Angiotensin receptors contribute to blood pressure homeostasis in salt-depleted SHR

SHIGEFUMI NAKAMURA, DAVID B. AVERILL, MARK C. CHAPPELL, DEBRA I. DIZ, K. BRIDGET BROSNIHAN, AND CARLOS M. FERRARIO

Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

Submitted 10 April 2002; accepted in final form 20 September 2002

Angiotensin receptors contribute to blood pressure homeostasis in salt-depleted SHR. Am J Physiol Regul Integr Comp Physiol 284: R164–R173, 2003; 10.1152/ajpregu.00210.2002.—This study evaluated the contribution of angiotensin peptides acting at various receptor subtypes to the arterial pressure and heart rate of adult 9-wk-old male conscious salt-depleted spontaneously hypertensive rats (SHR). Plasma ANG II and ANG I in salt-depleted SHR were elevated sevenfold compared with peptide levels measured in sodium-replete SHR, whereas plasma ANG-(1–7) was two-fold greater in salt-depleted SHR compared with salt-replete SHR. Losartan (32.5 μmol/kg), PD-123319 (0.12 μmol·kg⁻¹·min⁻¹), [d-Ala⁷]ANG-(1–7) (10 and 100 pmol/min), and a polyclonal ANG II antibody (0.08 mg/min) were infused intravenously alone or in combination. Combined blockade of AT₂ and AT(1–7) receptors significantly increased the blood pressure of losartan-treated SHR (+15 ± 1 mmHg; P < 0.01); this change did not differ from the blood pressure elevation produced by the sole blockade of AT₁, receptors (15 ± 4 mmHg). On the other hand, sole blockade of AT₂ receptors in losartan-treated SHR increased mean arterial pressure by 8 ± 1 mmHg (P < 0.05 vs. 5% dextrose in water as vehicle), and this increase was less than the pressor response produced by blockade of AT₁, receptors alone or combined blockade of AT₁, and AT₂ receptors. The ANG II antibody increased blood pressure to the greatest extent in salt-depleted SHR treated with only losartan (+11 ± 2 mmHg) and to the least extent in salt-depleted SHR previously treated with the combination of losartan, PD-123319, and [d-Ala⁷]ANG-(1–7) (+7 ± 1 mmHg; P < 0.01). Losartan significantly increased heart rate, whereas other combinations of receptor antagonists or the ANG II antibody did not alter heart rate. Our results demonstrate that ANG II and ANG-(1–7) act through non-AT₁ receptors to oppose the vasoconstrictor actions of ANG II in salt-depleted SHR. Combined blockade of AT₂ and AT(1–7) receptors and ANG II neutralization by the ANG II antibody reversed as much as 67% of the blood-pressure-lowering effect of losartan.

Address for reprint requests and other correspondence: D. B. Averill, Hypertension and Vascular Disease Center, Wake Forest Univ. School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157 (E-mail: daverill@wfubmc.edu).
measurements were made in an additional six SHR fed a normal salt diet (0.4% NaCl; Teklad TD99215) before and during the 2 days after instrumentation. Rats were housed individually in metabolic cages for 2 days before and 2 days after implantation of catheters to assess the effect of diuretic treatment. All procedures were performed in compliance with the policies implemented by the Animal Care and Use Committee of the Wake Forest University School of Medicine and in accordance with the "Guiding Principles for Research Involving Animals and Human Beings" as set forth by the American Physiological Society.

Animal Preparation

Two days before the experiments, rats in the salt-depletion group were anesthetized with halothane (1%; Ayerst Laboratories, Philadelphia, PA) in a 65%-35% mixture of room air and oxygen, respectively. Aseptic surgical procedures were used to implant plastic catheters (PE-50; Clay Adams, Becton Dickinson, Sparks, NJ) in a carotid artery and both jugular veins for subsequent recording of arterial pressure and infusion of drugs, respectively. The free ends of the catheters were exteriorized at the nape of the neck and occluded. Rats were treated postoperatively with penicillin G (30,000 U sc). After convalescence, rats were brought into a sound-attenuated room, and the arterial catheter was connected to a strain-gauge transducer (Uniflow Pressure Transducer, Baxter Healthcare, Irvine, CA) for the measurement of arterial blood pressure. The signal from the strain-gauge transducer was directed to an analog-to-digital converter (DT 2831, Data Translation, Marlboro, MA) and digitized at 1 kHz for beat-to-beat analysis of arterial pressure and heart rate as described in detail elsewhere (4). Venous lines were connected to infusion pumps (Pump 11, Harvard Apparatus, South Natick, MA). Animals were permitted a minimum of 60 min to adjust to the laboratory setting before experimental data were collected and stored for later offline analysis. In all experiments, 30 min of hemodynamic data were obtained as a baseline control before commencing drug treatments.

Experimental Plan

The pharmacological strategy consisted of determining the hemodynamic effects of either sequential or combined administrations of selective AT1 (losartan), AT2 (PD-123319), or AT1–7, ([D-Ala7]ANG-(1–7)) receptor antagonists and a purified high-affinity ANG II polyclonal antibody in experiments described below. The sequence of drug administration in these experiments is illustrated in Fig. 1. Control infusions of vehicle (15-min infusions of 5% dextrose in water at a rate of 0.2 ml/min) were done in sodium-depleted SHR pretreated with losartan (32.5 μmol/kg iv).

Hemodynamic effects of [D-Ala7]ANG-(1–7). The selective AT1–7 receptor antagonist [D-Ala7]ANG-(1–7) (34) was infused intravenously in conscious salt-depleted rats at a dose of either 10 (n = 8) or 100 pmol/min (n = 6) at a rate of 0.1 ml/min for up to 15 min. In the majority of salt-depleted SHR, [D-Ala7]ANG-(1–7) was administered 30 min after an intravenous injection of losartan at a dose of 32.5 μmol/kg (15 mg/kg). Arterial pressure and heart rate were monitored continuously in conscious SHR throughout the periods of drug administration and for an additional 25 min after cessation of the antagonist infusion.

Hemodynamic effects of PD-123319, alone or combined with [D-Ala7]ANG-(1–7). An additional 19 salt-depleted SHR, given an intravenous injection of losartan (32.5 μmol/kg) 30 min beforehand, received the selective AT2 receptor antagonist PD-123319 at a dose of 0.12 μmol·kg⁻¹·min⁻¹ (100 μg·kg⁻¹·min⁻¹) for 15 min either alone or in combination with [D-Ala7]ANG-(1–7) (10 pmol/min). The rate of intravenous infusion for each drug was 0.1 ml/min.

The dose of the AT2 receptor antagonist was derived from pilot experiments that assessed the plasma concentration of the AT2 receptor blocker PD-123319. In these experiments PD-123319 was infused intravenously at a rate of 0.12 μmol·kg⁻¹·min⁻¹ for 15 min. A 2-ml sample of arterial blood obtained at the end of the infusion period was processed for the determination of the plasma concentrations of the AT2 receptors.

Fig. 1. Schematic of the time course of pharmacological interventions employed in salt-depleted and salt-replete spontaneously hypertensive rats (SHR). All experimental groups had a minimum of 30 min of baseline (Control) recording of arterial blood pressure and heart rate. Losartan (Los) was injected over a period of 3–5 min. After losartan injection, rats were monitored for an additional 30 min before infusion of [D-Ala7]ANG-(1–7) (D-ALA), PD-123319 (PD), an ANG II antibody (ANG II Ab), denatured ANG II Ab (Denat Ab), or combinations of these agents. In addition, 5% dextrose in water (Veh DSW) was infused in salt-depleted, losartan-treated rats to assess whether infusion of 3 ml of fluid over a 15-min period altered blood pressure and heart rate. In some experimental groups, blood pressure and heart rate were monitored after termination of drug infusion (Postinfusion).
Angiotensin receptor antagonist using a radioreceptor assay developed in our laboratory and described in detail below.

Hemodynamic effect of the ANG II polyclonal antibody. A group of salt-depleted SHR (n = 8) was initially given losartan (32.5 μmol/kg iv), and 30 min later an ANG II polyclonal antibody was infused at a dose of 0.08 mg/min. A second group of salt-depleted SHR (n = 5) was pretreated with losartan as described above. Thirty minutes after administration of losartan, an intravenous infusion of [d-Ala7]ANG-(1–7) (10 pmol/min) was begun. At 10 min into the infusion period, the ANG II polyclonal antibody was infused via a second catheter at a dose of 0.08 mg/min while maintaining the infusion of [d-Ala7]ANG-(1–7) for an additional 10 min. A third group of salt-depleted losartan-treated SHR (n = 5) received a combined infusion of [d-Ala7]ANG-(1–7) (10 pmol/min) and PD-123319 (0.12 μmol·kg⁻¹·min⁻¹). Ten minutes into the combined infusion of the AT2 and AT(1–7) receptor antagonists, the ANG II antibody was infused via a second catheter at 0.08 mg/min. The effect of the ANG II polyclonal antibody on blood pressure of salt-replete SHR was also investigated. The ANG II polyclonal antibody was infused at 0.08 mg/min for 20 min, and aliquots of plasma were stored at −80°C until assayed for angiotensin peptides. Plasma was extracted on a Sep-Pak C18 column according to our previously published protocol (35). The sample was eluted, reconstituted, and split for the RIA of ANG I, ANG II, and ANG-(1–7). Samples were reconstituted in assay buffer (ANG II) or in Tris buffer with 0.1% BSA for ANG I and ANG-(1–7).

The recovery of radiolabeled ANG II added to the sample and followed through the extraction was 92% (n = 23). Samples were corrected for recovery. ANG I was measured using a modification of a commercially available New England Nuclear RIA kit (RIANEN, Dubuque, Billerica, MA). ANG II was measured using a Nichols Institute RIA (San Juan Capistrano, CA), and ANG-(1–7) was measured as described previously (35). The minimum detectable levels (MDLs) of the assays were 1.39 fmol/tube for ANG-(1–8), 3.81 fmol/tube for ANG II, and 1.93 fmol/tube for ANG I. Values at or below the MDL of the assay were assigned the value of the MDL for the respective peptide for statistical analysis. The intra-assay coefficient of variation was 18% for ANG I, 12% for ANG II, and 8% for ANG-(1–7).

To determine plasma ANG II concentrations in rats receiving the ANG II antibody, the plasma from arterial blood was initially acidified with 4% acetic acid (1:1 vol/vol) to promote dissociation of the antibody-peptide complex before extraction on a Sep-Pak C18 column. ANG II immunoreactivity was assessed by the RIA described above. HPLC identification of ANG II-derived fragments (ANG-(2–8), ANG-(3–8), and ANG-(4–8)) was performed on pooled samples injected onto a narrow-bore Nov-Pak C18 column (Waters, New Bedford, MA) under an isocratic condition of 27% mobile phase B (80% acetonitrile/0.1% heptafluorobutyric acid) at a flow rate of 0.35 ml/min. Fractions were collected at 1-min intervals and evaporated in a vacuum centrifuge, and RIAs were performed.

Materials. The polyclonal ANG II antibody utilized in these experiments was produced in our laboratory as described above. Losartan was kindly provided by Dr. R. Smith Parke-Davis (Ann Arbor, MI). [d-Ala7]ANG-(1–7) was purchased from Bachem (Torrance, CA).

Statistical Analysis

The effects of drug treatment within experimental groups were analyzed by two-way ANOVA with drug as one main effect and time as a repeated measure as the second main effect.
The hypotensive effect of losartan was accompanied by one-way ANOVA. Post hoc comparisons between levels of main effects were evaluated using a Fisher's predicted least significant difference test. Statistical analyses were performed using StatView software (Abacus Concepts, Berkeley, CA). Data are expressed as means ± SE, P values of <0.05 were considered statistically significant.

RESULTS

Mean arterial pressure and heart rate averaged 136 ± 2 mmHg and 425 ± 5 beats/min, respectively, in SHR subjected to sodium depletion over a 2-day period. The average baseline value for mean arterial pressure was significantly lower in salt-replete SHR than in salt-replete SHR (160 ± 4 mmHg; n = 6, P < 0.001), but the heart rate was no different in salt-replete SHR (437 ± 6 beats/min, n = 6). Short-term salt depletion did not significantly affect body weight (sodium-depleted SHR, 185 ± 2 g; salt-replete SHR, 185 ± 3 g). On the other hand, furosemide injection significantly (P < 0.0001) increased daily urine volume from 7.9 ± 0.2 ml/24 h before furosemide to 22.4 ± 0.7 ml/24 h after furosemide treatment.

Table 1 compares the plasma concentrations of angiotensin peptides in sodium-depleted SHR to that measured in salt-replete SHR. Salt depletion caused a >7-fold increase in the plasma concentrations of ANG I and ANG II and a twofold rise in the plasma levels of ANG-(1–7). The ANG I-to-ANG II ratio did not change because salt depletion caused similar increases in the plasma concentrations of ANG I and ANG II. On the other hand, the significant increases in the ANG I-to-ANG-(1–7) ratio and the ANG II-to-ANG-(1–7) ratio indicated that plasma levels of ANG-(1–7) did not increase to the same extent as ANG I and ANG II.

Effect of Angiotensin Receptor Blockade in Sodium-Depleted SHR

Thirty minutes after intravenous administration of losartan, mean arterial pressure had decreased significantly (P < 0.0001) from 139 ± 2 to 93 ± 2 mmHg (n = 60). The hypotensive effect of losartan was accompanied by reflex tachycardia (before losartan, 428 ± 5 beats/min; after losartan, 458 ± 5 beats/min; P < 0.0001).

To investigate the contributions of different angiotensin receptor subtypes to the prevailing level of blood pressure observed in salt-depleted SHR, we infused receptor subtype-selective antagonists for 15-min periods. Because the sodium depletion in these SHR may have made these rats sensitive to expansion of the plasma volume, we performed a set of time control experiments in which we infused 5% dextrose in water (the vehicle for antagonist infusions) at a rate of 0.2 ml/min for 15 min. In most experiments, drugs were infused at a rate of 0.1 ml/min, but in experiments utilizing dual infusions of antagonists (or antibodies), the infusion rate was 0.2 ml/min. By the end of the 15-min vehicle infusion, mean arterial pressure had changed by +0.4 ± 1.4 mmHg, and this was not statistically significant. Statistical analysis of blood pressure changes produced by antagonist or antibody infusion used this value of blood pressure change associated with vehicle infusion.

Figure 2 shows the averaged time course of the pressor responses in salt-depleted SHR given a 15-min infusion of [D-Ala7]ANG-(1–7) alone or in combination with preexisting AT1 blockade. [D-Ala7]ANG-(1–7) significantly increased blood pressure by the first minute after initiation of the infusion, and a steady-state increase in blood pressure was achieved by 5 min into the infusion period in rats either pretreated with losartan or in rats with no prior blockade of AT1 receptors. Equivalent maximal increases in mean arterial pressure were observed in both groups (no losartan, +13 ± 2 mmHg; losartan pretreatment, +15 ± 4 mmHg). In contrast, infusion of [D-Ala7]ANG-(1–7) significantly attenuated the antihypertensive effect of losartan (losartan alone, −46 ± 2 mmHg; losartan after [D-Ala7]ANG-(1–7), −34 ± 3 mmHg; P < 0.05).

To establish that the infusion dose of [D-Ala7]ANG-(1–7) (10 pmol/min) used in the majority of experiments had produced effective blockade of AT1 receptors, we infused [D-Ala7]ANG-(1–7) at 100 pmol/min in a separate group of losartan-pretreated, salt-depleted SHR. Both doses caused equivalent (F1,48 = 0.086) increases in mean arterial pressure with the same time

Table 1. Plasma angiotensin peptide concentrations in spontaneous hypertensive rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal (n = 8)</th>
<th>Salt Depletion (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma ANG I, fmol/ml</td>
<td>36.8 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>Plasma ANG II, fmol/ml</td>
<td>29.2 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Plasma ANG(1–7), fmol/ml</td>
<td>44.8 ± 8.8</td>
</tr>
<tr>
<td></td>
<td>Plasma ANG I/ANG II ratio</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Plasma ANG I/ANG(1–7) ratio</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Plasma ANG II/ANG-(1–7) ratio</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE. NS, not significant.
course. The maximal increase in mean arterial pressure was 15 ± 4 mmHg for the 10 pmol/min dose and 16 ± 3 mmHg for the 100 pmol/min dose. On the basis of these results, we concluded that [D-Ala7]ANG-(1–7) infusion at 10 pmol/min had effectively blocked the hemodynamic actions of ANG-(1–7).

Previous studies (8, 18, 37, 38) suggested that ANG II has a vasodepressor effect via AT2 receptors. Moreover, our investigations had demonstrated that ANG-(1–7) exerts a vasodepressor effect in low-salt SHR (19) or SHR treated chronically with an angiotensin-converting enzyme (ACE) inhibitor or losartan (20–22). Thus one goal of this study was to determine the relative contributions of ANG II acting at an AT2 receptor and ANG-(1–7) acting at an AT1 receptor as counterbalancing influences to the vasopressor actions of ANG II acting at the AT1 receptor in salt-depleted SHR. Figure 3A depicts the effects on the prevailing levels of mean arterial pressure in losartan-pretreated, salt-depleted SHR when 1) PD-123319 was infused to block AT2 receptors, 2) [D-Ala7]ANG-(1–7) was infused to block AT1 receptors, or 3) both antagonists were infused together. In each case, infusion of PD-123319 or [D-Ala7]ANG-(1–7) alone or together significantly (P < 0.05) increased mean arterial pressure to a new steady-state level by 5 min after initiating the antagonist infusion. Furthermore, the pressor response in each condition was maintained for as much as 15 min after the antagonist infusions were stopped. On the other hand, the pressor responses produced by blockade of AT2 or AT1 receptors alone or in combination were not associated with significant changes in heart rate (Fig. 3B).

To assess the relative contributions of vasodepressor actions exerted through AT2 and AT1 receptors, we analyzed the pressor responses produced by each antagonist alone or in combination as the maximal increase in mean arterial pressure observed in each rat during either the infusion period or the 25-min postinfusion period. Figure 4 shows that PD-123319 given alone caused an average maximal increase in mean arterial pressure of 8 ± 1 mmHg, whereas [D-Ala7]ANG-(1–7) alone or in combination with PD-123319 caused equivalent maximal increases in mean arterial pressure ([D-Ala7]ANG-(1–7), +15 ± 4 mmHg; PD-123319 + [D-Ala7]ANG-(1–7), +15 ± 1 mmHg). The average time of maximal increase in mean arterial pressure did not differ among the three treatment groups (PD-123319, 22 ± 4 min; [D-Ala7]ANG-(1–7), 17 ± 4 min; PD-123319 + [D-Ala7]ANG-(1–7), 14 ± 4 min).

Effect of ANG II Polyclonal Antibody in Salt-Replete and Salt-Depleted SHR

Blockade of AT2 and AT1 receptors did not fully reverse the antihypertensive effect of AT1 receptor blockade in the salt-depleted SHR. To evaluate a possible opposing residual effect of ANG II at non-AT1 receptors, an affinity-purified polyclonal ANG II antibody was infused into salt-depleted SHR pretreated...
with losartan and the combination of PD-123319 and [D-Ala7]ANG-(1–7). Responses to the ANG II antibody were also obtained in sodium-replete SHR in the absence of prior AT1 blockade.

Initially, we assessed the effects of the ANG II antibody in salt-replete SHR. Consistent with previous reports (30), the ANG II antibody reduced mean arterial pressure from 160 ± 4 to 150 ± 5 mmHg (P < 0.05). Heart rate did not change. Endogenous neutralization of ANG II elicited pressor responses in salt-depleted SHR treated with losartan alone, the combination of losartan and [D-Ala7]ANG-(1–7), or the combination of losartan, PD-123319, and [D-Ala7]ANG-(1–7). As shown in Fig. 5, infusion of the ANG II antibody to salt-depleted losartan-treated SHR produced an +11 ± 2 mmHg (paired t-test; P < 0.001) rise in mean arterial pressure from a baseline value of 83 ± 3 mmHg. Administration of the ANG II antibody in salt-depleted SHR after prior blockade of AT1 and AT(1–7) receptors increased mean arterial pressure from 104 ± 8 to 114 ± 7 mmHg (paired t-test; P < 0.05). Finally, ANG II antibody infusion in salt-depleted SHR that had already received losartan, PD-123319, and [D-Ala7]ANG-(1–7) increased mean arterial pressure from 102 ± 4 to 109 ± 3 mmHg (paired t-test; P < 0.01).

Changes in heart rate produced by the antibody in any of these conditions were not statistically significant.

To further ascertain the capacity of the ANG II antibody to neutralize (or “trap”) endogenous circulating levels of the ANG II, arterial blood was collected from salt-replete SHR and salt-depleted, losartan-treated SHR at the end of the 10-min administration of the ANG II antibody. Neutralization of endogenous ANG II was assessed by comparing the immunoreactive ANG II in blood obtained from rats that had not received the ANG II antibody vs. those in which the antibody was infused for 10 min. This comparison revealed a 16-fold increase in immunoreactive ANG II for salt-replete SHR and a 33-fold increase in immunoreactive ANG II for salt-depleted SHR (see Fig. 6A; note the log scale for ANG II concentration). Thus trapping of ANG II by the antibody differed in the salt-replete SHR compared with that obtained in the salt-depleted SHR. Chromatographic separation of the ANG II immunoreactivity from the pooled sample obtained from salt-depleted SHR (see Fig. 6B) showed that ANG II was the predominant product followed by ANG-(4–8), ANG-(2–8), and ANG-(3–8).

DISCUSSION

Endogenous activation of the renin-angiotensin system allowed assessment of the relative contributions of angiotensin receptors to the maintenance of arterial pressure in salt-depleted SHR. The counterbalancing hemodynamic actions of ANG II and ANG-(1–7) were examined utilizing a pharmacological strategy that combined the use of selective angiotensin receptor antagonists for AT1, AT2, and AT(1–7) receptors and a purified antibody that expressed high affinity and selectivity for ANG II. Our results confirmed previous suggestions that AT1-mediated vasoconstriction can be partially counteracted by a functionally active AT2 receptor in salt-restricted rats (8, 43). Our data also provide evidence that ANG-(1–7) exerts a physiologically important counterbalancing effect to the vasoconstrictor action of ANG II at AT1 receptors to the maintenance of arterial pressure in the condition of salt depletion or dietary salt restriction (19). The additional pressor effects produced by neutralization of endogenous ANG II after blockade of AT2 and AT(1–7) receptors may implicate a vasodilator action of ANG II at a receptor site distinct from AT1, AT2, and AT(1–7) receptor subtypes.
A variety of different factors may contribute to the blood pressure-lowering action of losartan that remained after blockade of AT₂ and AT₁ receptors. Li et al. (25) have shown in SHR that losartan antagonizes the vasoconstrictor effect of thromboxane A₂ (TxA₂) agonists at the TxA₂ receptor. Furthermore, Jaiswal et al. (23) found that losartan may inhibit thromboxane synthetase. This could have a dual effect: 1) reduced production of vasoconstrictor TxA₂ and 2) enhanced production of the vasodilator prostacyclin. These two actions of losartan could promote vasodepressor effects of losartan independent of AT₁ receptor blockade.

The novel biological actions of ANG-(1–7) at a site distinct from AT₁ or AT₂ receptors appear to engage vasodilator systems that include the production of prostaglandins (22), augmentation of bradykinin vascular responses (1, 24, 33), and release of nitric oxide (5, 24, 31). The demonstration of a pressor effect of [D-Ala⁷]ANG-(1–7) in salt-depleted SHR extends our findings in which 1) neutralization of endogenous ANG-(1–7) reversed the antihypertensive effects of renin-angiotensin system blockade (20–22) and 2) blockade of ANG-(1–7) stimulated a vasoconstrictor response in dietary salt-restricted SHR and [mRen-2]²⁷ transgenic hypertensive rats (19). Prior studies from our laboratory (5, 6, 19, 40) and from Tallant et al. (41) have demonstrated that [D-Ala⁷]ANG-(1–7) is a selective antagonist of the vasodilator and anti-growth-promoting actions of ANG-(1–7). The pressor effects of ANG-(1–7) monoclonal antibody were prevented by prior administration of [D-Ala⁷]ANG-(1–7) in SHR and [mRen-2]²⁷ transgenic hypertensive rats (19). Our results now show that the pressor response produced by systemic infusions of [D-Ala⁷]ANG-(1–7) is independent of the functional activity of AT₁ receptors because neither the time course nor the maximal amplitude of the blood pressure rise was altered by prior blockade of AT₁ receptors. Because the magnitude of the pressor response elicited by [D-Ala⁷]ANG-(1–7) in salt-depleted SHR was not modified by the administration of losartan beforehand, these data suggest no functional coupling between AT₁ and [D-Ala⁷]ANG-(1–7)-sensitive angiotensin receptors.

The mean arterial pressure of SHR subjected to the combination of salt restriction and diuretic administration was 24 mmHg lower than the blood pressure of the salt-replete cohort. This was associated with significant increases in plasma concentrations of ANG I, ANG II, and ANG-(1–7). It is interesting to note that blockade of the actions of ANG-(1–7) by [D-Ala⁷]ANG-(1–7) reversed this hypotensive effect of salt depletion by nearly 60%. The uncovering of this tonic depressor effect of ANG-(1–7) did not require coincident AT₁-mediated pressor actions of ANG II.

A variety of experimental strategies have been employed in attempts to uncover what role AT₂ receptors may play in the regulation of blood pressure. Manipulations have included stimulation of the renin-angio-

Fig. 6. A: comparison of ANG II concentration (plotted on a log scale) that was recovered from plasma of salt-replete and salt-depleted SHR that had either received an infusion of the ANG II Ab (Antibody) or no infusion (Control). In each dietary group (Na replete and Na deplete), infusion of the ANG II Ab dramatically increased the total plasma concentration of ANG II. B: high-pressure liquid chromatogram derived from the plasma obtained at the end of 10-min ANG II antibody infusion of salt-depleted SHR that had been administered losartan, [D-Ala⁷]ANG-(1–7), and PD-123319 before ANG II Ab infusion. The plasma was acid extracted to free ANG II that had bound to the antibody. The chromatogram demonstrates that the major immunoreactive peptide was ANG II, although lesser amounts of smaller angiotensin fragments were present.
tension system by low-salt diets, various models of hypertension (8, 32, 37, 38), activation of AT2 receptors by infusion of ANG II or AT2-selective agonists (7, 39), and AT2 knockout mice (18). The present study utilized sodium depletion as a way to profoundly activate the renin-angiotensin system with the outcome being significant elevations of ANG II, ANG I, and ANG-(1–7). In our sodium-depleted, losartan-treated SHR, we observed that AT2 receptor blockade produced only a modest elevation in blood pressure. Indeed, the apparent AT2-mediated vasodepressor effect in our study was similar in magnitude to the CGP-42112-induced enhancement of the depressor response to low-dose candesartan in SHR (3).

ANG II binds at AT1 and AT2 receptors with similar affinity (10, 36, 42, 44). The argument has been advanced that AT1 and AT2 receptors are in a tight balance with each other (10, 37). However, the suggestion that the antihypertensive effects of ANG II blockade are in part mediated by coupling of ANG II to the AT2 receptor may depend on the species studied, the state of water and salt balance, and the relative activity of counterbalancing vasodepressor systems. Furthermore, the selectivity of PD-123319 for other atypical ANG II receptor subtypes has not been excluded convincingly (26). For example, PD-123319 may act in the kidney (2) and brain (14) but not in the vascular system (5, 13) to partially block the effects of ANG-(1–7). Furthermore, it has been suggested that at high doses this AT2 antagonist may undergo in vivo biotransformation to a metabolite with AT1 receptor antagonist properties (45). Partial binding of a PD-123319 metabolite to AT1 receptors would have had no impact in our experiments because the rats had been pretreated with losartan. We cannot totally exclude, however, an effect of PD-123319 at vascular AT1 receptors, although in other studies there was no evidence for this interaction (19, 20).

The demonstration of a consistent pressor effect of the ANG II antibody in salt-depleted SHR subjected to various combinations of blockade of AT1, AT2, and AT1/AT2 receptors is a novel and unexpected finding. In contrast to the results obtained with [d-Ala7]ANG-(1–7), the ANG II antibody decreased blood pressure in salt-replete SHR with AT1 receptors unblocked, whereas this same ANG II antibody increased blood pressure in salt-depleted, losartan-pretreated SHR. This pressor effect persisted even when AT1(1–7) and AT2 receptors were blocked as well. ANG II antibody infusion was also associated with a 16- and 33-fold increase in recoverable ANG II in salt-replete and salt-depleted SHR, respectively. One might entertain the possibility that at high circulating levels of ANG II, some of this peptide is converted to ANG-(3–8), which might have vasodilatory actions (16). Another possible explanation for these results may be that ANG II exerts vasodepressor effects through an unidentified ANG II receptor. Further studies are needed to evaluate this possibility.

In conclusion, the powerful vasoconstrictor (pressor) effect of ANG II mediated by AT1 receptors is mitigated by important depressor actions of ANG II and ANG-(1–7) at other angiotensin receptors subtypes in salt-depleted SHR. The counterbalancing effects of these mitigating influences can be summarized in the following manner. The AT2-mediated actions of ANG II contributed to the blood pressure-lowering effects of AT1 blockade by ~17%. The apparent vasodepressor effects of ANG-(1–7) at the AT1(1–7) receptor and ANG II at a site distinct from the AT2 receptor amounted to 15 and 7 mmHg, respectively. Together, they contributed to the blood pressure-lowering effects of losartan by nearly 50%. Thus combined blockade of AT2 and AT1(1–7) receptors and ANG II neutralization could reverse as much as 67% of the blood pressure-lowering effect of losartan.

**Perspectives**

The contribution that the renin-angiotensin system makes in the regulation of fluid volume and sodium balance is undeniably one of the critical mechanisms of homeostasis. In hypertension, dysregulation of ANG II synthesis and activity has been uncovered by a variety of strategies directed either to reduce the production of ANG II from ANG I or block its coupling to AT1 receptors. Findings derived from advances in molecular biology and the advent of selective ANG II receptor antagonists suggest that the net vasoconstrictor and growth-promoting actions of ANG II are regulated, at least in part, through the binding of the ligand to the AT2 receptor. Our studies addressed one aspect of the potential interactions of ANG II through both the AT1 and AT2 receptors in a condition in which endogenous formation of ANG II is stimulated by salt depletion. Our experiments demonstrated that PD-123319 had negligible effects on blood pressure in AT1 receptor-blocked, salt-depleted SHR, whereas [d-Ala7]ANG-(1–7) significantly reversed the fall in blood pressure mediated by losartan. These findings add to the evidence that a depressor role of AT2 receptors is not the sole mechanism modulating the effects of ANG II in the presence of AT1 receptor blockade. Our studies further implicate ANG-(1–7) as a mechanism contributing to regulation of blood pressure and provide new insights into the biological effector mechanisms participating in the modulation of blood pressure. Strong evidence for biological actions of ANG-(1–7) comes from carefully controlled experiments in salt-depleted SHR where selective AT2 and AT1(1–7) receptor blockers were used alone or in combination with a specific ANG II antibody to dissect their relative contributions after AT1 blockade. The results demonstrate that the tonic depressor activity of ANG-(1–7) existed during AT1 receptor blockade is mediated by the peptide acting at a non-AT1/AT2 receptor. Taken all together, these data underscore the possibility that ANG-(1–7) has an intrinsic tonic function to act as a negative modulator in the vasopressor and growth-promoting actions of ANG II. In this context, the previously proposed concept of a deficit in the synthesis or activity of ANG-(1–7) gains further credence as a critically important mechanism.
in “low-renin” salt-sensitive hypertension. Moreover, enhanced production of ANG-(1–7) may underlie the mode of action of ACE inhibitors and ANG II antagonists as effective antihypertensive therapies.

This study was supported in part by National Heart, Lung, and Blood Institute Grants HL-50066, HL-56973, and PO1-HL-51952.

REFERENCES


