Acute coadministrations of an inhibitor of endopeptidase 24.11 (thiorphan) and a ligand (SC-46542) selective for the non–guanylate cyclase-linked atriopeptin binding sites increases urinary sodium excretion to a greater degree in conscious spontaneously hypertensive rats than in normotensive Wistar-Kyoto rats. In the present study, we examined the effects of chronic 10-day intravenous infusions of SC-46542 (des[Phe^{96},Gly^{97},Ala^{108},Gln^{161}] atriopeptin-(103–126)) (0.1 mg/kg/hr), thiorphan (1.5 mg/kg/hr), and atriopeptin-(103–126) (100 ng/hr) alone or in combination on direct recording of mean arterial pressure in conscious spontaneously hypertensive rats. During an 11-day time-control infusion of isotonic saline vehicle (100 μl/hr), mean arterial pressure remained stable. Chronic infusion of atriopeptin-(103–126) decreased mean arterial pressure progressively over the first 3 days; then mean arterial pressure progressively rose to control level over the following 3 days and remained at control level for the remainder of the experiment. Similarly, coinfusions of atriopeptin-(103–126) and SC-46542 or thiorphan, SC-46542 and thiorphan, or the triple infusion of atriopeptin-(103–126), SC-46542, and thiorphan had only transient effects on mean arterial pressure during 10-day infusions. SC-46542 alone had no effect on mean arterial pressure. Similarly, thiorphan alone had no effect on mean arterial pressure except at doses that blocked the acute pressor response to angiotensin I. Chronic infusions of atriopeptin-(103–126), SC-46542, and thiorphan alone or in combination are not effective long-term treatments for hypertension in spontaneously hypertensive rats. (Hypertension 1990; 16:642–647)

Atriopeptin (atrial natriuretic peptide) is a circulating hormone that is synthesized in, stored in, and released from cardiac atria and ventricles.1-2 Atriopeptin is also found in the adrenal medulla, lung, kidney, and brain.3-8 Other recent evidence indicates that renal tubular cells synthesize and secrete a 32-amino acid form of atriopeptin referred to as urodilatin.9 The biological effects of atriopeptin include diuresis, natriuresis, and decreased arterial pressure.1,2

Two subtypes of atriopeptin binding sites have been identified. A small proportion (1–30%) of binding sites are coupled to guanylate cyclase; however, the largest proportion (70–99%) of binding sites are not associated with any known second messenger system.10-13 Maack and colleagues14 have proposed that the non–guanylate cyclase-linked atriopeptin binding sites are specific for atriopeptin storage or clearance, serving as a buffer system to regulate plasma levels of atriopeptin. Atriopeptin is also metabolized by endopeptidase 24.11,15,16 Endopeptidase 24.11 is found in highest concentrations in the proximal tubule of the brush border membranes of the kidney.17 The cleavage of atriopeptin is prevented by inhibitors of endopeptidase 24.11, such as thiorphan.15 Thus, these two systems may represent important dual pathways for the regulation of circulating levels of atriopeptin.

The acute coadministration of a selective ligand for the non–guanylate cyclase-linked atriopeptin binding site (SC-46542) and an inhibitor of endopeptidase 24.11 (thiorphan) increases diuresis and natriuresis more than the administration of either compound alone in conscious Sprague-Dawley rats, Wistar-Kyoto (WKY) rats, and spontaneously hypertensive rats (SHR).18,19 Moreover, plasma atriopeptin immunoreactivity is increased more by the coadministration of SC-46542 and thiorphan than by either alone.18,19 In conscious Sprague-Dawley rats, the coadministration of SC-46542 and thiorphan greatly potentiates the depressor, diuretic, and natriuretic responses to a low dose infusion of atriopeptin.18 The natriuresis to coadministration of SC-46542 and thiorphan is greater in conscious SHR than WKY rats.19 This natriuresis is associated with a similar increase in urinary cyclic guanosine mono-
phosphate (cGMP) excretion in SHR and WKY rats, which is nephrogenous. Acutely, mean arterial pressure is not altered by coadministration of SC-46542 and thiorphan in conscious rats. However, the potentiated natriuresis, the increased urinary cGMP excretion, and the elevated plasma atriopeptin immunoreactivity in conscious SHR point to the possibility that the chronic administration of these compounds may reset the pressure/natriuresis mechanism of long-term arterial pressure control in SHR, leading to the normalization of arterial pressure. In the present study, we examined the effect of chronic (10 days) continuous intravenous administration of atriopeptin-(103-126), a selective ligand for the non-guanylate cyclase-linked atriopeptin binding site (SC-46542), and an inhibitor of endopeptidase 24.11 (thiorphan) alone and in combination on direct recording of mean arterial pressure in conscious SHR. We tested the hypothesis that chronic activation of the atriopeptin system, either by exogenous administration of atriopeptin or by blocking the degradation of atriopeptin or both, would have long-term antihypertensive effects in SHR.

Methods

Male SHR (n=85; body weight range, 275–325 g) 10–12 weeks of age were obtained from Harlan Sprague Dawley, Inc, Indianapolis, Ind., and were studied between 12 and 14 weeks of age. Rats were habituated for 3–4 days in individual experimental cages, which became their home cages for the duration of the study. Rats were anesthetized with methohexital sodium (30 mg/kg i.p. supplemented as needed with 10 mg/kg i.v. Brevital, Eli Lilly Co., Indianapolis, Ind.) or chloral hydrate (400 mg/kg, Sigma Chemical Co., St. Louis, Mo.), and catheters were implanted in a femoral artery and vein. The catheters were led to the back of the neck, exteriorized, and channeled through a tether and swivel system (Alice King Chatham, Los Angeles). SHR that did not resume normal food and water consumption were omitted from the study. Three to four days after catheter implantation a 100 μl/hr i.v. infusion (Harvard Pump 22, Harvard Apparatus, South Natick, Mass.) of isotonic saline was started for the duration of the 11-day experiment. Mean arterial pressure was measured continuously between 10:00 AM and 2:00 PM daily via a pressure transducer (model P23Db, Statham, Oxnard, Calif.), displayed on a chart recorder (model 3800, Gould Inc., Cleveland, Ohio), and recorded via an IBM PC AT using in-house designed and validated software. The patency of the venous catheter and the quality of the arterial pressure signal were verified before and after each 11-day experiment with an intravenous injection of angiotensin II (10 ng). Calibration of pressure recording equipment was conducted daily before and after each arterial pressure recording session.

The first experimental day (3–4 days after surgery) was a control arterial pressure recording day. On the second day SHR were studied in one of eight separate groups as follows: 1) saline vehicle control (100 μl/min) (n=7), 2) atriopeptin-(103-126) alone (100 ng/hr) (n=7), 3) coinfusion of atriopeptin-(103-126) (100 ng/hr) and SC-46542 (0.1 mg/kg/hr) (n=6), 4) SC-46542 alone (0.1 mg/kg/hr) (n=6), 5) thiorphan alone (1.5 mg/kg/hr) (n=7), 6) coinfusion of SC-46542 (0.1 mg/kg/hr) plus thiorphan (1.5 mg/kg/hr) (n=8), 7) coinfusion of atriopeptin-(103-126) (100 ng/hr) plus thiorphan (1.5 mg/kg/hr) (n=4), and 8) triple infusion of atriopeptin-(103-126) (100 ng/hr), thiorphan (1.5 mg/kg/hr), and SC-46542 (0.1 mg/kg/hr) (n=7). In each thiorphan experiment, the possibility of converting enzyme inhibition was tested by observing the pressor response to an intravenous injection of angiotensin I (50 ng).

In separate groups of SHR, vehicle, atriopeptin-(103-126), or atriopeptin-(103-126) plus thiorphan were infused as described above for 3 or 7 days. On day 3 or 7, blood samples were obtained for the measurement of plasma renin activity (PRA) and plasma atriopeptin immunoreactivity. Only a single blood sample was obtained from each group. PRA was determined using a 125I-angiotensin I radioimmunoassay kit (Dupont/New England Nuclear, Boston, Mass.). Plasma atriopeptin immunoreactivity was measured by an extraction method as previously described.

Thiorphan, SC-46542, and atriopeptin-(103-126) were synthesized in our laboratories (G.D. Searle Research and Development; Monsanto Corporate Research and Development, St. Louis, Mo.). Angiotensin I and II were obtained from Sigma. All compounds were prepared fresh daily. Stability of compounds was verified by high-performance liquid chromatography.

Data are presented as mean±SEM. Statistical analyses were performed using repeated-measures analysis of variance within each group for main effects and interactions, and Tukey's honestly significant difference test was used for pairwise comparisons between control days and experimental days. Statistical significance is defined as p<0.05.

Results

In time-control SHR, vehicle infusion alone had no effect on mean arterial pressure over 11 days of observation (Figure 1). Similarly, neither infusion of thiorphan alone nor infusion of SC-46542 alone had any effect on mean arterial pressure (Figure 1) or heart rate (Table 1). Heart rate was not measured during infusion of atriopeptin-(103-126) alone. The dose of thiorphan used did not alter the arterial pressure response to intravenous injection of angiotensin I (50 ng). Before thiorphan administration, infusion of angiotensin I increased (p<0.05) mean arterial pressure 35±4 mm Hg, and after 3 days of thiorphan infusion, angiotensin I increased (p<0.05) mean arterial pressure 30±6 mm Hg in the same SHR.

In contrast, during 10 days of continuous atriopeptin-(103-126) infusion in SHR, mean arterial pressure progressively decreased (p<0.05) over the first 3
days; however, mean arterial pressure then progressively rose to control level over the following 3 days and remained at control level for the remainder of the study (Figure 2). Similar to atriopeptin-(103-126) infusion alone, blockade of non-guanylate cyclase-linked atriopeptin binding sites with SC-46542 together with infusion of atriopeptin-(103-126) resulted in a transient antihypertensive effect (Figure 2). Mean arterial pressure remained below \( p<0.05 \) control level for 1–4 days but by day 5 was at control level and remained at control level for the duration of the study. No significant interaction was found between the SC-46542 and coininfusion of SC-46542 and atriopeptin-(103−126) groups (Figure 2).

Similarly, 10 days of coadministration of atriopeptin-(103−126) and thiorphan decreased \( p<0.05 \) mean arterial pressure for up to 6 days (Figure 3). After 6 days, mean arterial pressure returned toward control but did not attain control level in these SHR. The coinfusion of thiorphan and SC-46542 resulted in a sustained decrease \( p<0.05 \) in mean arterial pressure for up to 5 days followed by a gradual return toward control level; however, unlike the other groups mean arterial pressure remained below control level \( p<0.05 \) for duration of study.

**TABLE 1. Effects of Thiorphan, SC-46542, or Thiorphan and SC-46542 on Heart Rate in Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart Rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Thiorphan (n=7)</td>
<td>369±19</td>
</tr>
<tr>
<td>SC-46542 (n=6)</td>
<td>353±16</td>
</tr>
<tr>
<td>Thiorphan + SC-46542 (n=8)</td>
<td>376±17</td>
</tr>
</tbody>
</table>

**Figure 1.** Line graph showing 10 days infusion of SC-46542 alone, thiorphan alone, or saline vehicle control had no effect on mean arterial pressure relative to the control day (C) in conscious spontaneously hypertensive rats.

**Figure 2.** Line graph showing chronic infusion of atriopeptin (AP) or coinfusion of AP plus a selective ligand for non-guanylate cyclase-linked AP binding sites (SC-46542) had only transient antihypertensive effects in spontaneously hypertensive rats. Analysis of variance revealed a significant main effect of treatment \( p<0.05 \) but a nonsignificant interaction between groups \( p>0.05 \).

Relative to vehicle-infused SHR, atriopeptin-(103−126) or coinfusion of atriopeptin-(103−126) plus thiorphan had no effect on PRA or plasma immunoreactive atriopeptin on day 3 or 7 of compound administration (Table 2). Heart rate remained unchanged from day 1 to day 3 to day 7 during administration of thiorphan alone, SC-46542 alone, or coinfusion of thiorphan and SC-46542 (Table 1).

**Discussion**

For the past several years much effort has been expended to discover a therapeutically useful ana-
logue of atriopeptin for the treatment of hypertension. More recently, additional efforts have been directed at blocking non-guanylate cyclase-linked atriopeptin binding sites or inhibition of endopeptidase 24.11 as a means to regulate circulating levels of atriopeptin. Relatively few studies have examined long-term administration of atriopeptin, endopeptidase 24.11 inhibitors, or ligands selective for non–guanylate cyclase-linked atriopeptin binding sites. Atriopeptin has been reported to lower systolic pressure in SHR throughout 14 days of infusion.23 However, other reports have noted a transient antihypertensive effect of atriopeptin infusion. In two-kidney, one clip hypertensive rats atriopeptin infusion lowered systolic pressure gradually over the first 5 days, but systolic pressure returned to control group level over the following 7 days.24 Similarly, in 6-week-old SHR on high sodium diet and in 13-week-old SHR systolic pressure returned to control levels after 4–5 days of atriopeptin infusion.25 In all of these studies, atriopeptin was infused via osmotic minipump, and arterial pressure was estimated via the tail-cuff technique. Given the uncertainty of continuous infusion of atriopeptin using osmotic minipumps (not possible to check the rate of infusion on a daily basis) and the error associated with estimating arterial pressure via the indirect tail-cuff technique, we repeated the chronic atriopeptin infusion experiment using more direct procedures. In the present experiment, compounds were infused via an extracorporeal pump, and arterial pressure was measured via an indwelling catheter. The main hypothesis of this study was that long-term elevations in atriopeptin, such as by exogenous administration of atriopeptin or by blocking metabolic pathways of atriopeptin degradation (i.e., blocking clearance binding sites or inhibition of endopeptidase 24.11), would be effective long-term treatments of hypertension in SHR.

Similar to previous studies,22,24,25 continuous infusion of atriopeptin for 10 days produced a transient antihypertensive effect in conscious SHR. The mechanism for the transient antihypertensive response to chronic infusion of atriopeptin is not known. We tested the hypothesis that chronic infusion of atriopeptin may upregulate the non–guanylate cyclase-linked atriopeptin binding sites. These binding sites are not associated with any known second messenger system and have been proposed to be specific for atriopeptin storage or clearance.10–14 The acute administration of ligands selective for the non–guanylate cyclase-linked atriopeptin binding sites increases urinary sodium excretion and plasma atriopeptin immunoreactivity and potentiates the natriuretic and depressor responses to atriopeptin.14,18 Thus, an increase in the number of non–guanylate cyclase-linked atriopeptin binding sites would be expected to bind more atriopeptin, leaving less for antihypertensive effects. Accordingly, blocking the non–guanylate cyclase-linked atriopeptin binding sites would prevent uptake of atriopeptin, leaving more for antihypertensive effects. However, confusion of a ligand selective for the non–guanylate cyclase-linked atriopeptin binding sites (SC-46542) and atriopeptin-(103–126) did not result in a prolongation of the antihypertensive response. In contrast to atriopeptin infusion alone where mean arterial pressure decreased progressively over 3 days, mean arterial pressure decreased rapidly to its lowest point by day 1 in the present study, suggesting that there may have been an interaction between atriopeptin and SC-46542. Nevertheless, these results do not support the hypothesis that the transient antihypertensive effect of atriopeptin alone was due to an upregulation of non–guanylate cyclase-linked atriopeptin binding sites. The transient antihypertensive effect of atriopeptin-(103–126) was unrelated to changes in plasma immunoreactive atriopeptin, since plasma immunoreactive atriopeptin was similar on day 3 of infusion, when arterial pressure was lowest, and on day 7, when arterial pressure was back to control level. A previous study showed that chronic infusion of atriopeptin (100 ng/hour) had no effect on plasma levels of atriopeptin in conscious SHR, yet arterial pressure was reduced.26 Thus, it appears that the mechanism by which chronic infusion of atriopeptin reduces arterial pressure in SHR is not revealed by measuring plasma levels of atriopeptin.

Chronic blockade of non–guanylate cyclase-linked atriopeptin binding sites with SC-46542 and inhibition of atriopeptin degradation by endopeptidase 24.11 with thiorphan were also tested as possible antihypertensive therapeutic strategies in SHR. According to this approach, either preventing uptake of atriopeptin by the non–guanylate cyclase-linked atriopeptin sites or inhibiting the enzymatic hydrolysis of atriopeptin by endopeptidase 24.11 would be expected to increase endogenous atriopeptin levels, leading to decreased arterial pressure. Acute administration of SC-46542 potentiates the depressor response to atriopeptin.18 However, neither chronic

**Table 2. Effects of Vehicle, Atriopeptin-(103–126), or Atriopeptin and Thiorphan on Plasma Renin Activity and Atriopeptin Immunoreactivity in Conscious Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma renin activity (ng Ang I/ml/hr)</th>
<th>AP immunoreactivity (fmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>15 ±2(n=6)</td>
<td>16 ±6(n=4)</td>
</tr>
<tr>
<td>AP-(103–126)</td>
<td>14±3(n=6)</td>
<td>12±3(n=5)</td>
</tr>
<tr>
<td>AP+thiorphan</td>
<td>17±2(n=6)</td>
<td>14±2(n=6)</td>
</tr>
</tbody>
</table>

AP, atriopeptin or atrial natriuretic peptide; Ang I, angiotensin I; SHR, spontaneously hypertensive rats.
infusion of SC-46542 nor thiorphan resulted in decreased arterial pressure in SHR. Only rates of thiorphan infusion (15 mg/kg/hr) that inhibited converting enzyme (as determined by lack of pressor response to angiotensin I) decreased arterial pressure in SHR (unpublished observation). Thus, at the higher infusion rate of thiorphan it was difficult to differentiate between arterial pressure lowering due to endopeptidase 24.11 inhibition or converting enzyme inhibition.

Another strategy is to coinfuse SC-46542, thiorphan, and atriopeptin. The acute coadministration of SC-46542 and thiorphan resulted in greater increases in urinary sodium excretion, urinary cGMP excretion, and plasma immunoreactivity than administration of either alone. In fact, the long-term coinfusion of SC-46542 and thiorphan (but not single infusion) resulted in decreased arterial pressure in SHR. However, this antihypertensive response was not sustained. Coinfusion of SC-46542 and thiorphan decreased mean arterial pressure for up to 6 days. After 6 days, mean arterial pressure returned to control level in these SHR but never attained pretreatment values. Nevertheless, arterial pressure stabilized at a hypertensive level during days 7–10 of coinfusion of SC-46542 and thiorphan. The antihypertensive response to coinfusion of SC-46542 and thiorphan was unrelated to changes in plasma immunoreactive atriopeptin, as this measure was similar on day 3 and day 7 of administration. In addition, the lack of any changes in heart rate or PRA during infusion of SC-46542 and thiorphan suggests that the transient antihypertensive effects were not due to reflex increases in sympathetic nervous system activity. We hypothesized that the triple infusion of atriopeptin-(103–126), thiorphan, and SC-46542 would produce the most effective antihypertensive effects in SHR. The acute natriuretic and depressor response to atriopeptin is greatly potentiated by coadministration of SC-46542 and thiorphan. However, the coinfusion of atriopeptin, thiorphan, and SC-46542 for 10 days in conscious SHR resulted in an antihypertensive response similar to infusion of atriopeptin alone: mean arterial pressure decreased over the first 4 days of infusion then returned to control level. It is not clear why the triple infusion of these compounds did not produce the greatest or most prolonged antihypertensive responses. One observation of potential importance is that coinfusion of thiorphan and SC-46542, which was most effective in lowering mean arterial pressure, was the only group without atriopeptin infusion. Perhaps the exogenous administration of atriopeptin was the most important contributor to the tachyphylaxis. The tachyphylaxis in the thiorphan and SC-46542 group may be delayed; if mean arterial pressure had been followed longer, it would possibly have returned to control level. We conclude that infusions of atriopeptin-(103–126), a ligand selective for the non-guanylate cyclase-linked atriopeptin binding site (SC-46542), and an inhibitor of endopeptidase 24.11 (thiorphan) alone or in combination are not effective long-term treatments of hypertension in the spontaneously hypertensive rat. Although the atriopeptin system may be important in other pathophysiological conditions, the results of the present study are consistent with the lack of therapeutic efficacy in chronic sodium-retaining disorders, such as congestive heart failure, cirrhosis, and nephrosis.

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**KEY WORDS** • endopeptidase • atrial natriuretic peptides • blood pressure • spontaneously hypertensive rats
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