Atrial Natriuretic Factor–Potentiating and Antihypertensive Activity of SCH 34826
An Orally Active Neutral Metalloendopeptidase Inhibitor

Edmund J. Sybertz, Peter J.S. Chiu, Subbarao Vemulapalli, Robert Watkins, and Martin F. Haslanger

The effects of SCH 34826, an orally active neutral metalloendopeptidase inhibitor, on responses to atrial natriuretic factor-(103-125) or -(99-126) and on blood pressure were evaluated in rats. SCH 34826 (10, 30, and 90 mg/kg s.c. and 90 mg/kg p.o.) potentiated the antihypertensive action of atrial natriuretic factor (30 μg/kg i.v.) in conscious spontaneously hypertensive rats. SCH 34826 (90 mg/kg) also potentiated the diuretic and natriuretic responses to atrial natriuretic factor (30 μg/kg i.v.) as well as the plasma levels achieved after peptide injection. SCH 34826 significantly reduced blood pressure in the conscious deoxycorticosterone acetate–salt hypertensive rat, at doses of 90 mg/kg s.c. (-35±12 mm Hg), 10 mg/kg p.o. (-30±7 mm Hg), and 90 mg/kg p.o. (-45±6 mm Hg). SCH 34826 was devoid of acute antihypertensive activity in the spontaneously hypertensive rat but reduced blood pressure by day 3 of a 5-day treatment schedule. SCH 34826 (90 mg/kg s.c.) enhanced urine volume output in the deoxycorticosterone acetate–salt rat (2.78±0.6 vs. 1.27±0.3 ml/100 g/3 hr in vehicle-control rats, p<0.05). SCH 34826 (90 mg/kg s.c.) increased plasma levels of atrial natriuretic factor at 1 hour (753±89 vs. 451±79 pg/ml in vehicle-treated rats, p<0.05) but not 3 hours after dosing. The renal excretion of atrial natriuretic factor (3,092 ±1,089 vs. 21 ±6 pg/100 g/3 hr in vehicle-treated rats, p<0.05) and cyclic guanosine monophosphate (2,131 ±509 vs. 879±168 pg/100 g/3 hr in vehicle-treated rats, p<0.05) was markedly elevated by SCH 34826 in deoxycorticosterone acetate–salt rats. These studies suggest that neutral endopeptidase inhibition may represent a new approach to treatment of some forms of hypertension. (Hypertension 1990;15:152-161)

The atrial natriuretic factors (ANFs) are vasodilator, natriuretic, and diuretic peptides with a potential role in the physiological regulation of blood pressure and fluid and electrolyte homeostasis.1 ANF has a half-life in plasma of less than 3 minutes,2-5 and its metabolic fate has not been well characterized. Neutral metalloendopeptidase (NEP) (EC 3.4.24.11) is an enzyme that hydrolyzes peptide bonds on the amino terminal side of aromatic or hydrophobic amino acids.6 The enzyme is distributed in brain, lung, and kidney but is not widely distributed in any abundance in other tissues.7,8 Analysis of the amino acid sequence of ANF indicates that the hormone could be cleaved by NEP at the Cys105–Phe106 and Ser123–Phe124 bonds.8 Cleavage at either or both of these sites would result in inactivation of the hormone.1,9 NEP hydrolysis of ANF to inactive products has been shown by others.10-14

SCH 34826 (Figure 1) is an orally active prodrug of the NEP inhibitor SCH 32615.15 SCH 32615 inhibits purified kidney and brain NEP in a competitive fashion with a $K_i$ of 19 nM. It is essentially devoid of activity against a variety of other proteases including angiotensin converting enzyme (ACE) at concentrations up to 10 μM. SCH 34826 itself is inactive against NEP in vitro.

We used SCH 34826 and its active acid form SCH 32615 as a prototype NEP inhibitor to test the hypothesis that NEP inhibition would modify the biological actions to injection of pharmacological doses of ANF. In addition, the consequences of NEP inhibition with SCH 34826 on cardiovascular and renal function as well as on plasma and urine ANF and cyclic guanosine monophosphate (cGMP) levels were assessed in rats. The deoxycorticosterone acetate (DOCA)–salt rat is a volume-dependent model of hypertension in which plasma levels of ANF have been shown to be elevated.16
Methods

Potentiation of Hypotensive Responses to Atrial Natriuretic Factor

Male spontaneously hypertensive rats (SHR) (Taconic Farms, Germantown, New York) that weighed 270–350 g were anesthetized with ether, and the caudal artery was cannulated for direct measurement of blood pressure as previously described. A jugular vein was cannulated for intravenous administration of ANF. After a stabilization period of 90–120 minutes, the rats were challenged with ANF-(103–125) or -(99–126) injected intravenously as a bolus at a dose of 30 μg/kg, which is submaximum with respect to lowering blood pressure in the SHR in our laboratory. At 60–90 minutes after this first challenge with ANF, the rats were treated with SCH 34826, SCH 32615, or vehicle (aqueous 0.4% methylcellulose solution, 2 ml/kg body wt) subcutaneously and rechallenged with ANF 15 minutes later. In a separate study, SCH 34826 (90 mg/kg) or methylcellulose vehicle was administered orally and ANF challenges were performed 90 minutes later. Arterial blood pressure responses to ANF were evaluated.

Potentiation of the Renal Responses to Atrial Natriuretic Factor

Male SHR were anesthetized with ether. The carotid artery and jugular vein were cannulated. Rats were allowed to recover consciousness and 1 hour later were dosed with SCH 34826 (90 mg/kg s.c.) or methylcellulose vehicle. Fifteen minutes later, a 1 ml arterial blood sample was taken for measurement of plasma ANF levels. ANF-(99–126) (30 μg/kg i.v.) was administered as a bolus shortly thereafter, and additional blood samples were taken at 2, 10, and 30 minutes after injection of the peptide. Blood was replaced after each sample with 1 ml blood from a donor rat. Plasma ANF levels were measured by radioimmunoassay as described below.

In Vivo Angiotensin Converting Enzyme Inhibitory Activity

Male SHR were cannulated and prepared for direct measurement of arterial blood pressure as described above. After stabilization, rats were dosed orally with SCH 34826 (90 mg/kg) or methylcellulose vehicle.

Ninety minutes later, rats were challenged with intravenous injections of angiotensin I (1 μg/kg), angiotensin II (1 μg/kg), and bradykinin (1 and 3 μg/kg), and changes in mean arterial blood pressure were monitored.

Antihypertensive Activity of SCH 34826

Deoxycorticosterone acetate–salt hypertensive rats. Male Charles River caesarean-derived Sprague Dawley rats (175–200 g) (Wilmington, Massachusetts) were anesthetized with ether or methohexital sodium (Brevital, Eli Lilly and Co., Indianapolis, Indiana) (50 mg/kg i.p.). The left kidney was excised and removed. Two pellets (25 mg DOCA each) were implanted subcutaneously at the time of surgery. The rats were then given access to drinking water with 1% NaCl plus 0.2% KCl and standard laboratory rat chow. The rats were used in experiments 17–27 days after surgery, when a sustained hypertension of greater than 150 mm Hg (mean arterial pressure) was established. The rats weighed 180–270 g at the time of study.

On the day of study, with the rats under ether anesthesia, a cannula made of polyethylene (PE-50) fused to PE-10 tubing was advanced 9 cm into the aorta via the caudal artery as described elsewhere. Rats were placed into plastic restrainers where they recovered consciousness within 10 minutes. The rats

not change endogenous levels of plasma ANF (P.J.S. Chiu, unpublished observations). The rats were dosed subcutaneously with methylcellulose vehicle or SCH 34826 (90 mg/kg). Five minutes later, urine was collected for 10 minutes to obtain baseline values, followed by a bolus injection of ANF (30 μg/kg i.v.). ANF was administered in a volume of 1 ml/kg, which was flushed in with an equal volume of saline. The urine volume after ANF administration was registered, and urinary sodium was measured with a flame photometer (Radiometer, Copenhagen, Denmark).
were allowed 90–120 minutes from this time to recover fully as evidenced by a stable blood pressure. Blood pressure was measured with Gould Statham (Oxnard, California) pressure transducers connected to a Buxco (Sharon, Connecticut) analog-to-digital converter, which captured each pulse signal and derived heart rate. Analog blood pressure traces also were continuously displayed on Beckman 612 oscillographs (Censor Medics, Sacramento, California).

Drugs were administered as suspensions in 0.4% aqueous methylcellulose (2 ml/kg). Blood pressure was monitored before and for up to 4 hours after dosing with SCH 34826 or vehicle.

Spontaneously hypertensive rats. Male SHR weighing 270–350 g were prepared as described above for direct measurement of arterial blood pressure. Rats were dosed with SCH 34826 (90 mg/kg s.c.) twice daily for a 5-day period. Blood pressure was monitored for at least 1 hour before and for 4 hours after each day’s morning dose. The arterial catheters were sealed after the 4-hour observation period, and rats were returned to their cages.

Hemodynamic Actions in Deoxycorticosterone Acetate–Salt Rats

DOCA-salt hypertensive rats were prepared as described above. On the day of study, rats were anesthetized with ether. The abdominal aorta was cannulated for blood pressure measurement. A jugular vein was cannulated for indicator injection. A thermistor probe (YSI 511, Yellow Springs Instr. Co., Yellow Springs, Ohio) was inserted into a carotid artery and extended into the arch of the aorta. The venous catheters and probe were exteriorized at the back of the neck. Rats were placed in restrainers where they recovered consciousness. Cardiac output was measured as described previously with a Cardiotherm 500 (Columbus Instrs., Columbus, Ohio) output computer. Five percent dextrose in water, at room temperature, served as the indicator and was injected into the auricular cannula (100 μl plus 40 μl for the cannula dead space). Only 100 μl placement of this catheter in the right auricle is critical for accurate determinations of cardiac output. Catheter placement was previously verified anatomically by the contour of the analog cardiac output thermodilution curves. Curves were irregular and prolonged when the catheter was not located at the right auricle. In such instances, the catheter was repositioned and measurements were repeated. With appropriate catheter positioning, cardiac output curves were sharp, with a slight shoulder during the decline. Output measurements were made in duplicate and were averaged. Blood pressure, analog thermal dilution curve, and heart rate were recorded on an oscillographic recorder. Cardiac output measurements were made before and at hourly intervals after oral dosing with SCH 34826 (90 mg/kg) or vehicle.

Effect of SCH 34826 on Endogenous Atrial Natriuretic Factor and Cyclic Guanosine Monophosphate

Because the DOCA-salt rat demonstrated an acute reduction of arterial blood pressure in response to SCH 34826, we investigated in detail the effects of this acute treatment on plasma and urine ANF and cGMP as well as on urine sodium and volume excretion. DOCA-salt rats, individually housed in metabolic cages, were dosed subcutaneously with methylcellulose vehicle (2 ml/kg) or with SCH 34826 (90 mg/kg) shortly after receiving supplements of physiological saline, 1.5% of body weight, by gavage. Urine samples were collected for 3 hours. The rats were decapitated at 3 hours, and trunk blood was collected for determination of ANF with radioimmunoassay (RIA). Ten microliters 10 mM papaverine was added to 100 μl plasma or urine and stored at -70°C for cGMP measurements with RIA with the RIA kits supplied by Biomedical Technologies (Stoughton, Massachusetts). The urine sodium was measured with a flame photometer (Radiometer, Copenhagen, Denmark).

In a second study, the rats were decapitated 1 hour after administration of SCH 34826 (90 mg/kg s.c.), and plasma ANF levels were measured by radioimmunoassay.

Radioimmunoassay for Atrial Natriuretic Factor

Blood samples were collected by decapitation of the rats and collection of the trunk blood into chilled vacutainer tubes containing a mixture of EDTA (1.5 mg/ml), aprotinin (200 kallikrein inhibitor unit/ml), and soybean trypsin inhibitor (20 Nα-benzoyl-l-arginine ethyl ester unit/ml) with a volume of 100 μl each. Urine samples were similarly treated. The blood was immediately centrifuged at 3,000 rpm for 10 minutes. Both plasma and urine samples were stored at −20°C before measurements with RIA. A mixture of samples (1 ml plasma or 2 ml urine), 0.6 ml 8 M urea and 20 ml 1.5 M NaCl in disposable syringes, was passed through Sep-Pak C18 cartridges (Waters Assoc., Milford, Massachusetts), which had previously been activated by passing 5 ml methanol and 5 ml 8 M urea, followed by washing with 10 ml water. The sample-loaded cartridges were washed with 10 ml water and eluted with 8 ml 4% acetic acid in 90% ethanol. The eluants were blown down under nitrogen. The residues were reconstituted with 0.4 ml 0.02 M acetic acid and 0.6 ml assay buffer after lyophilization.

The extracted plasma or urine samples (100 μl) or standards were incubated with 100 μl antiserum (from Dr. Victor Dzau, Boston, Massachusetts) and 100 μl iodine-125–labeled rat ANF-(99–126) (3,500–4,500 cpm) in triplicate at 4°C for 24 hours. Free and bound ANF were separated using 0.5 ml dextrancoated charcoal. The samples were vortexed and incubated at 4°C for 15 minutes followed by centrifugation at 3,000 rpm for 15 minutes. The supernatants were counted in an automatic gamma counter. The nature of the immunoreactive species of ANF measured in plasma and urine was ascertained by high-performance liquid chromatography (HPLC) separation of the samples. Immunoreactivity in plasma and urine migrated as a single peak with...
retention time identical to that of ANF-(99–126) (data not shown). Recovery of ANF from rat plasma and urine averaged 63 and 45%, respectively. This methodology for measurement of plasma ANF has been described.

Drugs
The drugs used in these studies were ANF-(103–125) (Peninsula Labs., Belmont, California, or synthesized by Dr. Federico Gaeta, Schering Plough Research, Bloomfield, New Jersey), ANF-(99–126) (Peninsula Labs.), and SCH 34826 and SCH 32615 (both synthesized at Schering Plough Research). Angiotensin I, II, and bradykinin were obtained from Peninsula Labs.

Statistical Analyses
All values represent mean or mean±SEM. Data were analyzed statistically using a t test or analysis of variance with Duncan’s multiple range test or Dunnett’s test. Differences were considered significant at p<0.05.

Results

Potentiation of Hypotensive Response to Atrial Natriuretic Factor

Intravenous injection of ANF-(103–125) reduced blood pressure by approximately 15 mm Hg in conscious SHR (Figure 2). Blood pressure returned to baseline within 30–60 minutes of ANF challenge (not shown). Subcutaneous administration of SCH 34826 (10, 30, and 90 mg/kg s.c.) significantly enhanced the blood pressure response to ANF in both magnitude (Figure 2) and duration (not shown) of effect. The diacid of SCH 34826, SCH 32615, also potentiated ANF responses when administered subcutaneously at 30 mg/kg (Figure 2).

ANF-(99–126) has been demonstrated to be the major circulating form of the peptide in animals and humans. SCH 34826 (90 mg/kg s.c.) significantly potentiated the hypotensive response to intravenous injection of ANF-(99–126) in the SHR (Figure 2).

The effect of oral administration of SCH 34826 is demonstrated in Figure 3. As can be seen, SCH 34826 (90 mg/kg p.o.) significantly potentiated the hypotensive effect of ANF-(103–125) in the SHR in terms of both magnitude and duration of effect.

SCH 34826 and SCH 32615 did not alter baseline blood pressure relative to vehicle-control values in any of the experiments described above in the SHR (data not shown).

Potentiation of Renal Response to Atrial Natriuretic Factor

The renal potentiating activity of SCH 34826 was evaluated against a dose of 30 μg/kg i.v. ANF-(99–126) in conscious SHR. SCH 34826 (90 mg/kg s.c.) significantly augmented the diuretic and natriuretic response to ANF (30 μg/kg) (Figure 4). SCH

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All values represent mean or mean±SEM. Data were analyzed statistically using a t test or analysis of variance with Duncan’s multiple range test or Dunnett’s test. Differences were considered significant at p<0.05.

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Potentiation of Hypotensive Response to Atrial Natriuretic Factor

Intravenous injection of ANF-(103–125) reduced blood pressure by approximately 15 mm Hg in conscious SHR (Figure 2). Blood pressure returned to baseline within 30–60 minutes of ANF challenge (not shown). Subcutaneous administration of SCH 34826 (10, 30, and 90 mg/kg s.c.) significantly enhanced the blood pressure response to ANF in both magnitude (Figure 2) and duration (not shown) of effect. The diacid of SCH 34826, SCH 32615, also potentiated ANF responses when administered subcutaneously at 30 mg/kg (Figure 2).

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SCH 34826 and SCH 32615 did not alter baseline blood pressure relative to vehicle-control values in any of the experiments described above in the SHR (data not shown).

Potentiation of Renal Response to Atrial Natriuretic Factor

The renal potentiating activity of SCH 34826 was evaluated against a dose of 30 μg/kg i.v. ANF-(99–126) in conscious SHR. SCH 34826 (90 mg/kg s.c.) significantly augmented the diuretic and natriuretic response to ANF (30 μg/kg) (Figure 4). SCH
SCH 34826 significantly enhanced basal urine volume (196.4±31.5 vs. 67.8±20.0 µl/min/kg, p<0.05, Dunnett’s test) and tended to increase the baseline urine sodium excretion (6.10±2.19 vs. 2.11±1.15 meq/min/kg, p>0.05, Dunnett’s test).

**Potentiation of Plasma Levels of Atrial Natriuretic Factor After Injection of Peptide**

These studies were conducted to determine the influence of SCH 34826 on plasma levels of ANF achieved after injection of the peptide. In methylcellulose-treated rats, intravenous bolus injection of ANF-(99-126) (30 µg/kg) elicited a large increase in plasma ANF levels that returned to baseline over 10–30 minutes (Figure 5). SCH 34826 (90 mg/kg s.c.) markedly potentiated the plasma levels of ANF achieved after injection of the peptide to these conscious SHR rats (Figure 5).

**In Vivo Angiotensin Converting Enzyme Inhibitory Activity**

Table 1 summarizes the effects of SCH 34826 on blood pressure responses to angiotensin I, II, and bradykinin in conscious SHR. Oral administration of SCH 34826 (90 mg/kg) did not alter significantly the blood pressure responses to any of these vasoactive peptides.

**Antihypertensive Activity of SCH 34826 in the Deoxycorticosterone Acetate–Salt Rats**

The DOCA-salt rat is a volume-dependent model of hypertension associated with elevated plasma ANF levels. We assessed the influence of SCH 34826 on blood pressure in this model to determine if NEP inhibition would enhance the endogenous ANF activity and lower blood pressure. Subcutaneous administration of SCH 34826 (90 mg/kg) significantly reduced blood pressure in the DOCA-salt rat by 35±12 mm Hg versus 6±3 mm Hg in vehicle-control rats, (p<0.05, t-test).

SCH 34826 also significantly lowered blood pressure when administered orally at 10 and 90 mg/kg (Figure 6). A dose of 90 mg/kg produced a large and sustained reduction of blood pressure. Heart rate was not altered by either dose of SCH 34826, in spite of the reduction of blood pressure (data not shown).

**Hemodynamic Effects of SCH 34826 in Deoxycorticosterone Acetate–Salt Rats**

The effects of SCH 34826 on blood pressure, heart rate, cardiac output, and total peripheral resistance are illustrated in Figure 7. Oral administration of SCH 34826 (90 mg/kg) reduced arterial blood pressure for the 3-hour duration of study. Heart rate was not significantly affected by SCH 34826. Cardiac output was significantly reduced (p<0.05) at 1 hour, but not 2 and 3 hours after dose administration. Total peripheral resistance was unchanged relative to the vehicle group. The results suggest that the primary hemodynamic mechanism of blood pressure reduction is a fall in cardiac output.

**Effects of SCH 34826 on Renal Excretory Parameters and on Plasma and Urine Cyclic Guanosine Monophosphate and Atrial Natriuretic Factor in Deoxycorticosterone Acetate–Salt Rat**

These studies were performed to determine if antihypertensive doses of SCH 34826 effected an alteration in endogenous levels of ANF and cGMP, a biochemical marker for activation of the guanylate cyclase–coupled ANF receptor in the DOCA-salt rat. In addition, effects on basal excretion of sodium and volume were evaluated.

In the DOCA-salt rat, SCH 34826 (90 mg/kg s.c.) significantly enhanced the 3-hour excretion of urine volume (Table 2). Urine sodium excretion tended to be increased but was not significantly altered (Table 2). Plasma cGMP and ANF, when measured 3 hours after SCH 34826, were unchanged in these studies.

**Figure 4. Line graphs showing potentiation of renal response (change in urinary volume [ΔV] and change in urine sodium excretion [ΔU≤eqN]) to atrial natriuretic factor (ANF)-(103–125) in spontaneously hypertensive rats. Rats were dosed subcutaneously with 90 mg/kg SCH 34826 or methylcellulose (MC) vehicle control and challenged with ANF-(99–126) (30 µg/kg i.v.) 15 minutes later. Data are mean±SEM of number of rats indicated in parentheses. *p<0.05 versus MC vehicle–control group analysis of variance and Dunnett’s test.**
However, SCH 34826 markedly increased the urine excretion of both ANF and cGMP. A second study was performed to determine if changes in plasma ANF were evident at times earlier than 3 hours after dose administration. SCH 34826 (90 mg/kg s.c.) significantly increased plasma ANF levels 1 hour after drug administration in the DOCA-salt rat (753±89 vs. 451±79 pg/ml in vehicle-treated animals, p<0.05, analysis of variance and Dunnett's test).

### Antihypertensive Activity in Spontaneously Hypertensive Rats

SCH 34826 at a dose of 90 mg/kg s.c. did not lower blood pressure acutely in the SHR (Figure 8). SCH 34826 also did not affect plasma levels of ANF acutely in the SHR when measurements were made 3 hours after drug administration (40±46 pg/ml vs. 328±38 pg/ml in vehicle-control group, p>0.05, n=6 per group). We therefore evaluated the effects on arterial blood pressure of administering multiple doses of SCH 34826. SCH 34826 (90 mg/kg s.c.) twice daily elicited a modest but significant reduction of blood pressure that became apparent by day 3 and was evident through day 5 (Figure 8).

### Discussion

The results of the present studies demonstrate that the NEP inhibitor prodrug SCH 34826 and its active diacid SCH 32615 can modify the biological responses to ANF and elicit cardiovascular and renal changes in rodent models of hypertension. SCH 34826, when administered in doses of 10-90 mg/kg s.c., significantly enhanced the hypotensive response to intravenous injection of ANF. Because ANF-(99-126) and -(103-125) are both substrates for NEP,10-14 it is likely that the potentiation of responses is due to inhibition of the in vivo inactivation of the peptide by NEP. SCH 32615 is the active acid form of SCH 34826. SCH 32615 is at least as active as SCH 34826 in potentiating ANF responses. The similar activity of SCH 32615 and SCH 34826 in this test suggests that SCH 34826 undergoes efficient deesterification after subcutaneous administration. SCH 34826 is a prodrug form of SCH 32615 and was designed to facilitate oral bioavailability of the active acid.15 The oral ANF-potentiating activity of SCH 34826 was confirmed in that a 90 mg/kg dose significantly enhanced the hypotensive responses to the peptide. The magnitude of potentiation was similar to that achieved with subcutaneous dosing.

### Table 1. Effects of SCH 34826 on Blood Pressure Responses to Angiotensin I, II, and Bradykinin in Conscious Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>BL (mm Hg)</th>
<th>Δ Ang I (1 μg/kg)</th>
<th>Δ Ang II (1 μg/kg)</th>
<th>Δ BK (1 μg/kg)</th>
<th>Δ BK (3 μg/kg)</th>
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<tbody>
<tr>
<td>MC Vehicle</td>
<td>5</td>
<td>170±15</td>
<td>+55±6</td>
<td>+49±4</td>
<td>-16±3</td>
<td>-28±5</td>
</tr>
<tr>
<td>SCH 34826</td>
<td>5</td>
<td>173±8</td>
<td>+50±6</td>
<td>+46±6</td>
<td>-15±3</td>
<td>-25±2</td>
</tr>
<tr>
<td>(90 mg/kg p.o.)</td>
<td>5</td>
<td>170±15</td>
<td>+55±6</td>
<td>+49±4</td>
<td>-16±3</td>
<td>-28±5</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Rats were dosed orally with methylcellulose (MC) vehicle or SCH 34826 and challenged 90 minutes later with angiotensin I (Ang I), angiotensin II (Ang II), or bradykinin (BK) administered intravenously. None of the values in the SCH 34826 group is significantly different from those in the MC vehicle group. n, number of rats per group; BL, baseline value before challenge with angiotensin I and 90 minutes after dosing with SCH 34826 or vehicle.
ANF-(99–126) is the major circulating form of ANF. Our results with SCH 34826 demonstrate that NEP inhibition also potentiates the hypotensive effect of this circulating form of the peptide.

In addition to a reduction of blood pressure, another action of ANF is to enhance sodium and volume excretion from the kidneys. SCH 34826 potentiated the diuretic effect of a high dose of ANF-(99–126). The results support the contention that SCH 34826 can potentiate two major biological actions of ANF: diuresis and hypotension.

We determined plasma levels of ANF achieved in the presence and absence of SCH 34826 after injection of the dose of ANF (30 μg/kg) used in the above studies. As expected, very high levels of ANF were achieved after bolus injection of ANF. SCH 34826 further potentiated these high levels after injection of the peptide. These results confirm that the potentiation of biological responses to ANF by SCH 34826 is associated with significantly higher plasma levels of the peptide.

The cardiovascular consequences of NEP inhibition with SCH 34826 were evaluated by determining effects of the drug on baseline blood pressure in two experimental animal models of hypertension: The DOCA-salt rat and the SHR. The DOCA-salt rat is a volume-dependent form of hypertension in which plasma levels

![Figure 6](image)

**Figure 6.** Line graph showing antihypertensive activity of SCH 34826 in deoxycorticosterone acetate–salt rat. Rats were dosed orally with SCH 34826 or methylcellulose vehicle, and blood pressure (BP) was monitored for next 4 hours. Baseline BP at time of dosing is indicated next to each group. Data are presented as mean±SEM changes in arterial blood pressure (MAP). *p<0.05 versus change in vehicle-control group, analysis of variance and Duncan's test.

![Figure 7](image)

**Figure 7.** Line graphs showing hemodynamic changes produced by SCH 34826 in deoxycorticosterone acetate–salt hypertensive rats. Rats were dosed orally with methylcellulose vehicle or SCH 34826 (90 mg/kg), and blood pressure (MAP, BP), heart rate (HR), cardiac output (CO), and total peripheral resistance (TPR) were monitored for the next 4 hours. Data are mean±SEM. Baseline values are illustrated next to each treatment group. *p<0.05 versus change in vehicle group, analysis of variance and Dunnett's test.
TABLE 2. Effect of SCH 34826 on Plasma Atrial Natriuretic Factor and Cyclic Guanosine Monophosphate and on Urine Sodium, Volume, Atrial Natriuretic Factor, and Cyclic Guanosine Monophosphate in Conscious Desoxycorticosterone Acetate–Salt Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma ANF (pg/ml)</th>
<th>cGMP (pmol/ml)</th>
<th>Urine ANF (pg/100 g/3 hr)</th>
<th>cGMP (pmol/100 g/3 hr)</th>
<th>UV (ml/100 g/3 hr)</th>
<th>UN,V (meq/100 g/3 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC vehicle (5)</td>
<td>246±55</td>
<td>41±8</td>
<td>21±6</td>
<td>877±168</td>
<td>1.27±0.03</td>
<td>0.23±0.06</td>
</tr>
<tr>
<td>SCH 34826 (90 mg/kg s.c.) (5)</td>
<td>315±52</td>
<td>62±18</td>
<td>3,092±1,098*</td>
<td>2,131±509*</td>
<td>2.78±0.6'</td>
<td>0.42±0.13</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Rats were decapitated 3 hours after dosing with methyicellulose (MC) vehicle or SCH 34826. Number of rats per group is in parentheses. ANF, atrial natriuretic factor; cGMP, cyclic guanosine monophosphate; UV, urine volume; UN,V, urine sodium excretion. *p<0.05 versus response in vehicle-control rats.

of ANF are increased and blood volume expansion is a major contributor to the elevated pressure.16 The elevated ANF levels may reflect a compensatory response of the rat to maintain a normal blood pressure and plasma volume. We surmised that SCH 34826, by preventing degradation of ANF, would potentiate activity of the endogenous hormone and thereby lower blood pressure and elicit diuresis. SCH 34826 reduced blood pressure in the DOCA-salt rat. Onset of action was within 2–3 hours of dosing, and the effect was sustained for the 4-hour duration of study.

The hemodynamics of the antihypertensive response to SCH 34826 were evaluated in the DOCA-salt rat. The major hemodynamic change was a reduction in cardiac output. The fall in blood pressure was not associated with a reflex tachycardia or fall in total peripheral resistance. The mechanism for the decreased cardiac output remains to be established. SCH 34826 and its diacid are devoid of direct inotropic or chronotropic effects on the guinea pig heart in vitro (R.W. Watkins, unpublished observations), and hence, the action is unlikely to be due to a direct cardiodepressant effect. The hemodynamic pattern resembles that which we17 and others20–24 have observed previously with infusion of ANF.

The renal consequences of NEP inhibition with SCH 34826 in the DOCA-salt rat were evaluated. SCH 34826 enhanced volume excretion and tended to increase sodium excretion. These effects are consistent with potentiation of the effect of endogenous ANF on the kidney and are impressive, given the fact that blood pressure most likely was reduced in these rats and the natriuretic response to ANF is dependent on an adequate renal arterial perfusion pressure.25 Others have reported significant diuresis with the NEP inhibitors phosphoramidon and UK 69578.26,27

We measured plasma and urine ANF and cGMP to gain insight into the role of ANF in the mechanisms of antihypertensive and diuretic responses. Plasma ANF was significantly elevated by SCH 34826 when measurements were made at 1 hour subsequent to dosing. This effect is consistent with inhibition of metabolism of ANF by the inhibitor. However, plasma ANF levels were not significantly elevated after SCH 34826 when measurements were made 3 hours subsequent to dosing. It is possible that the fall
in blood pressure may have inhibited further release of ANF and offset an influence of inhibition of degradation on the steady-state plasma levels. The reduction of cardiac output by SCH 34826 is consistent with removal of stimuli for ANF release. Nevertheless, the fact that blood pressure remained depressed at a time when ANF levels in plasma had returned to control levels suggests a dissociation of the plasma ANF and blood pressure response to the inhibitor. It is possible that inhibition of ANF metabolism by NEP at some local site of cardiorenal control is critical to the hemodynamic response to SCH 34826 and that this action is not reflected in the steady-state plasma compartment of ANF. The localization of NEP and ANF receptors in the same organ (e.g., adrenal gland, renal glomeruli) is consistent with such a possibility.

Urine excretion of ANF and cGMP was markedly elevated 3 hours after dosing with SCH 34826. The urine excretion of ANF likely reflects an action of SCH 34826 on the proximal tubular brush border NEP. Thus, under circumstances where NEP is functionally active, ANF is filtered through the glomerulus and degraded by the brush border enzyme. When NEP is inhibited, ANF is not degraded and appears as intact peptide in the urine. These results further indicate that SCH 34826 interferes with the metabolism of endogenous ANF. However, it is unlikely that inhibition of NEP at this site contributes to the actions of SCH 34826 as the ANF is undergoing clearance from the body at this site. The rise in urine cGMP by SCH 34826 suggests that the inhibitor has enhanced the biochemical activity of endogenous ANF. cGMP is elevated on binding of ANF to one receptor and this second messenger serves as a useful in vivo biochemical marker of ANF receptor activation.

Urine cGMP was elevated at a time when plasma cGMP was unaffected by SCH 34826. This observation is consistent with the hypothesis that local changes in ANF (or cGMP) can be effected by NEP inhibition without alterations in the plasma compartment. The site of origin of the urine cGMP is not certain but may be from theglomerulus as both NEP and guanylate cyclase–linked ANF receptors are located at this site.

SCH 34826 did not lower blood pressure acutely in the SHR. Although plasma and ventricular ANF levels are elevated in the SHR, the model is not recognized as a volume-dependent form of hypertension. Hence, NEP-sensitive mechanisms may be masked by other counter-regulatory mechanisms of blood pressure control in SHR in the acute state or not as important to blood pressure homeostasis in the SHR as in the DOCA-salt rat. Moreover, the hypotensive response to ANF is reduced in SHR compared with DOCA-salt rats (E.J. Sybertz and P.J.S. Chiu, unpublished observations). It is possible that any of these factors may have precluded a demonstration of an acute antihypertensive effect of SCH 34826 and other inhibitors in this model.

We therefore evaluated whether subacute dosing with SCH 34826 would lower blood pressure in the SHR. SCH 34826 (90 mg/kg s.c.) twice daily elicited a moderate but significant reduction of blood pressure that became evident on day 3 and was sustained for the 5-day period of observation. The mechanisms of this delayed reduction of blood pressure remain to be established. The role of ANF in the response and the relation of this change to the acute reductions observed in the DOCA-salt rat also require further investigation. The observations resemble those of Janssen et al. in humans with continuous low dose infusion of ANF. In their study, ANF infusion at low doses, which caused modest changes in plasma levels of the peptide, resulted in a reduction of pressure in human hypertensive subjects. This reduction of blood pressure was not apparent until day 2 of the 5-day infusion.

NEP metabolizes numerous vasoactive peptides. Hence, the precise role of ANF in the hypotensive and diuretic response to SCH 34826 remains to be determined through the use of antibodies to or antagonists of the peptide or its receptor. At present, the renal and hemodynamic effect in volume-sensitive rats, the changes in ANF levels in plasma and urine, and the changes in urine cGMP are all supportive of an effect of SCH 34826 on ANF metabolism. The effects of SCH 34826 likely are not due to inhibition of degradation of enkephalins because naltrexone does not block the blood pressure response to a related NEP inhibitor, SCH 39370.

The effects are unlikely to have been caused by inhibition of bradykinin metabolism because SCH 34826, at a dose (90 mg/kg) that potentiates ANF and lowers blood pressure, did not change bradykinin responses (Table 1). The effects are not likely to have been caused by actions on ACE because SCH 34826 is devoid of activity on ACE in vivo and in vitro. Moreover, ACE inhibitors such as captopril and enalapril, which produce large alterations in bradykinin and angiotensin I pressor responses, do not lower blood pressure in the DOCA-salt rat under the conditions of these studies (E.J. Sybertz, unpublished observations)

Since submission of this work, several reports on ANF-potentiating activity of neutral endopeptidase inhibitors have appeared. Our results are consistent with these studies.

In conclusion, the results of the present studies demonstrate that NEP inhibition with SCH 34826 potentiates biological activity of ANF in vivo. In addition, SCH 34826 lowers blood pressure acutely and elicits diuresis in DOCA-salt hypertension. A reduction of blood pressure in SHR becomes evident only after several days of dosing. Based on this profile, NEP inhibition may represent a novel means for treatment of some forms of human hypertension. The role of ANF in the response remains to be confirmed.

References


KEY WORDS: endopeptidase inhibitor • atrial natriuretic factor • essential hypertension • antihypertensive agents
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