Dual Inhibition of Neutral Endopeptidase and Angiotensin-Converting Enzyme in Rats With Hypertension and Diabetes Mellitus

Tuula Tikkanen, Ilkka Tikkanen, Melinda D. Rockell, Terri J. Allen, Colin I. Johnston, Mark E. Cooper, Louise M. Burrell

Abstract—It has been suggested that combined inhibition of angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) may lower blood pressure more effectively than either treatment alone, independent of the degree of salt and volume status or the activity of the renin-angiotensin system. The effects of NEP inhibition in hypertension associated with diabetes mellitus are largely unknown. We therefore compared ACE inhibition, NEP inhibition, and dual NEP/ACE inhibition in diabetic hypertensive rats. Spontaneously hypertensive rats (SHR) aged 9 to 10 weeks were injected with either streptozotocin (45 mg/kg) or citrate buffer and randomized to receive either the ACE inhibitor captopril (25 mg/kg BID), the NEP inhibitor SCH 42495 (30 mg/kg BID), the dual NEP/ACE inhibitor S 21402 (25 or 50 mg/kg BID), or vehicle by gavage for 4 weeks. A group of diabetic SHR was also allocated to receive the combination of SCH 42495 (30 mg/kg BID) and captopril (25 mg/kg BID). The degree of renal NEP inhibition was determined by autoradiography, and plasma renin activity (PRA) was determined by radioimmunoassay. In diabetic SHR, the dual NEP/ACE inhibitor (50 mg/kg BID), as well as the combination of the NEP inhibitor and the ACE inhibitor, reduced systolic blood pressure more effectively than the ACE inhibitor \((P<0.001)\) or the NEP inhibitor \((P<0.001)\) alone. In nondiabetic SHR, the dual NEP/ACE inhibitor and the ACE inhibitor were equally effective, while the NEP inhibitor had only slight blood pressure–lowering effects. Relative heart weight decreased in parallel to the changes in blood pressure. Renal NEP was clearly inhibited \((70\% \text{ to } 92\%; P<0.001)\) by both the NEP inhibitor and the dual NEP/ACE inhibitor. Both the ACE inhibitor and the dual NEP/ACE inhibitor increased PRA, but the stimulating effect of dual NEP/ACE inhibition on PRA was less than that observed with ACE inhibition alone \((P<0.05)\). Albuminuria in diabetic SHR was lower during treatment with both the dual NEP/ACE inhibitor \((50 \text{ mg/kg BID})\) and the combination of NEP inhibition and ACE inhibition compared with vehicle treatment \((P<0.05)\). In conclusion, the present study shows that hypertension in SHR with streptozotocin-induced diabetes is modulated by natriuretic peptides and thus is sensitive to NEP inhibition. The increased efficacy of dual NEP/ACE inhibition on blood pressure in diabetic SHR, compared with ACE or NEP inhibition alone, suggests that this therapeutic approach may prove beneficial in the treatment of hypertension associated with diabetes mellitus and other forms of volume-dependent hypertension. \((\text{Hypertension. 1998;32:778-785.})\)

Key Words: natriuretic peptides ■ angiotensin ■ renal circulation ■ albuminuria ■ blood pressure

Inhibitors of angiotensin-converting enzyme (ACE) have proved effective in the treatment of hypertension associated with diabetes mellitus. As shown in a meta-analysis of clinical trials, ACE inhibitors reduce proteinuria and attenuate progression of renal failure in patients with diabetic nephropathy more effectively than treatment with conventional antihypertensive drugs. However, the antihypertensive efficacy of ACE inhibitors may be limited by the salt and water retention often found in diabetic patients. For maximal end-organ protection with antihypertensive drugs, effective control of blood pressure appears to be essential and quantitatively perhaps the most important factor. Inhibitors of neutral endopeptidase (NEP), a metalloendopeptidase involved in the degradation of a variety of vasoactive peptides, have been shown to potentiate the natriuretic, diuretic, and blood pressure–lowering effects of natriuretic peptides. In contrast to ACE inhibitors, NEP inhibitors lower blood pressure more effectively in salt- and volume-dependent than in renin-dependent forms of hypertension. Therefore, it has been proposed that the combination of ACE and NEP inhibition may be particularly useful in the treatment of all forms of hypertension. Co-inhibition of ACE and NEP is suggested to lower blood pressure in a broader range of conditions than inhibition of ACE or NEP alone, indepen-
Table 1. Body Weight, Glycemic Control, and Mean SBP

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Plasma Glucose, mmol/L</th>
<th>GHB, %</th>
<th>SBP, mm Hg</th>
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<tbody>
<tr>
<td>Control SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>15</td>
<td>274±9.3</td>
<td>6.5±0.2</td>
<td>5.0±0.1</td>
<td>189±1.0</td>
</tr>
<tr>
<td>ACEi</td>
<td>13</td>
<td>259±8.4</td>
<td>7.0±0.2</td>
<td>5.1±0.2</td>
<td>147±1.7‡</td>
</tr>
<tr>
<td>NEPi</td>
<td>12</td>
<td>275±5.6</td>
<td>7.1±0.2</td>
<td>5.0±0.2</td>
<td>171±1.3†‡</td>
</tr>
<tr>
<td>Dual NEP/ACEi (low dose)</td>
<td>9</td>
<td>262±2.8</td>
<td>6.4±0.2</td>
<td>5.3±0.2</td>
<td>166±2.2†</td>
</tr>
<tr>
<td>Dual NEP/ACEi (high dose)</td>
<td>9</td>
<td>295±2.8</td>
<td>7.1±0.1</td>
<td>4.8±0.2</td>
<td>149±1.5†‡</td>
</tr>
<tr>
<td>Diabetic SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>18</td>
<td>230±3.6*</td>
<td>31.4±0.9*</td>
<td>13.1±0.3*</td>
<td>175±1.4*</td>
</tr>
<tr>
<td>ACEi</td>
<td>15</td>
<td>219±8.8</td>
<td>31.6±0.9</td>
<td>13.2±0.6</td>
<td>150±1.2†</td>
</tr>
<tr>
<td>NEPi</td>
<td>19</td>
<td>219±6.5</td>
<td>31.4±1.2</td>
<td>13.5±0.4</td>
<td>158±1.2†</td>
</tr>
<tr>
<td>NEPi+ACEI</td>
<td>13</td>
<td>222±6.5</td>
<td>31.3±1.5</td>
<td>13.2±0.5</td>
<td>140±1.6†§</td>
</tr>
<tr>
<td>Dual NEP/ACEi (low dose)</td>
<td>13</td>
<td>214±5.0</td>
<td>33.4±1.0</td>
<td>13.8±0.5</td>
<td>155±1.4†‡</td>
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<tr>
<td>Dual NEP/ACEi (high dose)</td>
<td>10</td>
<td>234±11.0</td>
<td>32.5±1.0</td>
<td>13.0±0.5</td>
<td>139±2.5†‡</td>
</tr>
</tbody>
</table>

GHB indicates glycated hemoglobin. Other abbreviations are as in Figure 1. Body weight and GHB are from week 3.5. Plasma glucose is the mean of 2 glucose determinations measured at weeks 1 and 3.5. SBP is the mean of the 4 blood pressure determinations taken during the drug treatment period at weeks 1 to 4.

*P<0.001 vs vehicle-treated control SHR; †P<0.001 vs corresponding vehicle-treated groups (control or diabetic SHR); ‡P<0.001 vs NEP-treated, and dual NEP/ACEi (low dose)–treated diabetic SHR; §P<0.001 vs ACEi-treated, NEP-treated, and dual NEP/ACEi (low dose)–treated diabetic SHR.

The effects of NEP inhibition in hypertension associated with diabetes mellitus are largely unknown. In theory, enhanced vasodilating activity of natriuretic peptides after NEP inhibition may even promote glomerular hyperfiltration, leading to proteinuria and accelerated nephropathy in diabetic animals and humans.15–18 Recently dual inhibitors of NEP and ACE have been introduced for the treatment of hypertension and heart failure.15–18 Therefore, the antihypertensive, hormonal, and renal effects of NEP inhibition, ACE inhibition, and dual NEP/ACE inhibition in diabetic and nondiabetic spontaneously hypertensive rats (SHR) were evaluated.

Methods

Animals
Male SHR (n=146) were obtained from the Austin Research Laboratories, Austin and Repatriation Medical Centre, Heidelberg, Australia. To confirm the inbred status of SHR, all rats are regularly tested with polymorphic markers. Animals were housed at 23°C to 25°C in a 12-hour light/dark cycle with access to standard rat chow (0.4% to 0.6% NaCl, Norco) and normal water ad libitum. Experimental procedures involving animals were approved by the Austin and Repatriation Medical Center Animal Ethics Committee and conformed to the National Health and Medical Research Council of Australia guidelines for animal experimentation.

Drugs
S 21402, a dual NEP and ACE inhibitor (K<sub>i</sub> values of 1.7±0.3 and 4.2±0.5 nmol/L, respectively),22 and the selective NEP inhibitor SCH 42495 were gifts from IRIS (Courbevoie, France) and Schering-Plough Corp (Lafayette, NJ), respectively. The NEP inhibitor RB104 was kindly provided by Dr B.P. Roques (Paris, France). Aprotinin, Tris-HCl, phenanthroline, angiotensin I, captopril, and streptozotocin were obtained from Sigma Chemical Co, and Ultra- lente insulin was obtained from Novo-Nordisk. BSA was obtained from CSL Ltd. Human atrial natriuretic peptide (ANP) was obtained from Peninsula Laboratories Inc. All other reagents were purchased from BDH or Ajax Chemicals.

Induction of Diabetes
Rats aged 9 to 10 weeks and weighing between 200 and 250 g were fasted overnight and randomized to receive either streptozotocin at a dose of 45 mg/kg or citrate buffer (nondiabetic groups) by intravenous injection in the tail vein. Only diabetic animals with plasma glucose levels >17 mmol/L were included in the study. Long-acting insulin (Ultralente) was given to diabetic animals at a dose of 4 U SC daily to prevent ketoadiposis and to promote weight gain without rendering the animals euglycemic.

Drug Treatments
Streptozotocin-injected and citrate buffer–injected SHR were randomly allocated to receive by gavage the ACE inhibitor captopril (25 mg/kg BID in distilled water), the NEP inhibitor SCH 42495 (30 mg/kg BID in 5% arabic gum), the dual NEP/ACE inhibitor S 21402 at 2 different doses (25 mg/kg BID or 50 mg/kg BID in 5% arabic gum), or vehicle for 4 weeks starting on the day of streptozotocin or buffer injection. The choice of the 2 doses of the dual NEP/ACE inhibitor was based on previous findings suggesting that sufficient renal NEP inhibition, comparable to 30 mg/kg of the NEP inhibitor SCH 42495, may be obtained with 25 mg/kg of the dual NEP/ACE inhibitor.22,22 while 50 mg/kg of the dual NEP/ACE inhibitor was needed to provide equipotent angiotensin I pressor response inhibitory efficacy compared with 25 mg/kg of captopril (N. Farina and L.M. Burrell, unpublished data, 1998). A group of diabetic SHR was also allocated to receive the combination of SCH 42495 (30 mg/kg BID) and captopril (25 mg/kg BID) by gavage.

Laboratory Measurements
Systolic blood pressure (SBP) was measured weekly by tail-cuff plethysmography (38L flatbed recorder, model 229 amplifier, IITC Life Science) in conscious, lightly restrained rats.23 Body weight was measured weekly. At week 3, animals were placed in metabolic cages for 24 hours of urine collection and determination of water and food intake.

At week 4, trunk blood was collected into prechilled lithium heparin tubes and into tubes containing Na<sub>2</sub>EDTA and aprotinin (500
Figure 1. SBP in control (A) and diabetic (B) SHR. All drug treatments significantly decreased SBP compared with vehicle in both control SHR (P < 0.001 at all time points) and diabetic SHR (P < 0.001 at all time points). In control SHR, both the dual NEP/ACE inhibitor (high dose) and the ACE inhibitor lowered SBP more effectively than the NEP inhibitor (P < 0.001) or the low-dose dual NEP/ACE inhibitor (P < 0.001). In diabetic SHR, the dual NEP/ACE inhibitor (high dose) lowered SBP more than the ACE inhibitor (P < 0.01 at weeks 1 and 4; P < 0.001 at weeks 2 and 3), the NEP inhibitor (P < 0.001), or the low-dose dual NEP/ACE inhibitor (P < 0.001). The dual NEP/ACE inhibitor (high dose) and combined treatment with the NEP inhibitor and the ACE inhibitor were equally effective. The ACE inhibitor alone was slightly more effective than the NEP inhibitor (P < 0.05 at weeks 1, 2, and 4; P < 0.001 at week 3). ACEi indicates the ACE inhibitor captopril; NEPi, the NEP inhibitor SCH 42495; dual NEP/ACEi (low dose), the dual NEP/ACE inhibitor S 21402, 25 mg/kg BID; dual NEP/ACEi (high dose), the dual NEP/ACE inhibitor S 21402, 50 mg/kg BID; and NEPi + ACEi, the NEP inhibitor plus the ACE inhibitor.

In Vitro NEP Autoradiography

Intravenous NEP of the most potent radiolabeled inhibitor of NEP (K_i = 0.03 nmol/L), was used for these studies. It was iodinated by use of a minor modification of a previously published method. Briefly, the reagents for the iodination were dissolved in 0.2 mol/L phosphate buffer, pH 7.2, containing 5 mmol/L Na_2EDTA and 0.03% sodium azide. The p-nitrophenolic ester of RB104 (0.51 μg in 5.1 μL) was iodinated at room temperature with 1 mCi of Na^22I (Amersham Radiochemicals) and chloramine T (25 μg in 50 μL), and the reaction was stopped after 100 seconds with sodium metabisulfite (25 μg in 50 μL). The pH was then increased to 10 with 1 mol/L NaOH and left to stand for 5 minutes, and the pH was adjusted to <2 with 6 mol/L HCl. The reaction mixture was then transferred onto a Sep-Pak C18 cartridge that had been pretreated with 10 mL of 80% acetonitrile and 0.1% TFA and then 10 mL of 0.1% TFA. The reagents were eluted from the Sep-Pak cartridge with the use of 10 mL of 0.1% TFA followed by 50 mL methanol/water containing 0.1% TFA in a gradient from 40% methanol to 100% methanol. Fractions of the largest peaks were then tested for binding activity with renal autoradiography, and the best binding fractions with the lowest nonspecific binding were pooled and used for the present studies.

The technique for NEP autoradiography has previously been described. Briefly, slide-mounted sections (20 μm in thickness) were preincubated in 50 mmol/L Tris-HCl buffer, pH 7.4, for 50 minutes at room temperature and then incubated in Tris-HCl buffer, pH 7.4, containing 125 I-RB104 (~0.04 mCi, 75 000 counts/min) applied directly to each section for 2 hours at room temperature. Nonspecific binding was determined in the presence of 100 mmol/L EDTA and 2.5 mmol/L phenylalanine.

After incubation, sections were transferred through 4 successive 1-minute washes at 4°C in the appropriate buffer. After the 1-minute washes, sections were washed for 5 seconds in distilled water, dried under cold air, placed in x-ray cassettes, and exposed to Agfa Scopix CR3 x-ray film (Agfa Gevaert) for 2 days. Quantification of binding density was determined by computerized densitometry with the use of radioactive standards that were corrected for decay and fitted to calibration curves to convert the optical density of the autoradiographs to disintegrations per minute per square millimeter.

Statistical Analysis

Data were analyzed by ANOVA with and without repeated measures with the Statview SE + Graphics program (Brainpower). Comparisons of group means were performed by Fisher’s least significant difference method. Urinary albumin data were analyzed after logarithmic transformation. A P value <0.05 was viewed as statistically significant. Data are shown as mean ± SE unless otherwise indicated.

Results

Body Weight and Glycemic Control

Compared with nondiabetic SHR, diabetic SHR gained less weight (Table 1). No differences in plasma glucose or glycosylated hemoglobin levels were found between the diabetic groups (Table 1).
Blood Pressure

Mean SBP was higher in vehicle-treated nondiabetic SHR (189±1.0 mm Hg) than in vehicle-treated diabetic SHR (175±1.4 mm Hg) (P<0.001) (Table 1).

In diabetic SHR, the dual NEP/ACE inhibitor S 21402 (50 mg/kg BID) decreased SBP more than the ACE inhibitor captopril, while the NEP inhibitor SCH 42495 alone was slightly less effective than the ACE inhibitor (Figure 1B). The effect of the dual NEP/ACE inhibitor (50 mg/kg BID) on SBP was equal to that of combined treatment with the NEP inhibitor and the ACE inhibitor. The blood pressure-lowering effect of the dual NEP/ACE inhibitor was dose dependent.

In nondiabetic SHR, the dual NEP/ACE inhibitor (50 mg/kg BID) and the ACE inhibitor were equally effective in lowering blood pressure. The NEP inhibitor had a modest lowering effect on SBP (Figure 1A).

Heart Weight

Relative heart weight paralleled the changes in SBP. The dual NEP/ACE inhibitor showed a clear cardiac antihypertrophic effect in both diabetic and nondiabetic SHR (Figure 2).

In diabetic SHR, heart weight decreased most in rats treated with the dual NEP/ACE inhibitor (50 mg/kg BID). This effect was significant compared with rats receiving the NEP inhibitor alone (P<0.05), but the difference compared with other treatment groups did not reach statistical significance (Figure 2B).

In nondiabetic SHR, the dual NEP/ACE inhibitor (50 mg/kg BID) showed a cardiac antihypertrophic effect similar to that of the ACE inhibitor, while the NEP inhibitor alone decreased heart weight less effectively (Figure 2A).

PRA and ANP

There were no significant differences in PRA or plasma ANP levels between diabetic and nondiabetic SHR (Table 2).

Both the ACE inhibitor and the dual NEP/ACE inhibitor significantly increased PRA (Table 2). However, the stimulating effect of the dual NEP/ACE inhibitor on PRA was less than that observed with ACE inhibition alone. In contrast to the dual NEP/ACE inhibitor, the increase in PRA was not blunted in rats treated with the combination of the NEP inhibitor and the ACE inhibitor compared with treatment with the ACE inhibitor alone. The selective NEP inhibitor had no effect on PRA.

Plasma ANP levels decreased in ACE inhibitor–treated nondiabetic SHR compared with the corresponding vehicle group. Plasma ANP concentration was slightly increased in diabetic SHR treated with the lower dose of the dual NEP/ACE inhibitor but did not change in other groups treated.
Renal NEP was clearly inhibited by both the dual NEP/ACE inhibitor and the selective NEP inhibitor in nondiabetic and diabetic SHR (Table 2). In diabetic SHR, a more complete NEP inhibition (92% inhibition compared with nondiabetic SHR) was obtained with the use of complete NEP inhibition (92% inhibition compared with vehicle-treated diabetic SHR) was obtained with the use of the dual NEP/ACE inhibitor (high dose) or with the combination of NEP inhibition and ACE inhibition, urinary albumin excretion rate was lower than in the corresponding vehicle-treated groups (Figure 3). There were no significant differences in urinary sodium excretion at week 3 in the different groups. Plasma creatinine concentration was slightly higher (60±2.1 μmol/L) in diabetic SHR than in nondiabetic SHR (51±1.8 μmol/L) (P<0.001), but no changes were observed due to treatment with various drug regimens.

Renal NEP
Renal NEP concentration as assessed by 125I-RB104 binding was the same in nondiabetic and diabetic SHR (Table 2).

Renal NEP was clearly inhibited by both the dual NEP/ACE inhibitor and the selective NEP inhibitor in nondiabetic and diabetic SHR (Table 2). In diabetic SHR, a more complete NEP inhibition (92% inhibition compared with vehicle-treated diabetic SHR) was obtained with the use of the high dose (50 mg/kg BID) of the dual NEP/ACE inhibitor compared with the lower (25 mg/kg BID) dose (70% inhibition; P<0.05 versus high dose). No statistically significant differences in the degree of NEP inhibition were otherwise found among rats receiving the dual NEP/ACE inhibitor, the NEP inhibitor alone, or the combination of the NEP inhibitor and the ACE inhibitor.

Renal Effects
No drug treatment adversely effected kidney weight, renal excretory function, urinary albumin excretion rate, or GFR. As expected, kidney weight and urinary volume were increased in diabetic SHR compared with nondiabetic SHR (Table 3). In diabetic SHR, GFR was clearly increased but was not further changed in rats treated with either the NEP inhibitor or the dual ACE/NEP inhibitor (Table 3). Urinary albumin excretion rate was increased in diabetic SHR compared with nondiabetic SHR (P<0.01). In rats treated with the dual NEP/ACE inhibitor (high dose) or with the combination of NEP inhibition and ACE inhibition, urinary albumin excretion rate was lower than in the corresponding vehicle-treated groups (Figure 3). There were no significant differences in urinary sodium excretion at week 3 in the different groups. Plasma creatinine concentration was slightly higher (60±2.1 μmol/L) in diabetic SHR than in nondiabetic SHR (51±1.8 μmol/L) (P<0.001), but no changes were observed due to treatment with various drug regimens.

Discussion
The present study shows that combined NEP/ACE inhibition effectively lowers blood pressure and attenuates cardiac hypertrophy in both diabetic and nondiabetic SHR. Anti-hypertensive efficacy of the dual NEP/ACE inhibitor S 21402 in SHR is in accordance with previous reports,23,24 but this is the first study to explore the effects of dual NEP/ACE inhibition in experimental streptozotocin-induced diabetics. Dual NEP/ACE inhibition (50 mg/kg BID) lowered blood pressure more effectively than ACE inhibition or NEP inhibition alone in diabetic SHR. In nondiabetic SHR, the dual NEP/ACE inhibitor was as effective as the ACE inhibitor captopril, while the selective NEP inhibitor SCH 42495 had only a modest effect on blood pressure.

It has been shown that ACE inhibitors are most effective in renin-angiotensin–dependent forms of hypertension such as renovascular hypertension. By contrast, NEP inhibitors lower blood pressure only in salt- and volume-dependent hypertension models, notably in deoxycorticosterone acetate–salt hypertension, which is usually unresponsive to ACE inhibition.22,23 On the other hand, dual NEP/ACE inhibitors are suggested to lower blood pressure irrespective of the activity of the renin-angiotensin system or the degree of salt and water retention.14–18 Accordingly, the dual NEP/ACE inhibitor S 21402 has been shown to be effective in both renin-dependent renovascular and volume-dependent, renin-independent deoxycorticosterone acetate–salt hypertension.

As expected, kidney weight and urinary volume were increased in diabetic SHR compared with nondiabetic SHR (Table 3). In diabetic SHR, GFR was clearly increased but was not further changed in rats treated with either the NEP inhibitor or the dual ACE/NEP inhibitor (Table 3). Urinary albumin excretion rate was increased in diabetic SHR compared with nondiabetic SHR (P<0.01). In rats treated with the dual NEP/ACE inhibitor (high dose) or with the combination of NEP inhibition and ACE inhibition, urinary albumin excretion rate was lower than in the corresponding vehicle-treated groups (Figure 3). There were no significant differences in urinary sodium excretion at week 3 in the different groups. Plasma creatinine concentration was slightly higher (60±2.1 μmol/L) in diabetic SHR than in nondiabetic SHR (51±1.8 μmol/L) (P<0.001), but no changes were observed due to treatment with various drug regimens.

**TABLE 3. Relative Kidney Weight, 24-Hour Urinary Volume, and GFR**

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney Weight, mg/g</th>
<th>Urinary Volume, mL/24 h</th>
<th>GFR, mL/min</th>
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<tbody>
<tr>
<td>Control SHR</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vehicle</td>
<td>6.8±0.1</td>
<td>14±1</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>ACEi</td>
<td>7.2±0.1</td>
<td>16±1</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>NEPi</td>
<td>6.8±0.2</td>
<td>14±1</td>
<td>2.2±0.07</td>
</tr>
<tr>
<td>Dual NEP/ACEi (low)</td>
<td>7.2±0.1</td>
<td>17±2</td>
<td>2.2±0.05</td>
</tr>
<tr>
<td>Dual NEP/ACEi (high)</td>
<td>6.9±0.1</td>
<td>14±1</td>
<td>2.5±0.07</td>
</tr>
<tr>
<td>Diabetic SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10.1±0.2*</td>
<td>74±6*</td>
<td>2.8±0.1*</td>
</tr>
<tr>
<td>ACEi</td>
<td>10.9±0.2</td>
<td>66±11</td>
<td>2.8±0.2</td>
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<tr>
<td>NEPi</td>
<td>10.2±0.2</td>
<td>63±8</td>
<td>2.8±0.08</td>
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<td>NEPi+ACEi</td>
<td>10.4±0.2</td>
<td>66±7</td>
<td>2.8±0.07</td>
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<tr>
<td>Dual NEP/ACEi (low)</td>
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<td>75±12</td>
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<tr>
<td>Dual NEP/ACEi (high)</td>
<td>10.4±0.5</td>
<td>74±4</td>
<td>2.8±0.2</td>
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</tbody>
</table>

Abbreviations are as in Figure 1.

*P<0.001 vs vehicle-treated control SHR.

Figure 3. Urinary albumin excretion rate in control (A) and diabetic (B) SHR. *P<0.05 vs corresponding vehicle-treated control or diabetic group. Data for urinary albumin excretion rate are shown as geometric mean ×/× tolerance factors. Abbreviations are as in Figure 1.
as well as in SHR, a model of essential hypertension without activation of the renin-angiotensin system.\textsuperscript{15,18,34} The more potent blood pressure-lowering effect of the dual NEP/ACE inhibitor at the dose of 50 mg/kg BID compared with either captopril or selective NEP inhibition in diabetic SHR supports the concept that coinhibition of ACE and NEP lowers blood pressure in a broader range of conditions than inhibition of ACE or NEP alone.

Another important finding was that the selective NEP inhibitor SCH 42495 significantly reduced blood pressure and attenuated cardiac hypertrophy in diabetic SHR. Sensitivity of blood pressure to NEP inhibition in diabetic SHR suggests that the hypertension in this experimental model is partly salt and volume dependent. As has been previously reported, experimental diabetes is characterized by sodium retention associated with increased extracellular volume and suppression of plasma renin activity.\textsuperscript{24} This may also explain why the antihypertensive efficacy of the ACE inhibitor captopril was relatively less potent in diabetic SHR compared with nondiabetic SHR as judged by absolute decrease in blood pressure. Changes in renal NEP activity do not appear to explain the greater sensitivity of diabetic SHR to NEP inhibition or dual NEP/ACE inhibition since there was no detectable difference in renal NEP activity between diabetic and nondiabetic rats.

Compared with ACE inhibition, the blood pressure-lowering effect of the NEP inhibition was slightly weaker in diabetic SHR, but the favorable effect on cardiac weight was observed with both NEP and ACE inhibition. An interesting possibility therefore remains, not studied in the present experiment, that local cardiac effects are involved in the antihypertrophic action of NEP inhibition. The dual NEP/ACE inhibitor (50 mg/kg BID) was more effective than the selective NEP inhibitor in reducing heart weight in both nondiabetic and diabetic SHR.

This may relate to a specific, pressure-independent cardiac effect of ACE inhibition, but better blood pressure control cannot be excluded. In preliminary studies by our group, it has been shown in the nondiabetic context that dual NEP/ACE inhibition reduces left ventricular hypertrophy only in the setting of a reduction in blood pressure.\textsuperscript{35} A significant degree of renal NEP inhibition, as assessed by in vitro autoradiography, was achieved with both the low (25 mg/kg BID) and the high (50 mg/kg BID) dose of the dual NEP/ACE inhibitor S 21402 in the present study, in agreement with the earlier report\textsuperscript{18} showing complete abolition of urinary NEP activity even at lower doses of S 21402 (2.5 mg/kg PO). In diabetic SHR, however, renal NEP inhibition was significantly more complete in rats receiving the high (50 mg/kg BID) dose. Parallel to this, the antihypertensive efficacy was also greater with the higher S 21402 dose.

The exact degree of ACE inhibition with the dual NEP/ACE inhibitor S 21402 is more difficult to assess because this drug, like captopril, is a sulfhydryl group-containing compound that is rapidly oxidized under normal in vitro ACE assay conditions. Gonzales and coworkers\textsuperscript{18,34} reported that plasma ACE was maximally inhibited with 50 mg/kg of S 21402 in normotensive rats after acute oral administration. Correspondent to this result, the greatest decrease in blood pressure was obtained with 50 mg/kg of S 21402 in an acute dose-response study in SHR.\textsuperscript{34} In agreement with these observations, antihypertensive efficacy of S 21402 was significantly greater at the dose of 50 mg/kg (BID) compared with 25 mg/kg in both diabetic and nondiabetic SHR in the present study. The blood pressure-lowering effect of S 21402 (50 mg/kg BID) corresponded to that of combined treatment with the ACE inhibitor captopril (25 mg/kg BID) and the NEP inhibitor SCH 42495 (30 mg/kg BID) in diabetic SHR. Thus, the available data suggest that 50 mg/kg BID of S 21402 is sufficient for optimal ACE inhibition as well as for maximal antihypertensive efficacy in this experimental model.

In acute experiments the dual NEP/ACE inhibitor S 21402 has been shown to induce a dose-dependent increase in PRA due to a reduction in angiotensin II formation and a decrease in renal perfusion pressure.\textsuperscript{34} However, long-term treatment with a dual ACE/NEP inhibitor results in attenuated increase in PRA compared with ACE inhibition alone, as reported by Johnston and coworkers\textsuperscript{14} in SHR. In agreement with these findings, PRA increased less in dual NEP/ACE inhibitor-treated diabetic and nondiabetic SHR compared with ACE inhibitor–treated groups in the present study. One possible mechanism to explain the blunted activation of the renin-angiotensin system during dual NEP/ACE inhibition may relate to prevention of the degradation of natriuretic peptides in the kidney, which have been shown to suppress renin release.\textsuperscript{36} Further studies are required to explore whether these effects suppressing the rebound increase in PRA in response to interruption of the renin-angiotensin system add to the antihypertensive efficacy of dual NEP/ACE inhibition. However, treatment with the selective NEP inhibitor together with ACE inhibitor captopril did not attenuate the increase in PRA found with the ACE inhibitor alone. The difference between the combination therapy and the dual inhibitor S 21402 on PRA cannot be fully explained but may relate to differences in pharmacokinetic and enzyme inhibitory potencies between the 2 treatment regimens.

Plasma ANP levels were elevated in diabetic SHR treated with the low dose of the dual NEP/ACE inhibitor. Otherwise, plasma ANP levels did not change by dual NEP/ACE inhibition or by selective NEP inhibition under the conditions of the study. However, the dual NEP/ACE inhibitor has been shown to potentiate the natriuretic effects of ANP associated with increased urinary excretion of ANP and cGMP\textsuperscript{18,34} together with increase in plasma levels of cGMP.\textsuperscript{18}

Renal effects of NEP inhibition in experimental diabetes have not been studied previously. Inhibition of the actions of ANP by either an ANP antagonist or infusion of ANP antisera has been shown to ameliorate glomerular hyperfiltration in rats with streptozotocin-induced diabetes, suggesting a role for natriuretic peptides in the development of the glomerular hemodynamic changes in diabetes.\textsuperscript{19–21} In theory, enhanced activity of natriuretic peptides after NEP inhibition could further promote glomerular hyperfiltration in experimental diabetes. However, no changes in GFR were found in the present study in diabetic SHR treated with either the NEP inhibitor or the dual NEP/ACE inhibitor. Furthermore, neither treatment induced a rise in urinary albumin excretion, an
early functional marker of evolving diabetic nephropathy, during the 4-week treatment period. Indeed, the urinary albumin excretion rate was even lower in both diabetic and nondiabetic SHR treated with the dual NEP/ACE inhibitor (50 mg/kg BID) than in the corresponding vehicle-treated groups. A long-term study is now warranted to determine the effects of NEP inhibition on the course of experimental diabetic nephropathy.

ACE inhibitors have proved effective in the treatment of hypertension associated with diabetes mellitus. In patients with diabetic nephropathy, ACE inhibitors reduce proteinuria more effectively than treatment with conventional antihypertensive drugs, suggesting specific renoprotective effects by this class of drugs beyond blood pressure control. However, even ACE inhibitors do not provide complete protection against progression of diabetic nephropathy, nor does monotherapy with ACE inhibitors lower blood pressure in all diabetic patients. In diabetic SHR the ACE inhibitor enalapril attenuated but did not normalize progression of albuminuria. In the study of Lewis and coworkers, renal impairment still progressed in patients with diabetic nephropathy, especially if hypertensive, despite partial renoprotection observed in captopril-treated patients. The available data suggest that strict control of blood pressure is essential to confer the full renal and cardioprotective effects of antihypertensive drugs. Salt retention often found in diabetic patients not only attenuates the antihypertensive efficacy of ACE inhibition but may also interfere with the cardiac and renoprotective actions of ACE inhibitors. This study has suggested an additional blood pressure–lowering effect of dual NEP/ACE inhibition in SHR with streptozotocin-induced diabetes and may therefore have an important bearing on treatment of hypertension in diabetic patients. However, one must be cautious in extrapolating the present findings in rodents to humans.

In conclusion, this study shows that hypertension in SHR with streptozotocin-induced diabetes is sensitive to NEP inhibition and thus responsive to enhanced activity of natriuretic peptides. Treatment with the novel dual NEP/ACE inhibitor S 21402 resulted in superior antihypertensive efficacy and attenuation of cardiac hypertrophy together with a reduction in albuminuria without noticeable adverse renal effects during the 4-week treatment period in diabetic SHR. However, further studies are required to evaluate long-term effects of NEP inhibition with consequent increased activity of natriuretic peptides on the course of diabetic nephropathy. It appears that both angiotensin-dependent vasoconstriction and salt retention–associated volume factors are involved in the pathogenesis of hypertension in diabetic SHR. Dual NEP/ACE inhibitors may thus prove beneficial in the treatment of hypertension associated with diabetes mellitus.

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