP, and particularly BNP, under physiological conditions. While studies in normal animals and man have shown both a rise [7, 8] as well as no change [10, 11] in endogenous ANP concentrations during administration of NEP inhibitors, Hashimoto et al. [21] found that plasma BNP levels were unmeasurable in normal rats and no change was detected during NEP inhibition. These results led the authors to speculate that the clearance of ANP and BNP at physiological levels may be largely clearance-receptor mediated and that NEP plays a more significant role in the clearance of the peptides at supraphysiological levels. In addition, it has been suggested that NEP may not play such a pivotal role in the clearance of BNP compared with ANP, particularly at lower levels of circulating hormone. In a study in rats, NEP inhibition increased ANP but not BNP concentrations [22], while in hypertensive humans, endopeptidase inhibition induced a proportionately smaller increase in endogenous BNP than ANP [23]. The current results in sheep suggest that the enzyme NEP 24.11 not only has a similar affinity for both ANP and BNP, but also affects the clearance of both peptides in a significant and proportionately similar manner at physiological as well as pathophysiogical concentrations. These findings are consistent with data in vitro [24] showing that the enzyme has a similar affinity for both ANP and porcine (ovine [19]) BNP. However, these findings may not apply to humans and other species where the affinity of the enzyme for species-specific forms of BNP is likely to differ [6].

The rise in plasma ANP and BNP levels after NEP inhibition significantly activated guanylate cyclase, as judged by the increase in plasma cGMP in both normal and HF states. These cGMP increases in response to summative increments in natriuretic peptide levels were comparable to those previously observed during exogenous infusions of either ANP or BNP in both normal [25] and heart-failed (M. T. Rademaker, C. J. Charles,
Fig. 3. Renal response during 3h infusions of vehicle (open bars) and SCH 32615 (5mg/kg bolus and 3mg/kg/3h infusion) (hatched bars) in eight sheep before (non-paced) and during pacing-induced HF (paced). Significant differences between control and SCH 32615 data are shown by: *P<0.05, **P<0.01, ***P<0.001. Values are mean±SEM.

E. A. Espiner, M. G. Nicholls and A. M. Richards, unpublished work) sheep. Others have previously reported elevated cGMP concentrations in both normal and HF states during NEP inhibition [10]. However, although the absolute increase in plasma cGMP in the present study was significantly greater in HF compared with normal sheep, the percentage increase was only 70% of that in normal sheep and the nmol rise in cGMP produced per pmol rise in ANP and BNP in HF was half that observed in normal sheep. Evidence from studies both in vitro and in vivo has shown that prolonged exposure to ANP may lead to diminished activity of particulate guanylate cyclase and, thereby, to resistance against the effects of ANP. After protracted exposure to ANP in vitro, the production of cGMP by vascular smooth muscle cells was reduced [26], and the isolated rings of rat aorta became desensitized to the vasodilatory effect of ANP due to reduced cGMP production [27]. In addition, there is evidence of natriuretic peptide-receptor down-regulation by ANP [28]. Our cGMP findings would be consistent with these results.

In both normal and HF states, SCH 32615 induced significant falls in MAP associated with reduced cardiac filling pressures (LAP) and relative haemoconcentration (presumably by redistribution of plasma volume to extravascular space). The falls
in LAP are likely to be mediated by a decrease in circulating volume and hence venous return (as judged by the rise in haematocrit) and, in the setting of HF, an improvement in left ventricular function. Similar haemodynamic responses have been observed by others after NEP inhibition in experimental models and human HF [9, 10, 20], while studies in normal animals have reported increased haematocrit levels [14] and a tendency for arterial pressure to be reduced [10, 14]. These results are consistent with the effects reported during exogenous infusions of ANP and BNP in normal animals [25] and in HF [5, 29], and support the view that the effects of NEP inhibition are largely a consequence of augmented tissue and plasma levels of natriuretic peptides. We observed an increase in CO and reduction in CTPR only in HF animals. The CO response is dependent on the opposing contributions of the fall in ventricular filling pressures tending to depress CO, and the fall in systemic vascular resistance tending to increase it. In HF, the initial filling pressures are elevated and a moderate fall after therapy is therefore less likely to depress CO than in normal subjects, and is more than counterbalanced by the fall in CTPR.

A marked diuresis and natriuresis were observed in the HF animals during SCH 32615 treatment in the present study. A similar pattern of enhanced urine sodium excretion in HF after NEP inhibition has been reported previously [9–11]. In direct contrast to these results using NEP inhibitors is the blunted natriuretic response usually seen during exogenous ANP infusion in human HF compared with normal subjects [30]. Cavero et al. [31] found that NEP inhibition produced a greater natriuresis than infused ANP in dogs with experimental HF. Although the contribution of BNP was not assessed in these studies, the results suggest that inhibition of NEP 24.11 may increase sodium excretion by mechanisms other than elevating plasma natriuretic peptide concentrations, possibly by protecting these peptides from degradation within the kidney. This view is supported by results from Seymour et al. [32] 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 HF. By inhibiting endopeptidase within the glomerulus [33] and proximal tubule (particularly at the brush-border membranes where the enzyme is most concentrated [1]), NEP inhibi-
tors may increase the local concentration of natriuretic peptides at a number of intrarenal sites to enhance natriuresis. This mechanism may be more important in HF, where not only is proximal tubular fluid resorption enhanced, but circulating levels of both ANP and BNP are significantly higher. The observed increase in urine sodium excretion may also be mediated by glomerular as well as tubular mechanisms because it was associated with an increase in glomerular filtration rate (as evidenced by the rise in endogenous creatinine excretion). In HF, potentiation of ANP and BNP by NEP inhibition might also have promoted natriuresis by attenuating activated neuroendocrine anti-natriuretic factors such as raised plasma renin activity and angiotensin II levels, since we observed a significant reduction in plasma renin activity in HF animals. Because several other peptides are substrates for NEP 24.11 [34], the pharmacological effects of inhibitors of this enzyme may involve factors other than ANP and BNP.

In summary, the present study demonstrates that the enzyme NEP 24.11 contributes similarly to the clearance of ANP and BNP in sheep under both physiological and pathophysiological conditions. Thus, both ANP and BNP need to be taken into account when interpreting the actions of endopeptidase inhibitors which are likely to be species-specific. The enhanced responsiveness to NEP inhibition observed in sheep with HF, who have elevated plasma ANP and BNP concentrations, is consistent with the concept that NEP inhibition leads to potentiation of endogenous natriuretic peptide levels. Preventing the elimination of ANP and BNP in vivo through inhibition of their metabolic pathways may be of therapeutic value in HF.

ACKNOWLEDGMENTS

This study was supported by grants from the National Heart Foundation of New Zealand and The Health Research Council of New Zealand. We are grateful to the staff of the Christchurch School of Medicine Animal Laboratory for care of the animals and the staff of the Endocrine, Steroid and Biochemistry Laboratories for hormone and biochemical assays.

REFERENCES


28. Hirota Y, Tomita M, Takata S, Yoshimi H. Vascular receptor binding activities and cyclic GMP responses by synthetic human and rat atrial natriuretic
peptides (ANP) and receptor down-regulation by ANP. Biochim Biophys Res Commun 1985; 128: 538-46.


