Contribution of Angiotensin-(1–7) to Blood Pressure Regulation in Salt-Depleted Hypertensive Rats

Shridhar N. Iyer, David B. Averill, Mark C. Chappell, Kazuo Yamada, Alicia J. Allred, Carlos M. Ferrario

Abstract—We exposed 63 adult spontaneously hypertensive rats (SHR) and 10 (mRen-2)27 transgenic hypertensive rats to a 12-day regimen of either a normal diet (0.5%) or a low-salt diet (0.05%) to evaluate the hypothesis that the vasodepressor heptapeptide, angiotensin-(1–7) [Ang-(1–7)], buffers the pressor effects of angiotensin II during endogenous stimulation of the renin-angiotensin system. Catheters were inserted into a carotid artery and jugular vein under light anesthesia the day before the experiment. Separate groups of conscious instrumented SHR were given short-term infusions of an affinity-purified monoclonal Ang-(1–7) antibody or the neprilysin inhibitor SCH 39370. In addition, SHR and (mRen-2)27 rats were given the Ang-(1–7) receptor antagonist [D-Ala7]Ang-(1–7). Exposure to the low-salt diet increased plasma renin activity and elevated plasma levels of angiotensin I and angiotensin II in SHR by 81% and 68%, respectively, above values determined in SHR fed a normal salt diet. Concentrations of angiotensin I and angiotensin II were also higher in the kidney of salt-depleted SHR, whereas plasma and renal tissue levels of Ang-(1–7) were unchanged. Infusion of the Ang-(1–7) antibody produced dose-dependent pressor and tachycardic responses in salt-depleted SHR but no effect in SHR maintained on a normal-salt diet. A comparable cardiovascular response was produced in salt-depleted SHR given either SCH 39370 or [D-Ala7]Ang-(1–7). These agents had negligible effects on SHR fed a normal-salt diet. Blockade of Ang-(1–7) receptors produced a similar cardiovascular response in (mRen-2)27 transgenic hypertensive rats fed a low-salt diet. Injections of the heat-inactivated antibody or the subsequent infusion of the antibody to rats given [D-Ala7]Ang-(1–7) produced no additional effects. The data support the hypothesis that the hemodynamic effects of neurohormonal activation after salt restriction stimulate a tonic depressor action of Ang-(1–7). (Hypertension. 2000;36:417-422.)

Key Words: angiotensin II ■ blood pressure ■ hypertension, experimental ■ receptors, angiotensin ■ renin

In pursuing the characteristics of the vascular actions of angiotensin-(1–7) [Ang-(1–7)], we evaluated whether it may play a role in conditions in which the endogenous renin-angiotensin system is activated by reduced salt intake. As reviewed by Muntzel and Druke,1 the relationship between dietary salt intake and blood pressure remains imperfectly defined. Although salt depletion is a potent stimulus for the secretion of renin and the generation of angiotensin II (Ang II), the mechanism contributing to the failure of hyperreninemia to elevate blood pressure has not been convincingly explained. Sodium depletion achieved by diet or diuretics is often used as an adjunct to therapy with angiotensin-converting enzyme (ACE) inhibitors and Ang II receptor antagonists. Whereas these forms of antihypertensive therapy activate the renin-angiotensin system, dietary salt restriction or sodium depletion enhances the capacity of these medications to lower blood pressure. The interactions between salt restriction and blood pressure regulation are complex and engage multiple homeostatic mechanisms. Because Ang-(1–7) has vasodepressive2 and antiproliferative3 actions, we investigated in the present study the role of the heptapeptide in the condition of salt restriction by determining for the first time the effect of neutralizing the activity of Ang-(1–7) on the blood pressure of adult spontaneously hypertensive rats (SHR) after a 12-day exposure to dietary salt restriction with the use of agents that selectively interfere with either the activity or the production of the heptapeptide. The studies were complemented by an evaluation of the effect of blockade of Ang-(1–7) receptors in a transgenic model of high blood pressure. We used a 3-pronged pharmacological approach geared to (1) neutralize the activity of circulating Ang-(1–7) with a selective Ang-(1–7) monoclonal antibody [Ang-(1–7)-mAb], (2) inhibit the activity of neprilysin, an Ang-(1–7)-forming enzyme, and (3) block Ang-(1–7) receptors with a novel and selective Ang-(1–7) receptor antagonist.

Methods

Studies were performed in 63 ten-week-old male SHR (Charles River Laboratories Inc, Wilmington, Mass) and 10 (mRen-2)27 transgenic hypertensive rats housed in a room maintained at 22°C
with a 12-hour light/dark cycle. Rats were housed individually in metabolic cages (Nalgene, Fisher Scientific) and consumed a diet containing either 0.50% (n = 30) or 0.05% (n = 33) sodium chloride (Teklad) for 12 days. Baseline systolic blood pressure was recorded by tail-cuff plethysmography (Narco Biosystems).

On day 11 of the diet period, plastic catheters (PE-50, Clay Adams, Becton Dickinson) were implanted by using aseptic conditions and inhalation anesthesia (1% halothane, Ayerst Laboratories Inc). Prior to a carotid artery and jugular vein of 35 of the 63 SHR and 10 of the (mRen-2)27 transgenic hypertensive rats. The free ends of both catheters were tunneled dorsally and exteriorized between the shoulder blades. The other 28 SHR (n = 14 for each group of rats fed a normal- or low-salt diet) were decapitated, and trunk blood was obtained for measurements of plasma concentrations of angiotensin peptides. Kidneys were collected for measurements of tissue angiotensin levels of SHR assigned to either a normal- (n = 8) or a low-salt diet (n = 8). All experiments were performed as approved by the Animal Care and Use Committee of the Wake Forest University School of Medicine.

Animal Protocols

On the day of the experiment, blood pressure was recorded continuously with a solid-state strain-gauge transducer (Uniflow Pressure Transducer, Baxter Healthcare Corp) connected to the arterial catheter of conscious rats. The signal from the transducer amplifier was directed to an analog-to-digital converter for beat-by-beat analysis of arterial pressure and heart rate. Calibrated displays of systolic, diastolic, and mean arterial pressure and heart rate were imaged in a laser printer.

After an initial 30-minute stabilization period, arterial pressure and heart rate were recorded for 1 hour; then a specific Ang-(1–7)-mAb was delivered for 5 minutes at a rate of 50 µL·min⁻¹. Subsequent doses of the antibody were spaced at least 20 minutes apart. Peak changes in mean blood pressure and heart rate were recorded at the end of each infusion period. Control infusions of the heat-inactivated Ang-(1–7)-mAb (300 µg·min⁻¹) were performed in 3 of the rats fed a low-salt diet before the injection of the intact monoclonal antibody. In a second group of SHR fed either a normal- (n = 5) or low-salt (n = 5) diet, the specificity of the response produced by the enzyme activity of neprilysin, SCHR 39370, was inferred intravenously at doses between 0.5 and 30 mg·kg⁻¹.

The selective Ang-(1–7) antagonist, [D-α-Ala²]Ang-(1–7) (Bachem), was infused intravenously into a third group of SHR fed either a normal- (n = 6) or low-salt (n = 6) diet at doses ranging from 0.001 to 10 pmol·min⁻¹. Pilot experiments in 2 salt-depleted Sprague-Dawley (SD) rats showed that [D-α-Ala²]Ang-(1–7) produced a mean arterial pressure and heart rate at doses of 17 ± 9 mm Hg at doses of 0.5 and 10 pmol·min⁻¹. The specificity of the response produced by [D-α-Ala²]Ang-(1–7) was further evaluated by administration of the Ang-(1–7)-mAb at the highest dose tested (300 µg·min⁻¹) within 2 minutes after completion of the infusion of the antagonist given at its highest dose in 3 of 6 SHR fed a low-salt diet.

To verify whether the effects of endogenous neutralization of Ang-(1–7) may occur in a model of hypertension other than SHR, the Ang-(1–7) antagonist was given in the same manner and conditions to (mRen-2)27 transgenic hypertensive rats maintained with either a normal- (n = 5) or low-salt (n = 5) diet.

Plasma Renin Activity and Angiotensin Peptides

The activity of renin in plasma was measured by radioimmunoassay in all SHR exposed to the pharmacological protocol described above. The concentrations of angiotensins in plasma were determined in separate groups of SHR exposed to the same protocols of low-salt intake (n = 14) and normal-salt intake (n = 14) to avoid hemodynamic artifacts resulting from either the removal of blood (≥3 mL) in conscious rats, inhibition of Ang-(1–7) metabolism, or synthesis by the agents used in the physiological experiments. Rats were decapitated, and trunk blood was collected for measurements of plasma angiotensin peptides. In 8 of the SHR exposed to either a normal- or a low-salt diet, the kidneys were also rapidly removed for measurements of tissue angiotensin peptides.

Plasma and tissue concentrations of the angiotensin peptides were determined by radioimmunoassay, as described elsewhere. Trunk blood was collected into chilled Vacutainer blood collection tubes containing a mixture of peptidase inhibitors (EDTA, o-phenanthroline, p-chloromercuribenzoic acid, pepstatin A, and the rat renin inhibitor acetyl His-Pro-Phc-Val-Statine-Leu-Phe). After 20 minutes on ice, blood samples were centrifuged at 3000 rpm for 20 minutes, and aliquots of plasma were stored at −80°C until assayed for angiotensin peptides. To prevent activation of angiotensin-degrading enzymes, kidneys were quickly removed and immediately homogenized on ice in an acid ethanol (80% [vol/vol] 0.1N HCl) solution containing the following peptidase inhibitors: a renin inhibitor, EDTA, p-chloromercuribenzoic acid, o-phenanthroline, pepstatin A, and phenylmethylsulfonyl fluoride. Protein content of the homogenate was determined with a Bio-Rad Protein Reagent kit (Bio-Rad Laboratories).

Materials

Ang-(1–7)-mAb was produced in mouse ascites and purified by protein A affinity chromatography (Pharmacia Biotech), as described elsewhere, except that the antibody was dialyzed and concentrated in 10 mmol/L HEPES, pH 7.4, in 120 mmol/L sodium chloride. Preparation of the Ang-(1–7)-mAb has been described and extensively validated in detail elsewhere. [D-α-Ala²]Ang-(1–7) was obtained from Bachem; SCHR 39370 was a gift of E.J. Sybertz (Schering-Plough Research, Kenilworth, NJ). During the experiments, SCHR 39370 was initially dissolved in NaOH and diluted in the HEPES buffer to a pH of 7.4.

Statistical Analysis

All data are expressed as mean±SEM. The changes in arterial pressure and heart rate produced by the various maneuvers were analyzed by repeated-measures ANOVA, followed by the Scheffé post hoc test. A value of P≤0.05 was considered statistically significant.

Results

Direct measurements of arterial pressure confirmed that SHR remained hypertensive when placed on either a normal- or low-sodium diet (Table). Exposure to a low-salt diet caused an almost 3-fold elevation in plasma renin activity but no changes in arterial pressure or heart rate (Table). The increases in plasma renin activity were associated with significant increases in the levels of Ang I and Ang II in plasma (n = 14) and renal tissue (n = 8) but with no changes in the plasma and renal tissue concentrations of Ang-(1–7) (Figure 1).

Baseline mean arterial pressure and heart rate averaged 174±4 mm Hg and 392±17 bpm, respectively, in (mRen-2)27 transgenic hypertensive rats maintained on a normal-salt diet. At the completion of a 12-day low-salt diet, mean arterial pressure and heart rate were 161±3 mm Hg and 400±19 bpm, respectively.

### Baseline Hemodynamic and Plasma Renin Activity in Awake SHR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Salt</th>
<th>Low Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>200±6</td>
<td>212±9</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>138±4</td>
<td>147±6</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>167±4</td>
<td>174±7</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>371±7</td>
<td>376±15</td>
</tr>
<tr>
<td>Plasma renin activity, nmol</td>
<td>3.1±0.53</td>
<td>8.02±1.67*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of baseline hemodynamic values. *P<0.02 compared with rats fed a normal-salt diet.
Neutralization of endogenous Ang-(1–7) caused significant dose-dependent increases in the mean arterial pressure and heart rate of low-salt SHR (Figure 2). The peak rise in mean arterial pressure produced by the Ang-(1–7)-mAb averaged $20 \pm 3$ mm Hg above baseline values at doses between 200 and $300 \mu g \cdot min^{-1}$. In contrast, infusion of the same doses of the Ang-(1–7)-mAb had no effect on either the arterial pressure or the heart rate of SHR maintained on a normal-salt diet. Control infusions of the heat-inactivated antibody (300 $\mu g \cdot min^{-1}$) had no effect on the blood pressure or the heart rate of SHR fed a low-salt diet ($152 \pm 2$ mm Hg and $377 \pm 25$ bpm, respectively, before infusion versus $153 \pm 1$ mm Hg and $380 \pm 24$ bpm, respectively, after infusion; $P>0.05$).

Figure 2 also compares the effect of the neprilysin inhibitor, SCH 39370, on arterial pressure and heart rate of SHR fed normal- and low-salt diets. SCH 39370 produced a dose-dependent rise in the mean arterial pressure of SHR fed a low-salt diet that was of a magnitude not different from the rise obtained by the infusion of the Ang-(1–7)-mAb. Although tachycardia accompanied the SCH 39370–induced elevations in the arterial pressure of SHR fed a low-salt diet (Figure 2), these increases in heart rate did not differ from those measured in SHR fed a normal-salt diet receiving equivalent doses of the neprilysin inhibitor.

To further delineate the mechanism accounting for the pressor effects of endogenous neutralization of Ang-(1–7), the selective Ang-(1–7) antagonist, [D-Ala$^7$]Ang-(1–7), was infused at rates ranging from 0.001 to $10.0 \text{ pmol} \cdot \text{min}^{-1}$ into SHR and (mRen-2)27 rats placed on either a normal- or low-salt diet. [D-Ala$^7$]Ang-(1–7) at doses of 0.1 and $10.0 \text{ pmol}$ was shown in pilot experiments in SD rats fed a low-salt diet to produce a pressor response comparable to that obtained by infusion of the monoclonal Ang-(1–7) antibody and to abolish the vasodepressor response to intravenous infusion of the neprilysin inhibitor.
sions of 100 nmol of Ang-(1–7) in SD rats maintained on a normal-salt diet.

Infusion of [D-Ala\textsuperscript{7}]Ang-(1–7) caused a dose-dependent rise in mean blood pressure only in SHR and (mRen-2)\textsuperscript{27} hypertensive rats fed a low-salt diet (Figure 3). The pressor response was associated with tachycardia. The peak increases in mean arterial pressure produced by the infusion of [D-Ala\textsuperscript{7}]Ang-(1–7) in SHR were comparable to those observed after the highest doses of either Ang-(1–7)-mAb or SCH 39370. Furthermore, the administration of Ang-(1–7)-mAb (300 µg · min\textsuperscript{-1}) at the peak of the pressor response induced by 10 pmol · min\textsuperscript{-1} of [D-Ala\textsuperscript{7}]Ang-(1–7) had no further effect on the mean arterial pressure or heart rate of SHR and (mRen-2)\textsuperscript{27} transgenic hypertensive rats fed a low-salt diet. These data confirmed that the hemodynamic response produced by [D-Ala\textsuperscript{7}]Ang-(1–7) was attributable to blockade of the Ang-(1–7) receptors. In contrast, administration of the same doses of [D-Ala\textsuperscript{7}]Ang-(1–7) had no effect on the arterial pressure and heart rate of either SHR or (mRen-2)\textsuperscript{27} transgenic hypertensive rats maintained on a normal-salt diet.

Discussion

The present study evaluated the hypothesis that Ang-(1–7) may have a vasodepressor activity after salt depletion. To the best of our knowledge, this is the first study documenting a significant vasodepressor contribution of Ang-(1–7) to the regulation of arterial pressure and heart rate when the renin-angiotensin system has been stimulated by a low-salt diet. This interpretation is based on the results of 3 different maneuvers, which included neutralization of endogenous Ang-(1–7), inhibition of Ang-(1–7) formation, and Ang-(1–7) receptor blockade. The demonstration that infusion of the Ang-(1–7)-mAb had no subsequent pressor effect in low-salt–fed SHR given [D-Ala\textsuperscript{7}]Ang-(1–7) beforehand verified the specificity of the response mediated by the active antibody. Thus, 3 independent ways of preventing the actions of endogenous Ang-(1–7) showed that this vasodepressor peptide contributes to the hemodynamic adjustments associated with salt restriction in 2 different models of experimental hypertension. Ang-(1–7) may counterbalance not only the vasoconstrictor actions of Ang II but also the central neurogenic actions of Ang II, as evidenced by the concomitant increases in heart rate. The additional demonstration that Ang-(1–7) receptor blockade in (mRen-2)\textsuperscript{27} transgenic hypertensive rats was both qualitatively and quantitatively similar to that obtained in SHR fed a low-salt diet suggests that the response is not a trait unique to SHR.

Our findings agree with the report that Ang-(1–7) may contribute to the mechanisms by which reduced dietary salt intake potentiates the antihypertensive actions of ACE inhibitors and Ang II type 1 (AT\textsubscript{1}) receptor blockers.\textsuperscript{5,7} Past experiments showed that chronic inhibition of ACE augments plasma Ang-(1–7) levels in animal models of hypertension\textsuperscript{8,9} and in treated hypertensive subjects.\textsuperscript{10} Blockade of either Ang-(1–7) activity\textsuperscript{6} or synthesis\textsuperscript{7} reverses the antihypertensive effect of the combined treatment with lisinopril and losartan in SHR. Taken together, these findings favor the hypothesis that Ang-(1–7) is a part of a vasodepressor system opposing the hemodynamic effects of neurohormonal activation after salt restriction.\textsuperscript{11}

Among the various mechanisms that are activated in response to a change in sodium homeostasis, salt restriction causes transient contraction in plasma and, possibly, extracellular fluid volume,\textsuperscript{12} activation of the Ang II–aldosterone axis,\textsuperscript{13} a rise in vasopressin,\textsuperscript{14} and reflex compensation through neurogenic mechanisms.\textsuperscript{15} The long-term interactions of homeostatic mechanisms called into play in response to salt restriction remain imperfectly defined. Muntzel and Drueke\textsuperscript{1} emphasized that reduced sodium intake causes a heterogeneous response in blood pressure, with some individuals responding to sodium restriction with a fall in arterial pressure, others responding with no change, and others responding with a rise. In hyperreninemic essential and renovascular hypertensive subjects, salt restriction exacerbates hypertension,\textsuperscript{16} whereas in normotensive rats, blood pressure may be elevated by salt restriction.\textsuperscript{17} Nilsson et al\textsuperscript{18} and Gradin et al\textsuperscript{19} reported that long-term feeding of a low-salt diet lowers systolic blood pressure when given to SHR aged 5 to 6 weeks, whereas blood pressure is not modified in SHR with a more established form of hypertension.\textsuperscript{20} In keeping with these findings, a 12-day restriction in salt intake did not decrease the baseline blood pressure of SHR, even though plasma renin
activity and Ang II concentrations were higher than those determined in SHR consuming a normal-salt diet.

A similar hemodynamic response to the low-salt diet stimulus was observed in (mRen-2)27 transgenic hypertensive rats, inasmuch as their baseline blood pressures and heart rates were not affected by a 12-day low salt diet regimen.

An important issue is identifying the factors and systems that are activated by salt restriction and at the same time maintaining the prevailing level of blood pressure. In the situation of salt restriction and activation of the renin-angiotensin system, kinins, prostaglandins, and NO21 may have vascular actions that oppose the ability of Ang II to stimulate further vasoconstriction, in part via increases in sympathetic nerve activity. Downregulation of AT1 receptors may also account for the obtunding effect of increased Ang II activity on the blood pressure of salt-restricted animals.32 Although these may be some of the homeostatic factors operative in salt restriction, our data now show that Ang-(1–7) also contributes to buffer the hemodynamic effects associated with activation of the renin-angiotensin system by salt restriction.

The demonstration that plasma and renal tissue concentrations of Ang-(1–7) were not augmented in SHR fed a low-salt diet suggests that enhanced peptide formation may not account for the pressor effects produced by the inhibition of Ang-(1–7) activity. These novel findings imply that vascular sensitivity to Ang-(1–7) may be increased in the salt-restricted state so that the same concentrations exert a greater vasodepressor effect. Mechanisms of increased vascular sensitivity may relate to either upregulation of Ang-(1–7) receptors, amplification of Ang-(1–7) receptor–mediated vasodepressor signaling events, or both. Ang-(1–7) stimulates the release of vasodilator prostacyclin,23 augments the response to the vasodilator actions of kinin,24 and stimulates endothelium-dependent NO release.25 Salt restriction increases the expression of cyclooxygenase-2 mRNA in the kidney, a finding that would imply that increased vascular sensitivity to Ang-(1–7) in part results from a direct effect of the peptide on the release of vasodilator prostaglandins.26 Additional studies will be required, however, to ascertain the signaling mechanisms(s) that is affected by blockade of Ang-(1–7) activity in genetic models of hypertension.

This interpretation does not exclude the possibility that rapid metabolism of endogenous Ang-(1–7) may prevent detection of changes in peptide concentration either in plasma or tissue. Ang-(1–7) half-life in the circulation of SHR is ∼10 seconds, a value that is one fifth of that found for Ang II.27 The rapid destruction of the Ang-(1–7) peptide is due to its metabolism by ACE.28 Therefore, further studies are needed to assess the balance of Ang-(1–7) formation and degradation as important factors contributing to the effects of Ang-(1–7) in the setting of dietary salt restriction.

The 3-pronged pharmacological strategy used in these experiments suggests a role of Ang-(1–7) in the regulation of blood pressure in low salt–fed SHR and (mRen-2)27 transgenic hypertensive rats. The specificity of the monoclonal Ang-(1–7) antibody in neutralizing the actions of endogenous Ang-(1–7) has been reported in detail elsewhere.7 This antibody has a high affinity for Ang-(1–7) and no significant cross-reactivity with other angiotensin fragments (including Ang II), vasopressin, bradykinin, or substance P.7 Although neprilysin contributes to the metabolism of kinins, endothelin, and natriuretic peptides, the pressor effects obtained with inhibition were reported in previous experiments to (1) cause a 60% reduction in the circulating levels of Ang-(1–7) in SHR chronically treated with lisinopril and losartan7 and (2) not be modified by the preadministration of a kinin receptor antagonist or selective type A endothelin receptor antagonist.7

The mimicry of the response obtained by the administration of [D-Ala7]Ang-(1–7) provides further evidence for the participation of Ang-(1–7) because this receptor antagonist has no pharmacological selectivity for kinin, endothelin, and atrial natriuretic peptide receptors.4,29 Moreover, [D-Ala7]Ang-(1–7) has no intrinsic agonistic properties in salt-replete SHR30 and does not compete with the binding of [125I]-Ang II at AT1 or AT2 receptor subtypes.31 In bovine aortic endothelial cells, [D-Ala7]Ang-(1–7) blocked Ang-(1–7) binding, whereas AT1 and AT2 receptor blockers had no effect.31 In keeping with this interpretation, we now show that the subsequent infusion of the Ang-(1–7)-mAb had no additional effect on blood pressure in rats given [D-Ala7]Ang-(1–7). These findings are consistent with our previous demonstration that the actions of Ang-(1–7) are mediated by a unique non-AT1/AT2 receptor subtype.6

The tachycardic component of the pressor effects produced during inhibition of Ang-(1–7) synthesis or activity in SHR fed a low-salt diet suggests that the hemodynamic response had a neurogenic component. The coincident rise of blood pressure and heart rate might indicate that the baroreceptor reflex control of heart rate and sympathetic nerve activity was overridden because of Ang-(1–7) blockade. A more likely scenario would include a change in the balance of factors influencing the baroreceptor reflex. Ang-(1–7) acts in the nucleus tractus solitarii32 to augment the sensitivity of the baroreceptor reflex, whereas Ang II has the opposite effect.33 Xu et al34 found that Ang II has a pressure-independent effect to reset the baroreceptor reflex control of heart rate and sympathetic nerve activity to higher blood pressures in salt-depleted animals. Blockade of Ang-(1–7) may unmask the ability of Ang II to reduce baroreceptor reflex sensitivity and shift the baroreceptor reflex to higher blood pressures. Thus, the development of tachycardia during blockade of Ang-(1–7) in both low-salt SHR and (mRen-2)27 transgenic hypertensive rats is the first demonstration that Ang-(1–7) may have an important role in the regulation of sympathetic nerve activity in the situation of salt restriction.

The hemodynamic effects of endogenous neutralization of Ang-(1–7) were similar in (mRen-2)27 transgenic rats, which have an activated renin-angiotensin system. The absence of a hemodynamic response to neutralization of Ang-(1–7) in heterozygous (mRen+2)27 transgenic hypertensive rats maintained on a normal-salt diet is in keeping with the demonstration of increased plasma Ang II but not Ang-(1–7) levels in these animals.35 The report of Campbell et al56 of increased plasma Ang II and Ang-(1–7) in (mRen-2)27 transgenic rats was derived from observations of homozygous hypertensive animals in which lifetime therapy with lisinopril had been discontinued only 2 weeks before the experiment. The observation of increased plasma Ang-(1–7) levels in homozygous (mRen-2)27 animals may reflect a residual inhibition of
Ang-(1–7) metabolism, given the short time period of discontinuation of ACE therapy. Despite this caveat, both studies showed higher Ang II/Ang-(1–7) ratios in (mRen-2)27 hypertensive rats compared with SD rats, a finding that confirmed the existence of higher levels of Ang II for any corresponding level of Ang-(1–7). Therefore, in (mRen-2)27 transgenic hypertensive rats, the negative effects of blockade of Ang-(1–7) synthesis or activity confirm a diminished contribution of the heptapeptide in balancing the vasodepressor actions of Ang II in this model of high blood pressure.

In summary, inhibition of Ang-(1–7) functional pathways unmasked the depressor effects of the peptide after salt restriction in SHR and in (mRen-2)27 transgenic hypertensive rats. These findings provide a new explanation for the imperfectly defined relationship among blood pressure, the activation of the renin-angiotensin system, and salt restriction. The similarity of the responses obtained by the 3 different pharmacological approaches suggests that Ang-(1–7) contributes to the hemodynamic adjustment associated with the stimulus of a low-salt diet and that in SHR and in (mRen-2)27 hypertensive rats, the vasodepressor actions of Ang-(1–7) are mediated via a receptor that is [D-Ala7]Ang-(1–7) sensitive.

Acknowledgments

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References


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