Hemodynamic and Hormonal Effects of Neutral Endopeptidase Inhibitor SCH 39370 in Sheep

Christopher J. Charles, Eric A. Espiner, Vicky A. Cameron, A. Mark Richards, Timothy G. Yandle, and Edmund J. Sybertz

Whole body clearance of atrial natriuretic factor is due to both receptor uptake and enzymatic degradation initiated by neutral endopeptidase 24.11. The effects of neutral endopeptidase inhibition have been studied in seven sodium-replete sheep using SCH 39370, a specific and potent inhibitor of neutral endopeptidase, in the presence or absence of exogenous hormone [rat ANF-(101-126), 2.4 pmol/kg/min for 2 hours]. SCH 39370 alone (2.5 mg/kg bolus) increased plasma atrial natriuretic factor and plasma cyclic GMP levels, lowered arterial pressure for periods beyond changes in plasma atrial natriuretic factor or cyclic GMP, and suppressed both plasma aldosterone and cortisol levels when compared with vehicle injections. The effects of SCH 39370 were similar to or exceeded those of atrial natriuretic factor infusions, which induced significantly greater increases in plasma atrial natriuretic factor (p<0.01). Neither agent alone was natriuretic. When SCH 39370 and atrial natriuretic factor were given together, plasma cyclic GMP but not atrial natriuretic factor levels were increased (p=0.013) compared with atrial natriuretic factor infusion alone, and the half-life was prolonged (p=0.002) in the presence of SCH 39370. The hypotensive response was greater than that induced by atrial natriuretic factor alone (p=0.03) but not different from SCH 39370 alone. Inhibitory effects of SCH 39370 on aldosterone levels were similar in the presence or absence of exogenous atrial natriuretic factor. Although not excluding actions of neutral endopeptidase inhibitors mediated by non-atrial natriuretic factor pathways, these results are consistent with a prolonged effect of neutral endopeptidase inhibition to increase tissue atrial natriuretic factor activity and provide further evidence of the importance of neutral endopeptidase in atrial natriuretic factor physiology. (Hypertension 1991;17:643–651)

Atrial natriuretic factor (ANF), a circulating peptide with potent effects on renal, cardiovascular, and hormonal systems, appears to be an important new hormonal regulator of body fluid and blood pressure. Many of the actions of the hormone have therapeutic potential—particularly when normal pressure-volume relations are disrupted, as in hypertension, heart failure, and renal failure. However, techniques to increase blood or tissue hormone concentrations are made difficult by its polypeptide nature and rapid clearance from the circulation.1 Clearance of ANF from plasma is due to both specific receptor uptake2 and enzymatic degradation.3,4 Although the relative importance of these two pathways is unknown,5 evidence is accumulating6,7 that neutral endopeptidase (NEP) (EC 3.4.24.11) is crucial in initiating the hormone's conversion to the less active metabolite ANF-(99-105)/(106-126) (cleaved ANF).8,9 A variety of different NEP inhibitors appear to mimic10-12 or enhance13,14 the effect of intact ANF, but the precise extent and time course of these effects of hemodynamic and hormonal function remain to be determined. The importance of the prevailing plasma ANF concentration on the efficacy of NEP inhibitors is also unclear.

SCH 39370 (N-N-[1-(S)-carboxy-3-phenylpropyl]- (S)-phenylalanine)-(S)-isoserine) is a specific and potent inhibitor of NEP. The concentration of SCH 39370 required for 50% inhibition (IC50) of the hydrolysis of [3H]Leu-enkephalin is 11.2 nM. SCH 39370 completely prevents the degradation of 10 µM ANF-(99-126) by NEP at 100 nM with an IC50 value of 5 nM. In contrast, SCH 39370 shows no inhibitory
activity against several other proteases including angiotensin converting enzyme and carboxypeptidase A at 1 and 10 μM, respectively. We have evaluated the effects of NEP inhibition on the biological activity of both endogenous and exogenous ANF using bolus injections of SCH 39370 in conscious normal sheep.

Methods

Seven coopworth or merino ewes (body weight 31–48 kg) were housed in an air-conditioned, light-controlled room. The sheep were put under general anesthesia (induced by 20 mg/kg thiopental and maintained by a mixture of halothane, nitrous oxide, and oxygen), and the carotid artery was cannulated (16G Angiocath, Becton Dickinson, Sandy, Utah) for direct measurement of arterial pressure and heart rate. Three polyethylene catheters were placed in the jugular veins for infusion, blood sampling, and measurement of right atrial pressure. A Foley catheter (14G) was placed via the urethra in the urinary bladder. The animals were allowed to recover for at least 7 days before experiments commenced.

Each sheep was studied on four occasions at least 2 days apart. During the study, and commencing at least 5 days before experiments, the animals received a standard diet of sheep nuts and chaff supplemented with 60 mmol sodium administered orally each day as sodium chloride tablets. This provided a daily intake of 75 mmol sodium and 180 mmol potassium. Animals were held in metabolic cages with free access to water. After a 2-hour baseline hemodynamic recording period, rat ANF-(101–126) (Merck Sharpe & Dohme, West Point, Pa.) at 2.4 pmol/kg/min or 7.5 ng/kg/min in Haemaccel or control (Haemaccel alone) was administered intravenously commencing at 11:00 AM at a rate of 15 ml/hr for 2 hours. Haemaccel (Behring, Marburg, FRG) is an intravenous solution commonly used for plasma volume expansion consisting of 3.5% degraded gelatin polypeptides cross-linked via urea bridges with physiological concentrations of sodium, potassium, calcium, chloride, phosphate, and sulfate. Immediately before infusions commenced, an intravenous bolus injection (10 ml) of either SCH 39370 (2.5 mg/kg) or vehicle (1 M Tris-HCl, pH 7.4) was given in random order. Arterial pressure and right atrial pressure were measured continuously via the carotid and right atrial cannulae, respectively, using Statham pressure transducers (Spectramed Medical Products, Singapore) and an Astromed chart recorder (Astromed Inc., W. Warwick, R.I.). Heart rate was measured from the arterial trace at preset intervals. The pressure recordings were manually integrated over 5-minute time periods every 15 minutes for the duration of the infusion and at 30-minute intervals before and after infusion. Recordings were continued for 2 hours after infusion.

Urine was collected during the two 24-hour periods preceding infusion and then hourly at 1, 2, 3, and 4 hours from start of infusion. Volume was recorded before samples were analyzed for sodium, potassium, and creatinine excretion.

Venous blood was drawn at 60, 30, and 0 minutes before infusion and at 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes from start of infusion. The total blood volume withdrawn in each study did not exceed 200 ml. Venous blood was taken into chilled tubes and centrifuged, and the plasma was stored at −20°C (−80°C for ANF) before assay for ANF, cyclic guanosine 3′,5′-monophosphate (cyclic GMP), aldosterone, angiotensin II, and cortisol. Hematocrit was determined by the microhematocrit technique at the time of sampling. Additional samples were drawn on the two ANF infusion days at 1-minute intervals for 10 minutes immediately after infusion for determination of half-life of infused ANF in the presence or absence of SCH 39370. At the completion of the experiment, red blood cells were returned to the sheep in 0.9% saline. NEP was measured in a fluorometric assay using Glutaryl-Ala-Ala-Phe-amid methyl coumarain (AMC) (Enzyme Systems Products, Livermore, Calif.) as a substrate (unpublished results from our laboratory). Although this assay adequately measured NEP activity in human serum, it did not detect any activity in ovine serum. To assess the effects of SCH 39370 circulating in ovine serum in the present study, we added exogenous NEP activity to serum samples and monitored the inhibition of this activity by SCH 39370 in the sample. Twenty microliters of ovine kidney microvillar NEP preparation containing approximately 0.8 nmol/ml/min activity was added to 20 μl serum. To this tube and an identical control was added 30 μl 0.1 M Tris-HCl buffer, pH 7.6, and 10 μl substrate solution to give a final concentration of 0.5 mM. The NEP inhibitor phosphoramidon (final concentration 10 μM, Sigma Chemical Co., St. Louis, Mo.) was added to the control tube. Both tubes were incubated at 37°C for 30 minutes, at which time phosphoramidon was added to the sample tube. This was followed by a further 30-minute incubation in the presence of excess amnopeptidase M (Boehringer Mannheim, Mannheim, FRG) to release free AMC. The AMC released was measured fluorometrically after dilution with 3 ml buffer, and the NEP activity was calculated from the difference between the sample and control tubes.

Samples from individual sheep were assayed together to avoid interassay variation. The intra-assay coefficients of variation ranged from 5.5% for cyclic GMP to 7.2% for ANF. Half-life of infused ANF was calculated by manually fitting a straight line to the plot of log plasma ANF level against time and interpolating the time at which ANF had declined halfway between plateau and baseline. Metabolic clearance rate (MCR) was calculated from the formula

\[ \text{MCR} = \frac{\text{infusion rate}}{\text{mean level during infusion} - \text{basal level}} \]

The study protocol was approved by the Animal Ethics Committee of the Christchurch School of Medicine.
Effect of SCH 39370 Bolus Alone

Baseline Values

Statistics

Results

Effect of SCH 39370 Bolus Alone

Effect of SCH 39370 Bolus With Exogenous Atrial Natriuretic Factor

Table 1. Baseline Recordings

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Haemaccel</th>
<th>SCH+ Haemaccel</th>
<th>ANF</th>
<th>SCH+ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF (pmol/l)</td>
<td>19.3±6.7</td>
<td>16.6±4.2</td>
<td>15.9±2.9</td>
<td>16.1±4.0</td>
</tr>
<tr>
<td>Cyclic GMP (nmol/l)</td>
<td>11.5±4.0</td>
<td>6.5±0.96</td>
<td>6.9±0.67</td>
<td>7.9±1.3</td>
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<tr>
<td>Aldosterone (pmol/l)</td>
<td>581±106</td>
<td>482±95</td>
<td>529±70</td>
<td>461±107</td>
</tr>
<tr>
<td>Ang II (pmol/l)</td>
<td>17.0±2.0</td>
<td>13.7±2.4</td>
<td>12.9±1.0</td>
<td>12.6±2.4</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>208±36</td>
<td>213±38</td>
<td>185±21</td>
<td>163±20</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>98.4±4.6</td>
<td>109.3±3.6</td>
<td>106.4±3.1</td>
<td>106±3.1</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>78.2±4.5</td>
<td>89.3±2.8</td>
<td>87.5±3.2</td>
<td>86.4±3.2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>121±11.2</td>
<td>119±14</td>
<td>121±8.8</td>
<td>114±7.9</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SCH, SCH 39370; ANF, atrial natriuretic factor; cyclic GMP, cyclic guanosine 3',5' monophosphate; Ang II, angiotensin II; SAP, systolic arterial pressure; MAP, mean arterial pressure.
### Table 2. Right Atrial Pressure, Hematocrit, and Urine Data

<table>
<thead>
<tr>
<th>Measurement/treatment</th>
<th>Baseline</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>5.2±2.1</td>
<td>4.9±2.3</td>
<td>4.3±2.1</td>
<td>5.1±1.9</td>
<td>4.3±2.0</td>
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<tr>
<td>SCH+H</td>
<td>2.5±0.8</td>
<td>2.4±1.4</td>
<td>3.0±1.0</td>
<td>3.8±1.0</td>
<td>4.8±1.2</td>
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<tr>
<td>ANF*</td>
<td>2.7±1.5</td>
<td>1.6±1.9</td>
<td>4.3±1.2</td>
<td>3.1±1.6</td>
<td>3.2±1.5</td>
</tr>
<tr>
<td>SCH+ANF*</td>
<td>3.2±1.5</td>
<td>0.6±1.1</td>
<td>0.3±1.3</td>
<td>1.2±0.8</td>
<td>0.6±1.1</td>
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<tr>
<td>Hematocrit (%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>31.4±1.5</td>
<td>30.7±1.3</td>
<td>31.2±1.7</td>
<td>31.4±1.8</td>
<td>31.4±1.8</td>
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<tr>
<td>SCH+H</td>
<td>31.3±1.7</td>
<td>29.7±2.3</td>
<td>31.1±2.2</td>
<td>30.4±2.5</td>
<td>30.4±2.2</td>
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<tr>
<td>ANF</td>
<td>30.2±2.1</td>
<td>29.5±1.9</td>
<td>30.8±2.0</td>
<td>30.5±2.0</td>
<td>29.1±1.9</td>
</tr>
<tr>
<td>SCH+ANF</td>
<td>29.0±1.9</td>
<td>29.7±1.6</td>
<td>30.5±1.9</td>
<td>30.7±1.8</td>
<td>29.9±1.7</td>
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<tr>
<td>Urinary rate (ml/min)</td>
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<tr>
<td>H†</td>
<td>0.69±0.18</td>
<td>1.16±0.33</td>
<td>0.79±0.29</td>
<td>1.64±0.97</td>
<td>0.71±0.27</td>
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<tr>
<td>SCH+H†</td>
<td>0.95±0.18</td>
<td>0.88±0.13</td>
<td>0.88±0.21</td>
<td>1.02±0.44</td>
<td>1.49±0.92</td>
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<tr>
<td>ANF†</td>
<td>1.10±0.17</td>
<td>1.08±0.15</td>
<td>1.45±0.27</td>
<td>1.34±0.34</td>
<td>1.32±0.29</td>
</tr>
<tr>
<td>SCH+ANF†</td>
<td>0.80±0.19</td>
<td>2.49±0.91</td>
<td>1.60±0.66</td>
<td>0.72±0.20</td>
<td>0.59±0.19</td>
</tr>
<tr>
<td>Urinary sodium (μmol/min)</td>
<td></td>
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<td></td>
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<tr>
<td>H‡</td>
<td>35±10</td>
<td>73±33</td>
<td>58±29</td>
<td>73±35</td>
<td>57±34</td>
</tr>
<tr>
<td>SCH+H‡</td>
<td>60±9</td>
<td>44±11</td>
<td>62±17</td>
<td>85±43</td>
<td>87±46</td>
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<tr>
<td>ANF‡</td>
<td>47±10</td>
<td>71±18</td>
<td>82±23</td>
<td>85±30</td>
<td>86±27</td>
</tr>
<tr>
<td>SCH+ANF‡</td>
<td>52±11</td>
<td>164±61</td>
<td>131±51</td>
<td>73±23</td>
<td>50±19</td>
</tr>
<tr>
<td>Urinary potassium (μmol/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H</td>
<td>78±20</td>
<td>109±34</td>
<td>73±30</td>
<td>53±20</td>
<td>58±22</td>
</tr>
<tr>
<td>SCH+H</td>
<td>83±19</td>
<td>111±31</td>
<td>97±40</td>
<td>60±20</td>
<td>57±19</td>
</tr>
<tr>
<td>ANF</td>
<td>85±19</td>
<td>109±32</td>
<td>79±28</td>
<td>79±22</td>
<td>80±19</td>
</tr>
<tr>
<td>SCH+ANF</td>
<td>74±19</td>
<td>257±118</td>
<td>82±25</td>
<td>56±19</td>
<td>58±21</td>
</tr>
<tr>
<td>Urinary creatinine (μmol/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>5.1±0.8</td>
<td>4.6±0.7</td>
<td>4.0±1.3</td>
<td>4.4±1.2</td>
<td>4.1±1.0</td>
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<tr>
<td>SCH+H</td>
<td>5.1±0.6</td>
<td>5.5±0.8</td>
<td>4.9±1.0</td>
<td>4.5±1.2</td>
<td>4.4±1.2</td>
</tr>
<tr>
<td>ANF</td>
<td>5.7±0.6</td>
<td>5.4±0.7</td>
<td>4.3±0.8</td>
<td>5.4±1.1</td>
<td>5.5±0.5</td>
</tr>
<tr>
<td>SCH+ANF</td>
<td>4.4±0.5</td>
<td>12.8±6.7</td>
<td>4.4±0.9</td>
<td>4.0±0.9</td>
<td>4.2±1.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM. H, Haemaccel; SCH, SCH 39370; ANF, atrial natriuretic factor.

*Treatment difference (two-way analysis of variance [ANOVA]), p=0.05.
†Baseline difference (two-way ANOVA), p=0.001.
‡Baseline difference (two-way ANOVA), p=0.042.
§Change from baseline with time (one-way ANOVA), p=0.049.

together, the elevation in plasma cyclic GMP was almost double that observed when ANF was given alone (Figure 4, p=0.013). In the presence of ANF, SCH 39370 in the serum inhibited exogenous NEP in a manner similar to SCH 39370 alone, namely, 60% at 30 minutes and 20% at 120 minutes (Table 3), a significant inhibition of NEP compared with ANF alone (p<0.001).

There was a subtle initial fall in systolic arterial pressure at the onset of ANF infusions. When ANF infusion was combined with SCH 39370 pretreatment, the fall in systolic arterial pressure was consistently greater than that induced by ANF alone (Figure 5, p=0.027) but did not again be significantly different from SCH 39370 alone. As noted with SCH 39370 alone, these vasodepressor effects of SCH 39370 were observed some 3-4 hours after the bolus injection and at a time when plasma ANF and cyclic GMP levels had returned to baseline levels. Heart rate remained stable in response to ANF infusion alone, but with SCH 39370 plus ANF, heart rate continued to rise throughout the study (Figure 5). At the study's completion, heart rate was 20% higher than levels observed with ANF alone (p=0.016). Right atrial pressure was lower when ANF infusion was combined with SCH 39370 bolus (Table 2, p=0.05). There was a trend for hematocrit
Figure 1. Line graphs showing plasma atrial natriuretic factor (ANF) and cyclic guanosine 3',5'-monophosphate (cGMP) levels before and after a bolus of SCH 39370 (SCH) (●) or control (○). In all sheep a control infusion (Haemaccel) was given from 0 to 2 hours after bolus. Values are shown as mean percentage change from baseline ±SEM for seven separate sheep. Baseline values represent mean of three measurements made within 1 hour of commencing infusions. Levels were significantly elevated for both ANF (p=0.03) and cGMP (p=0.016) after SCH bolus.

to increase when SCH 39370 and ANF were given together (Table 2, NS).

Neither urinary volume nor sodium excretion rate appeared to be affected by infusion of ANF alone (Table 2), but when ANF was combined with SCH 39370, both urine volume (p=0.08) and sodium excretion (p=0.10) tended to be higher (Table 2). When analyzed by one-way ANOVA, sodium excretion was significantly increased above baseline (p=0.049) after infusion of SCH 39370 plus ANF. There was no significant effect on excretion rates of either potassium or creatinine (Table 2).

Plasma aldosterone levels fell progressively on both ANF (p=0.002 by one-way ANOVA) and SCH 39370 plus ANF treatment days to values 50% of baseline at 2 hours and then returned toward preinfusion levels after termination of the ANF infusion (Figure 6). There was no significant difference in values between the two study days. Inhibition of aldosterone occurred without significant changes in plasma angiotensin II, which along with plasma cortisol, was similar with or without SCH 39370 pretreatment (Figure 6).

**Discussion**

These studies show that a single dose of the NEP inhibitor SCH 39370, given to normal sheep consuming a normal sodium intake, significantly increased plasma ANF and plasma cyclic GMP, reduced blood pressure, and reduced plasma aldosterone and cortisol levels. Previous reports of the effects of NEP inhibitors in normotensive states are controversial. Early reports in rats showed no effect of NEP inhibitors on endogenous plasma ANF levels, whereas...
more recent studies, particularly in humans,\textsuperscript{10-12} clearly indicate that NEP inhibitors may increase plasma ANF levels and promote ANF bioactivity at normal (physiological) levels of the hormone. These findings are confirmed in the present study where a single dose of SCH 39370 induced a significant increase (within 15 minutes) of both plasma ANF and cyclic GMP, both of which were sustained for at least 3 hours. The changes in plasma ANF and cyclic GMP parallel the time course of the NEP inhibitory effects of SCH 39370 in serum samples drawn at 30 and 120 minutes after injection.

Compared with constant infusions of ANF, sufficient to raise plasma ANF levels threefold, SCH 39370 bolus induced a similar pattern of aldosterone inhibition and was more effective in lowering arterial pressure even though plasma levels of both ANF and cyclic GMP were lower than those induced by ANF infusions alone ($p=0.01$ for ANF). Although SCH 39370 may also act through non-ANF-dependent pathways, these results suggest that changes in ANF activity at the cell membrane level (in keeping with inhibition of the hormone’s degradation) are more important than increments in plasma hormone concentrations per se. Otherwise, the action of SCH 39370 and ANF infusion were similar. Neither agent alone was natriuretic nor was any significant effect observed on hematocrit or right atrial pressure under these experimental conditions. Interestingly, both SCH 39370 and ANF treatments were associated with a significant fall in plasma cortisol levels. Similar trends have been observed during ANF infusions by others,\textsuperscript{21} sometimes with rebound rise after cessation of ANF.\textsuperscript{22} Although ANF has been shown to inhibit corticotropin releasing factor release in hypothalamic cells\textsuperscript{23} as well as adrenocorticotropic hormone secretion from pituitary cells,\textsuperscript{24} further work is required to clarify such an action of ANF in vivo under physiological conditions.

Based on previous reports,\textsuperscript{13,14,25} we expected proportionately greater effects of SCH 39370 when plasma ANF levels were raised by exogenous infusions of the hormone. Although we did observe a significantly greater hypotensive action (including a significant fall in right atrial pressure) with SCH 39370 plus ANF compared with ANF alone, both the percentage fall in arterial pressure (10%) and inhibition of aldosterone (50–60%) induced by SCH...
FIGURE 5. Line graphs showing hemodynamic response to SCH 39370 (SCH) bolus (●) or control (○) combined with atrial natriuretic factor (ANF) infusion. In all sheep an ANF infusion (2.4 pmol/kg/min) was given from 0 to 2 hours after bolus. Values are shown as mean percentage change from baseline±SEM for seven separate sheep. Baseline values represent mean of four measurements made within 1.5 hours of commencing infusions. Systolic and mean arterial pressures were significantly reduced during SCH 39370 plus ANF (p=0.027 for both), and heart rate was significantly elevated (p=0.016).

FIGURE 6. Line graphs showing hormonal response to SCH 39370 (SCH) bolus (●) or control (○) combined with atrial natriuretic factor (ANF) infusion. In all sheep an ANF infusion (2.4 pmol/kg/min) was given from 0 to 2 hours after bolus. Values are shown as mean percentage change from baseline±SEM for seven separate sheep. Baseline values represent mean of three measurements made within 1 hour of commencing infusions.

SCH 39370 were similar in the presence or absence of ANF infusions. Furthermore, the increment in plasma cyclic GMP associated with SCH 39370 plus ANF, over and above that of ANF alone, was similar to that observed after a bolus of SCH 39370. Thus, these findings do not suggest a potentiation of ANF’s activity by NEP inhibitors, at least in the context of threefold plasma ANF elevations induced in these studies, but rather additive effects of the two treatments. In the present study, the MCR of infused ANF was not altered by SCH 39370, yet the half-life was significantly prolonged when measured at 120 minutes after SCH 39370 bolus, at which time SCH 39370 was still active in the serum as shown by the inhibition of exogenous NEP activity. From this we conclude that the volume of distribution (V_D) for ANF was increased—presumably because there was greater exposure of the hormone to additional vascular clearance sites. This “physiological regulation” of ANF clearance has been noted by others—for example, when V_D is acutely reduced by sudden increases in plasma angiotensin II. The higher level of plasma ANF (see Figure 4) at 120 minutes, immediately before termination of the infusion, is consistent with increasing saturation of this pool, as predicted by the relations that govern V_D, MCR, and half-life. Alternatively, it is possible that, during ANF plus SCH 39370 infusion, there was a fall in endogenous ANF secretion that masked a true reduction in MCR. Our observations with SCH 39370 contrast with those of Almeida et al in rats where the ANF “C”-receptor blocker [C-ANF-(4-23)] induced a proportional fall in both V_D and MCR without greatly affecting the half-life of [125I]-ANF. Previous studies showed a relatively minor impact of SCH 39370 alone on pharmacokinetic parameters of radiolabeled tracer quantities of ANF injected into rats. However, when SCH 39370 was combined with C-ANF-(4-23) treatment, half-life was greatly prolonged. Taken together, the SCH 39370 findings in vivo are consistent with inhibition of ANF uptake and metabolism by mechanisms other than those mediated by “cleansing” receptors. The precise mechanism of action of NEP inhibitors in vivo is still to be clarified, but it appears likely that inhibition of ANF degradation by membrane-bound NEP enzyme in close proximity to “B” receptors plays a major part.
As noted above, the biological consequences of such inhibition (e.g., prolonged and continuing hypotension well beyond any effect on plasma cyclic GMP or ANF) is consistent with this view. The acute effects of NEP inhibitors on plasma ANF and cyclic GMP are similar both in sheep, which lack detectable NEP activity in plasma (unpublished observations from our laboratory), and in humans where plasma NEP activity is readily detectable.29 Thus, it appears likely that local tissue NEP activity rather than circulating enzyme is the important regulator of the hormone’s degradation.

Previous workers, using higher doses of NEP inhibitors, have shown prominent renal effects—particularly natriuresis.14,25,30 In the present study, natriuresis was only observed as a change from baseline with time on the SCH 39370 plus ANF day, with no significant treatment differences when the two study days were compared. However, major fluctuations in basal sodium excretion in normal sheep make interpretation difficult, at least in the setting of small elevations in plasma ANF levels in isovolemic sheep. Previous studies in sheep15 using similar ANF infusions for 24-hour periods have shown a transient early natriuresis within the first 8 hours of the infusion period. The present results are consistent with a previous study13 where SCH 39370 was found to have no natriuretic activity in deoxycorticosterone acetate–salt rats. It is possible that the reduction in arterial pressure (and thus renal perfusion pressure) counterbalanced any natriuretic effects of SCH 39370 in the present study.

Inhibition of the renin-angiotensin-aldosterone system by ANF has been well documented in humans and experimental animals, but there are few published studies of the effects of NEP inhibitors. One study,12 using an oral compound (UK 79300), showed that renin suppression occurred in normal human volunteers. In the present study, both the pattern and the time course of hormone response strongly suggests that aldosterone secretion was inhibited by SCH 39370 (both alone and in combination with ANF). Because there was no concomitant change in plasma angiotensin II, these results are consistent with an increase in ANF inhibitory activity at the level of the adrenal glomerulosa. However, because arterial pressure fell below control values by 10% in the presence of SCH 39370, but plasma angiotensin II remained unchanged, it is possible that there was also a relative inhibition of renin secretion. In any event, inhibition of aldosterone by SCH 39370 appears to be similar to that induced by ANF alone and not further affected by a threefold rise in plasma ANF concentration. It remains to be seen whether these biological actions of NEP inhibition are related to colocation of NEP and ANF receptors within the adrenocortical tissue.

In conclusion, single doses of SCH 39370 in normal sheep induce a significant rise in plasma ANF and cyclic GMP and promote biological effects consistent with a prolonged tissue action of the hormone. These findings provide further evidence of the importance of NEP in ANF physiology.

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