Angiotensin-(1-7) Contributes to the Antihypertensive Effects of Blockade of the Renin-Angiotensin System

Shridhar N Iyer, Carlos M. Ferrario, Mark C. Chappell

Abstract—Angiotensin-converting enzyme (ACE) inhibition alone or in combination with the angiotensin type-I receptor (AT₁) antagonist losartan augments circulating levels of the bioactive peptide angiotensin-(1-7) [Ang-(1-7)]. Hence, we determined whether Ang-(1-7) contributes to the hypotensive effects produced by the combined administration of lisinopril and losartan in spontaneously hypertensive rats by blocking the peptide’s synthesis with either of two structurally different nephrilysin inhibitors. Intravenous administration of CGS 24592 (30 mg/kg) to rats in which blood pressure was normalized by 9 days of therapy with lisinopril and losartan elicited an elevation of mean arterial pressure that was sustained throughout the infusion period and for 20 minutes thereafter. The hypertensive response was associated with a 62% reduction in circulating levels of Ang-(1-7) and no change in plasma angiotensin II (Ang II). Intravenous infusion of one other nephrilysin inhibitor (SCH 39370, 30 mg/kg) produced an increase in mean blood pressure of a magnitude similar to that found with CGS 24592. Pretreatment with the nonselective antagonist [Sar²Thr⁷]-Ang II abolished any additional pressor effects of either nephrilysin inhibitor in spontaneously hypertensive rats treated with lisinopril or losartan. However, neither the endothelin A antagonist BQ123 nor the kinin B₂ antagonist HOE 140 had an effect on basal blood pressure or altered the pressor or heart rate effects of the nephrilysin inhibitors. These data suggest that inhibition of Ang-(1-7) formation in rats exposed to the combined blockade of Ang II production and activity is associated with a reversal of the antihypertensive actions produced by these therapies. Thus, endogenous Ang-(1-7) functions as a vasodilator hormone in this form of genetic hypertension (Hypertension. 1998;31[part 2]:356-361.)

Key Words: angiotensin-(1-7) • nephrilysin • angiotensin-converting enzyme • lisinopril • losartan • SHR • hypertension

Angiotensin-(1-7) [Ang-(1-7)], one of several bioactive end products of the renin-angiotensin system, may function as an antihypertensive hormone by virtue of its potent natriuretic and diuretic actions³⁴ and vasodepressor effects in the coronary and mesenteric vascular beds.⁵-⁷ Moreover, low concentrations of Ang-(1-7) enhance the vasodilator actions of bradykinin in both intact rats⁸ and isolated canine coronary artery rings.⁹ The receptor mediating the vascular actions of Ang-(1-7) has been tentatively characterized as a non-angiotensin type-I receptor (AT₁)/angiotensin type-II receptor (AT₂) subtype.¹¹⁻¹²

Production of Ang-(1-7) from angiotensin I (Ang I) is determined by the action of tissue endopeptidases,¹³ neutral endopeptidase 24.11 (EC 3.4.24.11, nephrilysin) account for the generation of the peptide in the systemic and renal circulation.¹⁴ Whereas the enzymatic pathway involved in the generation of Ang-(1-7) is distinct from that of Ang II, angiotensin converting enzyme (ACE) EC 3.4.15.1 cleaves the heptapeptide into inactive products.¹⁵ This finding explains the observation in man and animals of a 5- to 25-fold increase in the plasma concentration of Ang-(1-7) during chronic treatment with ACE inhibitors.¹⁶⁻²⁰ Because previous studies suggest that endogenous neutralization of Ang-(1-7) mitigates the antihypertensive actions of the combined administration of lisinopril and losartan,¹¹ we have now explored the effects of acute inhibition of Ang-(1-7) synthesis in spontaneously hypertensive rats (SHRs) after their blood pressure was normalized by these therapies. The physiological consequences of endogenous Ang-(1-7) inhibition were determined by the comparative effect of infusion of two specific inhibitors of nephrilysin at a time when circulating levels of Ang-(1-7) were increased significantly by chronic therapy with lisinopril and losartan.¹¹

Methods

Animals

Adult male SHRs (12 weeks of age, 350 g) were purchased from Charles River Animals had free access to tap water and rat chow (Purina Mills Inc.) providing an intake of 17 mmol sodium/28 mmol potassium per 100 g each day. The rats were housed under a 12-hour light/dark cycle in an AAALAC-approved facility and were treated for 9 days with lisinopril (20 mg/kg/24 hours) and losartan (10 mg/kg/24 hours) administered in the drinking water, as described previously.¹¹ Systolic blood pressure was measured in conscious animals by a tail-cuff method (Narco Bio-Systems).
Experimental Protocol

On day 8 of the treatment period, a plastic catheter (PE-50, Clay Adams, Becton Dickinson) was implanted under aseptic conditions into a carotid artery. A second PE-50 catheter was implanted into a jugular vein. The free ends of the catheters were tunneled cephalad and extended at the nape of the neck. The procedures were performed under sterile conditions using inhalation anesthesia with 1% halothane (Ayerst Laboratories Inc.) in 95% O2/5% room air. Measurements of arterial pressure and heart rate were obtained in freely moving conscious rats between 9:00 AM and 12:00 noon the day after the surgery. During the experiments, arterial pressure was recorded continuously using a strain gauge transducer (Uniflow Pressure Transducer, Baxter Healthcare Corp.) connected to the arterial line. The electronic signal was directed to an analog-to-digital converter for beat-by-beat analysis of arterial pressure and heart rate as described in detail elsewhere.11 Calibrated displays of systolic, diastolic, and mean blood pressure and heart rate were imaged using a laser printer.

After a 90-minute recording of baseline hemodynamics, the animals were infused with either of two specific neprilysin inhibitors. Six SHRs received CGS 24592 (IC50 = 2 μmol/L for neprilysin)20 at a dose of 30 mg/kg, whereas five other SHRs were infused with SCH 39370 (IC50 = 11 nmol/L) at a dose of 30 mg/kg. A third group of SHRs (n = 4) were given corresponding infusions of the vehicles (see Materials section, below). All agents were infused intravenously at a rate of 100 μL/min for 10 minutes. Samples of arterial blood (1 mL) were collected for peptide measurements at the completion of the experiment to ascertain the effect of the drugs or vehicle on the plasma concentrations of Ang II and Ang (1–7). Blood samples were collected into chilled tubes containing a mixture of pepptidase inhibitors and chelating agents as described by us previously.19 Plasma samples were stored at –80°C before extraction and assayed (see below).

Assays

Immunoreactive plasma levels of Ang-1–7 and Ang II were measured by radioimmunoassay (RIA) as described by us elsewhere.27,28 Plasma was extracted on C18 Sep-Pak columns (Waters Corporation) activated with acetone, methanol, water, and 0.1% heptfluorobutyric acid (HFBA, Pierce)/water. Plasma was thawed on ice, diluted with 0.1% HFBA and acetomethine, methanol, water, and 0.1% heptfluorobutynic acid (HFBA, NaOH and diluted with phosphate-buffered saline (PBS), the vehicle

Statistics

Statistical differences in arterial pressure and heart rate resulting from experimental manipulations were evaluated by repeated-measures ANOVA followed by Scheffé's post hoc test. Changes in the plasma concentrations of angiotensin peptides were analyzed by the unpaired t test. All data were presented as the mean ± SEM and the criterion for statistical significance were set at a value of P < 0.05.

Results

Eight days of treatment with the combination of Isnopril and losartan caused a decrease in systolic blood pressure from 180 ± 3 mm Hg to 104 ± 5 mm Hg (P < 0.05) in SHRs at 12 weeks of age. Intravenous infusion of the potent neprilysin inhibitor CGS 24592 (IC50 = 2 μmol/L) caused a rapid rise in mean arterial blood pressure (from 81 ± 3 mm Hg to 66 ± 4 mm Hg) that peaked at approximately 5 minutes after the start of the infusion (Fig 1). At the peak of the pressor response, mean arterial pressure had risen by 15 ± 3 mm Hg (P < 0.05), a 20% increase above baseline values. CGS 24529 caused an increase in diastolic pressure by 18 ± 3 mm Hg (from 74 ± 1 mm Hg to 92 ± 2 mm Hg). Whereas systolic blood pressure rose by 13 ± 1 mm Hg (from 90 ± 3 mm Hg to 103 ± 4 mm Hg), arterial pressure remained elevated throughout the 20-minute recording period without a significant change in heart rate (365 ± 15 beats per minute [bpm] and 385 ± 7 bpm before and after CGS 24592 infusion, respectively).

Materials

The neprilysin inhibitors SCH 39370 and CGS 24592 were provided by Dr. P. J. Syberts of Schering Plow Research and Dr. A. J. Trapani of Novartis Corporation. The inhibitors were initially dissolved in NaOH and diluted with phosphate-buffered saline (PBS), the vehicle

Figure 1. Time course of changes in mean arterial pressure (MAP, top) and heart rate (HR, bottom) during infusion of either the neprilysin inhibitor (CGS 24592, n=6) or vehicle (PBS, n=4) CGS 24592 (30 mg/kg) or PBS was infused intravenously for a period of 10 minutes and blood pressure was monitored for an additional 20 minutes after cessation of either the CGS 24592 or PBS infusion.
Effect of CGS 24592 on Plasma Angiotensins

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ang-(1-7) (pmol/L)</th>
<th>Ang II (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated SHRs</td>
<td>249±49</td>
<td>33±5</td>
</tr>
<tr>
<td>CGS-24526-treated SHRs</td>
<td>95±8*</td>
<td>41±8</td>
</tr>
</tbody>
</table>

Plasma concentrations of Ang-(1-7) and Ang II in SHRs, meditated with the combination of losartan and lisinopril for 8 days, 20 minutes after the administration of either vehicle (n=4) or a nephrilysin inhibitor (CGS 24592, n=6)

Data are mean±SEM

*P<0.01

The effectiveness of CGS 24592 as an inhibitor of Ang-(1-7) formation was evaluated by measurements of plasma angiotensin levels in treated SHRs given either the vehicle or the nephrilysin inhibitor. Table 1 shows that CGS 24592 was associated with plasma concentrations of Ang-(1-7) that were 62% below the values determined in vehicle-treated SHRs (P<0.01). Plasma Ang II levels were not different (P<0.05) in SHRs infused with vehicle and CGS 24592 (Table 1).

Figure 2 shows the effect of pretreatment of SHRs with [Sar',Thr']-Ang II before administration of CGS 24592. Infusion of [Sar',Thr']-Ang II elicited a statistically significant increase in mean blood pressure to a peak value of 98±4 mm Hg from pretreatment levels of 83±3 mm Hg (P<0.05), however, heart rate did not change (342±6 bpm and 337±5 bpm before and after [Sar',Thr']-Ang II infusion, respectively).

The subsequent administration of CGS 24592 did not cause an additional elevation in mean arterial pressure but heart rate increased to an average of 366±10 bpm (Fig 2).

A second structurally different nephrilysin inhibitor was used to evaluate the specificity of the hemodynamic response of CGS 24592. The carboxylic ester compound SCH 39370 also displays selective and potent inhibitory properties against nephrilysin (K i=11 nmol/L). As shown in the upper panel of Fig 3, treatment with SCH 39370 increased mean arterial blood pressure from 89±6 mm Hg to 110±3 mm Hg (P<0.01). The increase in mean blood pressure with SCH 39370 was of a magnitude not different than that obtained with CGS 24592. On the other hand, the pressor response produced by SCH 39370 was associated with significant tachycardia averaging 406±13 bpm versus 381±15 bpm (P<0.05).

Infusion of [Sar',Thr']-Ang II before administration of SCH 39370 abolished any additional increase in blood pressure produced by the nephrilysin inhibitor (Fig 4) but did not block the increase in heart rate (445±15 bpm). Thus, administration of the competitive nonselective Ang II receptor blocker obtundd any further elevation in arterial pressure produced by two structurally different nephrilysin inhibitors having equivalent effects on blood pressure but not heart rate.

The potential contribution of endogenous activation of other vasoactive hormones due to inhibition of the catalytic activity of nephrilysin was evaluated by injection of selective receptor antagonists of bradykinin and endothelin-A before the infusion of a nephrilysin inhibitor in chronically treated SHRs. Injection of HOE 140 had no effect on the baseline mean arterial blood pressure (79±3 mm Hg and 80±4 mm Hg before and after HOE 140 injection, respectively) and heart rate.
Figure 4. Effects of the [Sar1-Thr5]-Ang II antagonist (80 nmol/kg/min) on the cardiovascular responses to the neprilysin inhibitor (SCH 39370, n=5). [Sar1-Thr5]-Ang II was infused for a period of 10 minutes. Five minutes after cessation of [Sar1-Thr5]-Ang II infusion, SCH 39370 was infused for a period of 10 minutes and blood pressure was monitored for an additional 20 minutes.

Figure 5. Effects of pretreatment with bradykinin antagonist (HOE 140, 0.1 mg/kg, n=3) and endothelin-A receptor antagonist (BQ123, 1 mg/kg, n=4) on the change in mean arterial pressure (top) and heart rate (bottom) before and after infusion of the neprilysin inhibitor SCH 39370.

Figure 6. Effects of the [Sar1-Thr5]-Ang II antagonist (80 nmol/kg/min) on the cardiovascular responses to the neprilysin inhibitor (SCH 39370, n=5). [Sar1-Thr5]-Ang II was infused for a period of 10 minutes. Five minutes after cessation of [Sar1-Thr5]-Ang II infusion, SCH 39370 was infused for a period of 10 minutes and blood pressure was monitored for an additional 20 minutes.

Discussion

The present study showed that acute blockade of Ang-(1-7) formation with two different neprilysin inhibitors reverses the hypertensive effects of combined losartan/losartan treatment in SHR. The hypertensive actions of the neprilysin inhibitor CGS 24592 were associated with a significant reduction in circulating levels of Ang-(1-7) but no changes in plasma Ang II concentrations. The hypertensive effects of both neprilysin inhibitors were not evident in the presence of Ang II receptor blockade with [Sar1-Thr5]-Ang II. The pressor effects of acute neprilysin inhibition in this model of genetic hypertension extend previous findings showing that endogenous neutralization of Ang-(1-7) with a selective monoclonal antibody reversed the antihypertensive effect of the combined treatment with losartan and losartan. The similarity of the effects obtained by either neutralization of endogenous Ang-(1-7) or inhibition of Ang-(1-7) synthesis in chronically treated SHRs indicates that Ang-(1-7) exerts a depressor function and mediates, in part, the antihypertensive effects associated with combined ACE inhibition and Ang II receptor blockade.

To our knowledge, this is the first study that addresses the acute effects of neprilysin inhibition in lisinopril/losartan-treated animals. This combination therapy normalized the blood pressure of SHRs and markedly elevated plasma levels of Ang-(1-7). High levels of plasma Ang-(1-7) are due to several factors. Inhibition of ACE contributes to the increases in plasma Ang-(1-7) concentration through both increased availability of the Ang I substrate and inhibition of Ang-(1-7) metabolism. An additional, albeit smaller, contribution may originate from the conversion of excess circulating Ang II into Ang-(1-7) as a result of AT1 receptor blockade. The two structurally different specific inhibitors of neprilysin caused a partial reversal of the sustained antihypertensive effect of the combined ACE inhibition and AT1 receptor blockade. The hypertensive response was accompanied by a 62% reduction in the circulating levels of Ang-(1-7), a finding that agrees with the previous demonstration that neprilysin is a major enzymatic pathway for the in vivo generation of Ang-(1-7) in both Wistar Kyoto rats and SHR. The absence of an additional pressor response to the neprilysin inhibitors by
the prior administration of [Sar, Thr]-Ang II suggests that the hypertensive response was related to blockade of an angiotensin receptor that may mediate a vasodepressor effect. Thus, the combination of hormone assay measurements and angiotensin receptor blockade suggest that the reversal of the antihypertensive action of the combined therapy is accounted for by the removal of a vasodepressor action of high circulating levels of Ang-(1-7). The similarity of the effects obtained with the nephrilysin inhibitors and the administration of a specific monoclonal Ang-(1-7) antibody supports this interpretation.

As suggested by us previously, the combination of losartan and losartan augments the vasodilator actions of Ang-(1-7) such that inhibition of peptide synthesis translates into a partial reversal of the antihypertensive effects of the drug regimen. Whether chronic inhibition of nephrilysin will be associated with a sustained inhibition of Ang-(1-7) formation and reduced expression of the antihypertensive effects of the drug regimen was not determined in these experiments. The demonstration that acute nephrilysin inhibition elevates blood pressure does provide a model to investigate the effects of long-term inhibition. However, alternate pathways exist for the generation of Ang-(1-7), which may complicate the results of chronic nephrilysin inhibition. Nevertheless, in the acute setting of these experiments, our data show that the nephrilysin actions of Ang-(1-7) contributed to the maintenance of the antihypertensive action of a chronic therapy resulting from the combination of ACE inhibition and AT1 receptor blockade.

The involvement of other vasoactive autacoids whose metabolism is dependent on the catalytic activity of nephrilysin is unlikely. Blockade of endothelin and kinin receptors did not prevent the hemodynamic response produced by inhibition of nephrilysin. Moreover, the effects of Ang-(1-7) are mediated by an angiotensin receptor. Our data exclude AT1 receptors because SHRs were chronically treated with losartan. It is also unlikely that high Ang-(1-7) concentrations (>200 pmol/L) may stimulate AT1 receptors because the peptide exhibits such a low affinity for the vascular receptor (IC50 >1 μmol/L). Treatment with the AT2 antagonist PD 123319 did not block the pressor actions of [Sar, Thr]-Ang II, whereas we also showed that Ang-(1-7) exhibits very low affinity (IC50 >5 μmol/L) for Ang II binding at the AT1 receptor. Our studies and those of others suggest that Ang-(1-7) interacts with a non-AT1/AT2 receptor that is sensitive to the [Sar, Thr]-Ang II antagonist.

The increases in blood pressure resulting from the two nephrilysin inhibitors were blocked by [Sar, Thr]-Ang II, however, the antagonist did not attenuate the increase in heart rate. Preliminary studies indicated that the β-adrenergic antagonist metoprolol blocked the increase in heart rate but not the pressor response to SCH 39370 (unpublished observations). Additional studies will be required to assess the specificity of the cardiac rate response produced by inhibition of the nephrilysin.

Nephrilysin inhibitors have been used as antihypertensive agents with equivocal results. In addition, mixed ACE and nephrilysin inhibitors are currently being evaluated for antihypertensive properties. Our findings point to the potential pitfalls of this therapeutic approach, especially in conditions in which AT1 receptor function may be impaired or blocked. In view of the wide use of ACE inhibitors and AT1 blockers, the data obtained in these experiments suggest a need for a further understanding of the mechanisms that contribute to the antihypertensive actions of these agents.

In conclusion, the results from the present study reinforce the concept that not only do different hormonal systems contribute to the regulation of blood pressure, but peptide products within a single system such as Ang II and Ang-(1-7) exhibit counter regulatory actions. The vasodepressor actions of Ang-(1-7) in this model of hypertension reveal the complex role that nephrilysin has in the generation and metabolism of vasoactive hormones.

Acknowledgments

Studies were supported by Grants HL-56973 and HL-51952 from the National Heart, Lung, and Blood Institute of the National Institutes of Health.

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Hypertension. 1998;31:356-361
doi: 10.1161/01.HYP.31.1.356

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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