Potentiation of Renal Effects of Atrial Natriuretic Factor-(99–126) by SQ 29,072

Andrea A. Seymour, Susan A. Fennell, and Joel N. Swerdel

Depressor and renal activities of atrial natriuretic factor-(99–126) were determined in conscious, unrestrained spontaneously hypertensive rats treated with a neutral endopeptidase inhibitor, SQ 29,072 (7-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]heptanoic acid). SQ 29,072 (100 μmol/kg i.v.) prolonged the transient depressor effects of the peptide for as long as 2 hours. During the first hour after 3, 10, and 30 nmol/kg atrial natriuretic factor, urinary excretion of cyclic 3'5' guanosine monophosphate was significantly increased by 9.2±3.4, 13.0±2.2, and 12.7±4.2 nmol/kg/hr, respectively, in vehicle-treated rats and by 26.9±7.9, 52.1±11.1, and 46.4±12.2 nmol/kg/hr, respectively, in rats given 100 μmol/kg SQ 29,072. During the first hour after 3 and 10 nmol/kg atrial natriuretic factor-(99–126), the sodium loss was 161±56 and 139±42 meq/kg/hr in vehicle-treated rats and was significantly greater (694±316 and 1,038±135 meq/kg/hr) in rats given 100 μmol/kg SQ 29,072. After administration of 3, 10, and 30 μmol/kg SQ 29,072, the area over the curves of the depressor responses to 3 nmol/kg of the peptide increased from 297±70 to 306±108, 440±143, and 669±186 mm Hg·min, respectively, while the concurrent natriuretic responses rose from 161±56 to 250±88, 332±142, and 694±316 meq/kg/hr. In summary, the neutral endopeptidase inhibitor SQ 29,072 increased the magnitudes and especially the durations of the depressor, natriuretic, and cyclic guanosine monophosphate responses to exogenous atrial natriuretic factor-(99–126) in conscious spontaneously hypertensive rats, presumably by inhibition of degradation of atrial natriuretic factor in vivo. In conclusion, neutral endopeptidase inhibition offers an important new technique for enhancement and prolongation of the biological lifetime of atrial natriuretic factor. (Hypertension 1989;14:87–97)

Neutral endopeptidase (NEP) is an enzyme that cleaves the α-amino bond of hydrophobic amino acids provided that the susceptible bond does not involve the C- or N-terminal amino acid. Recently, several laboratories have demonstrated that isolated preparations of kidney tissue, a rich source of NEP, degraded atrial natriuretic factor (ANF) in vitro. Furthermore, the enzyme responsible for ANF degradation by rat renal cortex copurified with NEP (protease 3.4.24.11) activity was sensitive to known NEP inhibitors and possessed the physical properties of NEP.

There are several potential sites at which NEP may cleave ANF-(99–126), a 28-amino acid peptide containing a 17-member ring formed by a disulfide bridge between Cys108 and Cys121 (structure reviewed in Reference 10). Although NEP theoretically could hydrolyze the Ser123-Phe124 bond in the C-terminal extension as well as several bonds within the ring, a number of investigators have reported that a major ring-opened metabolite was formed in vitro by cleavage of the Cys108-Phe124 bond. Three such ring-opened peptides, which were produced by in vitro hydrolysis of the Cys108-Phe124 bond of rat ANF-(99–126) (unpublished observations, N. Delaney), ANF-(103–126), and ANF-(103–123), possessed diminished vasorelaxant activity in vitro (unpublished observations, J. Bergey, and Reference 11) and were inactive at doses as high as 100 nmol/kg i.v. in conscious spontaneously hypertensive rats (SHR) (unpublished observations, A.A. Seymour, and Reference 12). These results were consistent with previous findings that disruption of the ANF ring structure by other means markedly reduced biological activity and suggested that degradation of ANF by NEP may inactivate the peptide.

In another series of experiments in conscious SHR, the depressor activities of rat ANF-(99–126), (103–126), and (103–123) and human ANF-(99–126) and (105–126) were potentiated by SQ 29,072 (7-[[2-(mercaptomethyl)-1-oxo-3-phenylpro-
pyl]amino]heptanoic acid), a novel inhibitor of NEP. Since the only peptide bonds in rat ANF-(103–123) that are potentially susceptible to hydrolysis by NEP are adjacent to hydrophobic amino acids located within the loop formed by the disulfide bridge (i.e., Phe\textsuperscript{106}, Ile\textsuperscript{110}, Ile\textsuperscript{113}, or Leu\textsuperscript{119}), the activity of SQ 29,072 was consistent with prevention of enzymatic hydrolysis of one or more of the peptide bonds within the ANF ring. These studies provided additional evidence that NEP may metabolize and inactivate ANF in vivo.

Demonstration that SQ 29,072 enhances renal as well as vascular activity of ANF would strengthen the earlier conclusion that inhibition of NEP prevented metabolism of ANF in vivo. To accomplish that goal, the effects of SQ 29,072 on the natriuretic, diuretic, kaliuretic, and cyclic 3\'5\' guanosine monophosphate (cGMP) responses to exogenous rat ANF-(99–126) were determined in unrestrained conscious SHR in the present study. In a previous series of experiments, SQ 29,072, a specific inhibitor of NEP (K\textsubscript{i} = 26 nM), which is virtually inactive against angiotensin converting enzyme (K\textsubscript{i} = 260 \textmu M) (unpublished observations, M. Asaad), potentiated the depressor activity of ANF in conscious SHR, but did not affect the depressor responses to bradykinin or the pressor effects of angiotensin I, angiotensin II, or arginine vasopressin. In those studies, the complete depressor dose–response relation for exogenous ANF and the dose-related potentiation of that activity by SQ 29,072 were characterized in conscious SHR, a model in which ANF produced a larger depressor response than in normotensive rats. The present experiments extended that earlier work by examination of the renal responses to depressor doses of ANF-(99–126) in conscious, unrestrained SHR and provided additional evidence that a specific NEP inhibitor may prevent inactivation of ANF in vivo.

Materials and Methods

Male SHR (Taconic Farms, Germantown, New York) weighing ~300 g were housed individually in a room maintained on a 12-hour light/dark cycle and were allowed free access to normal rat chow and tap water. Two and a half weeks before a study, each rat was anesthetized with 50 mg/kg i.p. of pentobarbital, and modified Weeks' catheters\textsuperscript{18} were implanted in the abdominal aorta and vena cavae. Two weeks later, the rats were anesthetized and a cannula was surgically inserted into the urinary bladder. The free ends of all catheters were routed subcutaneously to a fitting at the back of the neck, passed through a protective spring covering and anchored to a swivel above the cage. This arrangement allowed measurement of blood pressure and collection of urine from conscious unrestrained rats studied in their home cages. Each rat was tested on alternate days, provided that the catheters remained functional. All procedures in conscious rats were conducted in accordance with American Association for Accreditation for Laboratory Animal Care (AAALAC) guidelines.

On the day of an experiment, the arterial catheter was connected to a Gould-Statham transducer (Spectramed, Oxnard, California) so that mean arterial pressure (MAP) could be monitored with a Grass recorder (Grass Instr. Co., Quincy, Massachusetts). Throughout the day, saline (0.9% NaCl) was administered intra-arterially (~10 \textmu l/min) from a pressurized reservoir to assure patency of the catheter. In addition, hydration was maintained by a continuous intravenous infusion (37 \textmu l/min) of an isosmotic solution of dextrose and 0.21% NaCl via an Ismatec roller pump (Cole-Parmer Instr. Co., Chicago, Illinois). Two hours after beginning the intra-arterial and intravenous infusions, baseline measurements were obtained during three consecutive 20-minute periods. During each 20-minute interval, MAP was measured and urine was collected into tared test tubes. After the control samples were obtained, urine collections were discontinued and each rat was treated with SQ 29,072 dissolved in 1 ml/kg 2% NaHCO\textsubscript{3} in phosphate buffer or an equivalent volume of the vehicle alone. Thirty minutes later, rat ANF-(99–126) (Peninsula Labs., Inc., Belmont, California) was injected intravenously and urine collections were resumed.

In six groups of SHR, each rat was treated with the NaHCO\textsubscript{3} vehicle or with 100 \mu mol/kg SQ 29,072 30 minutes before administration of a single dose of 3, 10, or 30 nmol/kg i.v. ANF-(99–126). In previous studies,\textsuperscript{15} this dose of SQ 29,972 maximally potentiated the depressor activity of ANF-(99–126) in conscious SHR. In three additional groups of SHR, a lower dose of 3, 10, or 30 \mu mol/kg SQ 29,072 was injected 30 minutes before delivery of a submaximal dose of 3 nmol/kg i.v. ANF-(99–126). The effects of 3, 10, 30, and 100 \mu mol/kg i.v. SQ 29,072 on the renal responses to 3 nmol/kg ANF-(99–126) were analyzed as a group for construction of four-point dose–response curves.

The depressor responses to ANF were expressed as the area over the blood pressure curve (AOC) integrated from the time at which MAP reached 50% of the maximal response until MAP had recovered by 50%. Within that time frame, MAP was measured at 5-minute intervals and AOC was calculated by trapezoidal estimation. Urine volume was determined gravimetrically and urinary concentrations of sodium and potassium were ascertained with ion-selective electrodes (Nova Biomedical, Waltham, Massachusetts). Urine volume flow (UV) was expressed as microliters per kilogram per minute and the rates of urinary excretion of sodium and potassium (U\textsubscript{Na,V} and U\textsubscript{K,V}, respectively) are given in units of micromolar equivalents per kilogram per minute. To take into account the duration as well as the magnitude of the renal responses, the amounts of urine, sodium, and potassium that were excreted during the hour of control measurements and during the first hour after ANF-(99–126) administration
TABLE 1. Rate of Urinary Sodium Excretion in Response to Atrial Natriuretic Factor-(99-126) in Conscious Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>ANF (nmol/kg)</th>
<th>SQ 29,072 (μmol/kg)</th>
<th>Control U\textsubscript{Na,V} (μeq/kg/min)</th>
<th>ΔU\textsubscript{Na,V} (μeq/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>2.5±0.5</td>
<td>5.0±1.3*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.2±0.7</td>
<td>10.7±4.4*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.9±0.3</td>
<td>13.6±5.7*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.0±0.2</td>
<td>12.6±4.3*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.6±0.5</td>
<td>11.0±4.9*</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.0±0.5</td>
<td>6.4±1.9*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.4±2.7</td>
<td>20.0±5.1*</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.1±0.8</td>
<td>7.4±3.4*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.8±0.3</td>
<td>11.5±6.3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ANF, atrial natriuretic factor; U\textsubscript{Na,V}, rate of urinary sodium excretion. *p<0.05 compared with intragroup baseline. **p<0.05 compared with vehicle-treated spontaneously hypertensive rats.

were calculated for each rat and were expressed as microliters per kilogram per hour or microequivalents per kilogram per hour as appropriate. The differences between the control and treatment values were ascertained for each rat and the average values for the changes from control were used to construct dose–response curves for each parameter (Figures 1, 2, and 4).

Urinary cGMP excretion (U\textsubscript{cGMP,V}) was measured in the experiments in which 3, 10, and 30 nmol/kg ANF-(99-126) were administered to rats treated with vehicle or 100 μmol/kg SQ 29,072. A portion of each urine sample collected in those experiments was frozen immediately and stored at −70°C. At a later time, urinary cGMP concentrations were measured using a radioimmunoassay kit (New England Nuclear Research Products, Boston, Massachusetts). Excretion rates of cGMP were normalized for body weight and are expressed as picomoles per kilogram per hour. (Hourly excretion rates were determined as described above).

The measurements of renal excretory function obtained during the three control periods were averaged and are presented as a single baseline value in Tables 1–4. To account for slight differences among the baseline measurements obtained in the different groups of rats, the responses to ANF are given as the changes from the average control values (Tables 1–4). All data were analyzed on a VAX mainframe computer using RSI procedures (BBN Research Systems, Bolt Beranek and Newman, Inc., Cambridge, Massachusetts). Intragroup comparisons were made by using two-way analysis of variance or a paired t test as appropriate. Significant differences (p<0.05) among different groups were identified by using a one-way analysis of variance or the unpaired t test. The values given in the Results section represent the mean±SEM.

Results

Doses of 3, 10, and 30 nmol/kg ANF-(99-126) significantly increased sodium excretion within 20 minutes of administration in conscious SHR treated with vehicle (Table 1). In each case, sodium excretion returned to values that were not significantly different from baseline within 40 minutes. Although there were no statistically significant differences among the natriuretic responses measured 20 minutes after the three doses of ANF-(99–126) in vehicle-treated SHR, the rate of sodium excretion tended to rise as the dose of ANF-(99–126) was increased (Table 1).

In the rats treated with 100 μmol/kg SQ 29,072, the peak natriuretic responses to each dose of ANF-(99–126) tended to be greater than those obtained in the vehicle-treated rats. Furthermore, sodium excretion remained significantly elevated for 60–100 minutes after the ANF-(99–126) challenge in the SHR treated with 100 μmol/kg SQ 29,072 (Table 1). For comparison of the effects of vehicle and SQ 29,072 on the responses to ANF-(99–126), the amounts of sodium excreted during the hour of control collections and during the first hour after ANF injections were calculated (see Materials and Methods). Doses of 3, 10, and 30 nmol/kg ANF-(99–126) significantly increased hourly sodium loss from 141±25 to 302±68 μeq/kg/hr (p<0.05), from 122±30 to 261±63 μeq/kg/hr (p<0.05), and from 126±47 to 332±106 μeq/kg/hr (p<0.05), respectively, in the vehicle-treated rats. In the SHR treated with 100 μmol/kg SQ 29,072, hourly sodium excretion rose significantly from 98±31 to 792±305 μeq/kg/hr (p<0.05), from 323±163 to 1,361±237 μeq/kg/hr (p<0.05), and from 106±17 to 690±234 μeq/kg/hr (p<0.05) after administration of 3, 10, and 30 nmol/kg i.v. ANF-(99–126), respectively. In SHR treated with either vehicle or 100 μmol/kg SQ 29,072, the amounts of sodium excreted during the second hour after administration of ANF-(99–126) were not significantly different from the amounts of sodium lost during the hour of control measurements.

To compare the effects of vehicle with the effects of SQ 29,072 administration on sodium excretion,
pretreated with 100 μmol/kg LV. SQ 29,072 (•) phosphate (cGMP) excretion (U NaV and U NaV arrow up, respectively) were somewhat larger than the natriuresis produced by 30 nmol/kg ANF-(99-126) (206±96 μeq/kg/hr) in vehicle-treated SHR. Therefore, 100 μmol/kg SQ 29,072 increased the natriuretic potential of a given dose of ANF-(99-126) by greater than 10-fold.

All doses of ANF-(99-126) significantly increased urinary cGMP excretion for 40-60 minutes in the SHR treated with vehicle (Table 2). The amount of cGMP excreted in the urine rose significantly from 1.0±0.2 to 10.2±3.4 nmol/kg/hr (p<0.05), from 2.2±0.4 to 15.6±2.3 nmol/kg/hr (p<0.05), and from 1.2±0.4 to 14.0±0.4 nmol/kg/hr (p<0.05) during the first hour after administration of 3, 10, and 30 nmol/kg ANF-(99-126), respectively. During the second hour, urinary cGMP excretion rates [1.4±0.3, 2.7±0.6, and 2.0±0.5 nmol/kg/hr after 3, 10, and 30 nmol/kg ANF-(99-126), respectively] were not significantly different from the baseline values in the vehicle-treated SHR.

Pretreatment with 100 μmol/kg SQ 29,072 potentiated the urinary cGMP excretion stimulated by ANF-(99-126) by increasing both the magnitudes and the durations of the responses (Table 2) such that urinary cGMP excretion remained significantly elevated throughout the 2-hour experiment. Such a sustained increase in cGMP was not produced by any dose of ANF-(99-126) in the vehicle-treated SHR. In SHR treated with 100 μmol/kg SQ 29,072, the hourly cGMP excretions were significantly elevated (p<0.05) during both the first and second hours after ANF-(99-126), increasing from 1.3±0.2 to 28.2±8.0 and 8.1±1.6 nmol/kg/hr after 3 nmol/kg, from 1.7±0.5 to 53.9±11.6 and 14.2±3.3 nmol/kg/hr after 10 nmol/kg, and from 1.6±0.5 to 48.0±12.5 and 18.0±3.2 nmol/kg/hr after 30 nmol/kg ANF-(99-126). Compared with the rats receiving vehicle, the increases in urinary cGMP excretion measured during both the first and second hours

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Urinary sodium and cyclic guanosine monophosphate (cGMP) excretion (U NaV and U cGMP arrow up, respectively) were measured during an hour of control saline infusions and at hourly intervals after administration of 3, 10, or 30 nmol/kg i.v. atrial natriuretic factor (ANF)-(99-126) in conscious spontaneously hypertensive rats (SHR). Increases above control values in sodium excretion measured during first hour after 3 and 10 nmol/kg ANF-(99-126) were significantly greater (*p<0.05) in SHR treated with vehicle (○) than in SHR treated with vehicle (•), vehicle-treated spontaneously hypertensive rats.

| TABLE 2. Rate of Urinary Excretion of Cyclic Guanosine Monophosphate in Response to Atrial Natriuretic Factor-(99-126) in Conscious Spontaneously Hypertensive Rats |
|-----------------|-----------------|-----------------|-----------------|
| ANF (nmol/kg) | SQ 29,072 (μmol/kg) | Control | ΔU cGMP (pmol/kg/min) |
|                |                  | U cGMP arrow up |                  |                  |                  |                  |
|                |                  | (pmol/kg/min)   |                  |                  |                  |                  |
| 3              | 0                | 17±4            | 103±32*         | 48±18            | 2±4             | 13±3             | 8±5             | 5               |
| 10             | 0                | 25±4            | 464±184*        | 339±93*          | 186±35†         | 67±33            | 77±10†          | 5               |
| 10             | 100              | 36±7            | 380±86*         | 138±44*          | 49±23           | 23±8             | 1±6             | 5±2             | 6               |
| 10             | 100              | 29±9            | 892±277†        | 1128±168†        | 587±183†        | 364±98†          | 158±42†         | 100±34†         | 7               |
| 30             | 0                | 21±7            | 408±150*        | 179±51*          | 50±13†          | 23±8             | 4±3             | 1±5             | 6               |
| 30             | 100              | 27±9            | 585±185*        | 1134±254†        | 602±267†        | 334±67†          | 280±63†         | 207±100†        | 6               |

Values are mean±SEM. ANF, atrial natriuretic factor; U cGMP arrow up, rate of urinary excretion of cyclic guanosine monophosphate.

*p<0.05 compared with intragroup baseline.

†p<0.05 compared with vehicle-treated spontaneously hypertensive rats.
TABLE 3. Rate of Urinary Flow In Response to Atrial Natriuretic Factor-(99-126) in Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>ANF</th>
<th>SQ 29,072</th>
<th>Control UV (µl/kg/min)</th>
<th>ΔUV (µl/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>40'</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>162±20</td>
<td>115±26*</td>
</tr>
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<td>340±57*</td>
</tr>
<tr>
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<td>0</td>
<td>141±21</td>
<td>209±51*</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>141±26</td>
<td>247±100*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ANF, atrial natriuretic factor; UV, urine volume flow.

*p<0.05 compared with intragroup baseline.
†p<0.05 compared with vehicle-treated spontaneously hypertensive rats.

after administration of each dose of ANF were significantly greater in the SHR treated with 100 µmol/kg SQ 29,072 (Figure 1).

ANF-(99–126) also transiently increased urine volume within the first 20 minutes after dosing in the vehicle-treated rats (Table 3). During the first hour after 3, 10, and 30 nmol/kg ANF-(99–126), the hourly rate of urine flow was significantly elevated from 9.4±1.2 to 12.0±1.6 ml/kg/hr, from 8.1±2.1 to 11.6±2.3 ml/kg/hr (p<0.05), and from 8.5±1.3 to 14.1±2.5 ml/kg/hr (p<0.05), respectively, in SHR pretreated with vehicle. In the rats receiving 100 µmol/kg SQ 29,072, urine volume rose from 9.0±1.7 to 18.1±3.8 ml/kg/hr (p<0.05), from 13.8±2.7 to 26.7±3.4 ml/kg/hr (p<0.05), and from 8.5±1.6 to 17.6±3.2 ml/kg/hr (p<0.05) in response to 3, 10, and 30 nmol/kg ANF-(99–126), respectively. When expressed as the changes from control levels, the diuretic responses during the first hour after 3 and 10 nmol/kg ANF-(99–126) were significantly greater in SHR treated with 100 µmol/kg SQ 29,072 than in rats receiving vehicle (Figure 2). As shown in Table 3, this potentiation was a consequence of the increases in both the magnitude and the duration of the diuretic response.

During the first 20 minutes after each dose of ANF-(99–126), the rates of potassium excretion were significantly elevated to similar levels in both vehicle- and SQ 29,072-treated rats (Table 4). Prior treatment with 100 µmol/kg SQ 29,072 significantly prolonged the kaliuretic responses to both 3 and 10 nmol/kg ANF-(99–126) (Table 4). During the first hour after 3, 10, and 30 nmol/kg ANF-(99–126), hourly potassium excretion rose from 208±40 to 249±38 µeq/kg/hr (p<0.05), from 163±45 to 185±40 µeq/kg/hr (p<0.05), and from 121±16 to 196±30 µeq/kg/hr (p<0.05), respectively, in rats treated with vehicle. After administration of 100 µmol/kg SQ 29,072, doses of 3, 10, and 30 nmol/kg ANF-(99–126) increased hourly potassium excretion from 139±21 to 272±61 µeq/kg/hr, from 192±46 to 323±48 µeq/kg/hr (p<0.05), and from 96±21 to 183±48 µeq/kg/hr (p<0.05), respectively. Compared with the kaliuretic responses to 3 and 10 nmol/kg ANF-(99–126) in SHR pretreated with vehicle, the increases in potassium excretion during the first hour were significantly potentiated by 100 µmol/kg SQ 29,072 (Figure 2), primarily by improving the durations of the response (Table 4).

In the SHR receiving 3, 10, and 30 nmol/kg of ANF-(99–126), baseline MAP were 156±5, 157±11,
and 151±6 mm Hg before vehicle treatment and were 142±9, 153±6, and 145±4 mm Hg before administration of 100 μmol/kg SQ 29,072. Neither vehicle nor 100 μmol/kg SQ 29,072 significantly affected MAP during the first 30 minutes after treatment, indicating that a stable baseline value had been attained before injection of the exogenous ANF-(99-126). The changes in MAP measured at 20-minute intervals after each dose of ANF-(99-126) are shown in Figure 3. In the three groups of SHR treated with vehicle, doses of 3, 10, and 30 nmol/kg ANF-(99-126) maximally lowered MAP by -15±2 mm Hg at 22±9 minutes, by -15±1 mm Hg at 10±2 minutes and by -12±2 mm Hg at 7±2 minutes, respectively. In the SHR treated with 100 μmol/kg SQ 29,072, the greatest depressor responses measured 20 minutes after administration of 10 and 30 nmol/kg ANF-(99-126) (-21±3 and -26±6 mm Hg, respectively) were only slightly greater than in the vehicle-treated rats. During the first 20 minutes after 3 nmol/kg ANF-(99-126) in SHR treated with SQ 29,072, MAP fell by -2 to -10 mm Hg in four of five rats. (In the fifth rat, MAP rose by 15 mm Hg so that the immediate response to ANF was not apparent.) In contrast to vehicle-treated SHR in which MAP was lowest within 22 minutes of ANF administration, MAP reached 130±6 mm Hg (-14±2 mm Hg) at 80 minutes after 3 nmol/kg ANF-(99-126), 132±8 mm Hg (-19±4 mm Hg) at 80 minutes after 10 nmol/kg and 126±8 mm Hg (-24±2 mm Hg) at 100 minutes after 30 nmol/kg ANF-(99-126) in SHR receiving 100 μmol/kg SQ 29,072. After all doses of ANF-(99-126), these antihypertensive effects were sustained throughout the 2-hour period. Because accurate calculation of the AOC of the depressor response depends on 50% recovery of blood pressure in each rat, that parameter could not be determined for the rats treated with 100 μmol/kg SQ 29,072.

In view of the profound effects of 100 μmol/kg SQ 29,072 on the depressor and renal responses to ANF-(99-126), the effects of lower doses of the inhibitor on the activity of a submaximal dose of ANF-(99-126) (3 nmol/kg) were determined in three additional groups of SHR. Before treatment with 3, 10, or 30 μmol/kg SQ 29,072, baseline MAP was 166±10, 160±12, and 156±3 mm Hg, respectively, and were not affected by any dose of SQ 29,072. The depressor responses to 3 nmol/kg ANF-(99-126) were -15±2 mm Hg in SHR treated with vehicle (data given above) and -19±3, -17±3, and -26±6 mm Hg in SHR treated with 3, 10, and 30 μmol/kg SQ 29,072, respectively. In addition, the durations of the depressor responses tended to increase such that the AOC were progressively enhanced by doses of 3, 10, and 30 μmol/kg SQ 29,072 (Figure 4).

Although all doses of SQ 29,072 approximately doubled the natriuretic responses measured 20 minutes after administration of 3 nmol/kg ANF-(99-126) (Table 1), there were no statistically significant differences among the treatment groups at that time due to the variability of the responses. After 40 minutes, the natriuretic responses to 3 nmol/kg ANF-(99-126) were significantly greater in SHR treated with 30 and 100 μmol/kg SQ 29,072 than in rats treated with vehicle (Table 1). When the amounts of sodium excreted during the first hour after ANF-(99-126) were calculated, sodium excretion rose significantly (p<0.05) from 141±25 to 302±68 μeq/kg/hr, from 252±43 to 501±117 μeq/kg/hr, from 54±18 to 386±156 μeq/kg/hr, from 60±10 to 524±168 μeq/kg/hr, and from 98±31 to 792±305 μeq/kg/hr in SHR treated with vehicle or 3, 10, 30, and 100 μmol/kg i.v. SQ 29,072, respectively. (Data for vehicle and 100 μmol/kg SQ 29,072 also given above.) As shown in Figure 4, the rises in hourly sodium excretion produced by 3 nmol/kg i.v. ANF-(99-126) were clearly enhanced in a dose-related manner by increasing doses of SQ 29,072.

As observed for the natriuretic responses, the diuretic and kaliuretic responses measured during the first 20 minutes after injection of 3 nmol/kg ANF-(99-126) (Tables 3 and 4) were not signifi-
FIGURE 3. Mean arterial pressure (MAP) was measured at 20-minute intervals after injection of 3, 10, and 30 nmol/kg i.v. atrial natriuretic factor (ANF)-(99-126) (top, middle, and bottom graphs, respectively) in conscious spontaneously hypertensive rats (SHR). ANF-(99-126) significantly (*p<0.05) reduced blood pressure within each group of rats for at least 20 minutes after administration. For each dose of ANF-(99-126), pretreatment with 100 pmol/kg i.v. SQ 29,072 (●) significantly (*p<0.05) potentiated the depressor response compared with vehicle treatment (○).

FIGURE 4. Compared with vehicle treatment (○), increasing doses of SQ 29,072 (●) significantly (*p<0.05) enhanced the area over the blood pressure curve (AOC) of the depressor responses (top) and the natriuretic responses (bottom) to 3 nmol/kg i.v. atrial natriuretic factor (ANF)-(99-126). UNaV, rate of urinary excretion of sodium.

Discussion

The depressor, natriuretic, diuretic, and kaliuretic effects of ANF, which have been characterized in numerous laboratory and clinical studies (see Reference 19 for review), are typically short-lived and until recently have been sustained only by continuous infusions of the peptide. The present study investigated the possibility that regulation of the degradation or inactivation of the peptide may provide an important alternative technique for prolongation of the biological half-life of ANF.

The work leading to the present experiments included identification of an important enzymatic pathway by which ANF may be inactivated in vivo. Several laboratories have reported recently that ANF is a substrate for neutral endopeptidase, a metalloendopeptidase present in renal brush border membrane. Furthermore, renal preparations were shown to degrade ANF in vitro by the action of an enzyme that shares the physical properties and inhibitor sensitivity that are characteristic of NEP.

Although NEP could potentially cleave ANF at several sites, the principal metabolite of ANF-(99-126) produced in vitro was identified as the ring-opened peptide resulting from cleavage of the Cys-Phe bond (unpublished observations, N. Delaney, and Reference 11). Three such ring-opened metabolites were shown to possess reduced vasorelaxant activity (unpublished observations, J. Bergey, and Reference 11). These three metabolites also lacked...
were measured since earlier studies have indicated ANF increased cGMP production in isolated renal glomeruli and collecting ducts, structures which SQ 29,072 potentiated the antihypertensive activity of ANF. This conclusion was supported by evidence that biologically active ANFs stimulated cGMP levels in a variety of iso-
terceptive endothelial cells, vascular smooth muscle cells, and isolated aortic rings. In addition, ANF increased cGMP production in isolated renal glomeruli and collecting ducts, structures that are the primary sites of ANF binding in the kidney.

Earlier studies have also demonstrated that the stimulation of cGMP production by exogenous ANF increased both plasma and urinary cGMP levels in rats, dogs, and humans. In anesthe-
tized dogs, urinary cGMP excretion surpassed the filtered load of the cyclic nucleotide during intrarenal infusions of ANF-(101-126), and arterial cGMP load exceeded renal venous efflux, suggesting that urinary cGMP was derived from both renal and nonrenal sources. Because other workers have found that the time course of urinary cGMP excretion coincided more closely with the biological responses to ANF than did plasma cGMP levels, the rate of urinary cGMP excretion was believed to most accurately reflect ANF activity in vivo. For technical reasons, it was not possible to measure plasma or urinary ANF at 20-minute intervals in the present experiments; therefore, urinary cGMP was used as an index of ANF activity. Since it has been demonstrated that SQ 29,072 enhanced the urinary cGMP response to ANF-(99-126) in a pattern consistent with the depressor and renal responses, the effects of the inhibitor may be more confidently related to specific potentiation of ANF.

The present results differ slightly from the earlier study of SQ 29,072 in nonhydrated SHR. In those nonhydrated rats treated with 100 μmol/kg SQ 29,072, the depressor responses to ANF-(99-126) were not maintained for as long as those in the hydrated rats used in the present experiments. The most obvious difference between those two studies was the continuous infusion of 37 μl/min i.v. saline, which was necessary for measurement of renal function in the present experiments. Although previous investigators have shown that rapid volume expansion increases plasma levels of ANF in SHR, the effects of the continuous saline infusion used in the present work has yet to be established. Therefore, it was not clear whether such sustained depressor responses in the hydrated SHR reflected potentiation of the exogenous ANF bolus or potentiation of endogenous ANF released by the slow volume expansion. One may also speculate that the higher doses of exogenous ANF may have saturated the putative ANF clearance receptors so that a greater proportion of endogenously released ANF would have been available to the biological receptors, especially when degradation of the peptide was attenuated by the NEP inhibitor.

Finally, the prolonged depressor effects obtained in the presence of 100 μmol/kg SQ 29,072 may result from potentiation of some other depressor peptide...
susceptible to NEP cleavage and capable of stimulating cGMP excretion. In a preliminary study, bradykinin, a known substrate for NEP in vitro, released endothelium-derived relaxing factor and thereby stimulated soluble guanylate cyclase activity and cGMP production in rat mesangial cells. Although the effects of bradykinin on urinary cGMP excretion in vivo have not been demonstrated, previous studies have shown that SQ 29,072 did not potentiate the depressor activity of bolus injections of bradykinin in conscious SHR. Based on the best information currently available, the effects of SQ 29,072 measured in the present study are most consistent with potentiation of the exogenous ANF bolus and perhaps endogenously released ANF.

In addition to sustaining the depressor activity of ANF-(99–126), SQ 29,072 prolonged the natriuretic responses in a manner dependent on both the dose of the inhibitor and the dose of ANF. In the presence of 100 μmol/kg SQ 29,072, sodium excretion was maximal for at least 40 minutes after a single dose of ANF-(99–126) and was greater than baseline for as long as 100 minutes. In more recent studies (unpublished observations, A.A. Seymour), 100 μmol/kg SQ 29,072 induced only a slight, statistically insignificant rise in baseline sodium excretion within the first 30 minutes. Therefore, it appeared that the present results were a consequence of the potentiation of exogenous ANF by SQ 29,072.

The attenuation of the natriuresis at the later time points coincided with the full development of the depressor activity and presumably the greatest decrease in renal perfusion pressure. Since the renal effects of ANF are known to be suppressed by reduction of renal artery pressure in normotensive animals, one may assume that the renal responses to ANF in SHR would also be blunted as perfusion pressure approached the lower limits of glomerular autoregulation. This conclusion remains tentative until the crucial pressure at which the renal effects of ANF are attenuated in SHR has been established. Nevertheless, SQ 29,072 clearly enhanced both the magnitude and duration of the natriuretic responses to the lower doses of ANF-(99–126) in the conscious SHR.

In the presence of SQ 29,072, the diuretic and kaliuretic responses to ANF-(99–126) appeared to be dissociated from natriuretic effects. That is, sodium excretion was significantly elevated for much longer times than were urine volume and potassium excretion. These differences were especially obvious in the SHR receiving 30 nmol/kg ANF-(99–126) in which SQ 29,072 sustained the natriuresis for 100 minutes while the inhibitor had no apparent effect on the diuretic and kaliuretic responses. These findings are consistent with the proposal that ANF increases sodium excretion by inhibition of sodium transport in the collecting ducts (see Reference 46 for review) as well as by changing tubular flow and sodium delivery to the distal tubule, conditions known to influence potassium transport. Any changes in tubular flow due to alterations in renal hemodynamics are more likely to be sustained under conditions in which renal perfusion pressure is maintained or decreased only transiently, that is, in the absence of SQ 29,072 in the present experiments. Should the inhibition of collecting duct sodium transport by ANF proceed independently of the changes in flow, sodium excretion could increase without a concurrent rise in potassium excretion or urine volume.

The usefulness of blocking ANF degradation became evident when the effectiveness of ANF-(99–126) in vehicle-treated SHR was compared with its activity in SHR receiving SQ 29,072. The magnitude and duration of the renal responses to 3 nmol/kg ANF-(99–126) exceeded the responses to any dose of ANF-(99–126) tested in the vehicle-treated SHR. Indeed, the natriuretic response to 3 nmol/kg ANF-(99–126) in SHR treated with 10 μmol/kg SQ 29,072 resembled the pattern of natriuresis elicited by 30 nmol/kg ANF-(99–126) in vehicle-treated SHR.

In summary, the NEP inhibitor SQ 29,072 significantly potentiated the depressor and natriuretic responses to exogenous ANF-(99–126) in conscious SHR. In addition, SQ 29,072 markedly enhanced the urinary cGMP excretion response to ANF-(99–126) indicating that the inhibitor specifically prolonged ANF activity in vivo. These results provide new evidence that SQ 29,072 enhances the renal and depressor responses to ANF in a manner consistent with inhibition of a degradative pathway. In conclusion, inhibitors of NEP offer an important new approach for enhancement of the in vivo activity of ANF.

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