Effects of nitric oxide synthase inhibition on angiotensin receptors and metabolism in the pregnant hypertensive rat

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ABSTRACT

Endothelial dysfunction and a consequent decrease in nitric oxide production have been implicated in the pathogenesis of pre-eclampsia. A prominent feature of the pre-eclamptic syndrome is a loss of the pregnancy-induced refractoriness to infused pressor agents, such as angiotensin. In this study, we sought to determine whether a decrease in nitric oxide production might be linked via changes in angiotensin II receptors and angiotensin II metabolism to changes in pressor sensitivity to infused angiotensin II. Pregnant and non-pregnant spontaneously hypertensive rats (SHRs) were randomly allocated to receive 5 mg kg\(^{-1}\) day\(^{-1}\) N\(^\circ\)G-nitro-L-arginine methyl ester (L-NAME) in the drinking water or drinking water alone from days 7 to 14 of gestation. Steady-state metabolic clearance studies of angiotensin II were then performed, or tissues were harvested for angiotensin II receptor studies. Treatment with L-NAME caused an increase in systolic pressure (\(P < 0.001\)) in both pregnant and non-pregnant rats, while urinary protein excretion increased only in the pregnant SHRs (\(P < 0.001\)). Plasma angiotensin II levels were significantly increased in the L-NAME-treated SHRs compared with controls (non-pregnant, \(P < 0.0005\); pregnant, \(P < 0.01\)). The metabolic clearance rate of angiotensin II was decreased by L-NAME treatment in non-pregnant SHRs (\(P < 0.05\)), but was increased by L-NAME treatment in the pregnant rats (\(P < 0.01\)). In the aorta, the angiotensin II receptor number increased after treatment with L-NAME in both non-pregnant (\(P < 0.0005\)) and pregnant (\(P < 0.05\)) SHRs, and the dissociation constant increased in the non-pregnant SHRs (\(P < 0.005\)). Thus treatment of SHRs with L-NAME increased blood pressure, as well as the circulating angiotensin II concentration and vascular angiotensin II receptor expression. However, treatment with L-NAME did not affect pressor sensitivity to infused angiotensin II. We conclude, therefore, that although a decrease in nitric oxide production is associated with changes in angiotensin II concentrations and receptor numbers, it does not induce changes in pressor sensitivity to infused angiotensin II in the SHR.

INTRODUCTION

Pre-eclampsia remains a major source of maternal and foetal morbidity and mortality for which, despite much research, no definitive cause has been found. It has been suggested by a number of workers that this disease process results, at least in part, from endothelial dysfunction [1]. In particular, it has been suggested that this endothelial dysfunction results in a reduced capacity to synthesize the endothelium-derived relaxing factor nitric oxide (NO) and, as a consequence, a failure of vasodilatation [2,3].

Key words: angiotensin II metabolism, angiotensin II receptors, hypertension, NO synthase inhibition, pregnancy.
Abbreviations: ANG II, angiotensin II; MCR, metabolic clearance rate; L-NAME, N\(^\circ\)G-nitro-L-arginine methyl ester; SHR, spontaneously hypertensive rat.
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Women with pre-eclampsia, or those who are destined to develop this disorder, also display an increase in pressor responsiveness to infused angiotensin II (ANG II) [4-5]. It is possible that this phenomenon is secondary to a decrease in NO production, as administration of NO synthase inhibitors in experimental animals has been reported to increase pressor sensitivity to infused ANG II [6,7].

The mechanisms by which an increase in pressor sensitivity to exogenous ANG II could occur are not understood, but may be related to an increase in ANG II receptor density or a decrease in ANG II metabolism. The concept that an increase in ANG II receptor density underlies the increase in pressor sensitivity arose from studies of platelet ANG II receptors in pregnant women, with the platelet receptor being considered a model for the vascular ANG II receptor. In normotensive pregnant women, sensitivity to infused ANG II decreased, as did platelet ANG II receptor numbers [8-13]. In women with pre-eclampsia, a condition in which pressor sensitivity to ANG II is increased, platelet ANG II receptor numbers were also increased [8-13], suggesting that this might be the explanation. However, in studies in normotensive pregnant rats and sheep, vascular ANG II receptor numbers were found to be increased [14,15] at times when there was desensitization to infused ANG II [16-18]. The metabolic clearance rates (MCRs) of ANG II were unchanged or decreased [14,19,20], suggesting that, at least in normotensive pregnancy, a post-receptor mechanism might be responsible for the loss of the pressor response to ANG II.

Unlike women with essential hypertension, who have an increased incidence of pre-eclampsia, the spontaneously hypertensive rat (SHR) does not develop a superimposed pre-eclamptic syndrome. We postulated that treatment with a nitric oxide synthase inhibitor, such as Nω-nitro-arginine methyl ester (L-NAME), would reduce NO production in the SHR, cause accelerated hypertension and proteinuria and increase sensitivity to infused ANG II. It would, therefore, provide a model in which to study the mechanisms responsible for the increased pressor sensitivity to ANG II in pregnancies complicated by chronic hypertension and a superimposed pre-eclamptic syndrome.

METHODS

Animals and protocol
Female SHRs, aged 14 weeks, were housed four to a box in an air-conditioned room with a 12 h light/dark cycle and allowed access ad libitum to standard rat chow (Doust and Rabbidge, Sydney, Australia) and drinking water. Each box of rats was exposed to a breeder male for 72 h, and gestation was dated from the third night. Those SHRs that did not become pregnant served as age-matched non-pregnant controls.

Systolic pressures were measured every second day by tail cuff plethysmography, and the rats were weighed twice weekly. On day 7–10 of gestation, when the SHRs were 15 weeks of age, they were randomly allocated to receive L-NAME 5 mg·kg⁻¹·day⁻¹ in the drinking water or drinking water alone. On day 12–15 of gestation the SHRs were placed in individual metabolism cages and urine was collected for 48 h to determine endogenous creatinine clearance, and sodium and protein excretion. On day 14–17 of gestation, the SHRs underwent metabolic clearance studies for ANG II or had blood collected and tissues harvested for ANG II receptor studies. Day 14–17 of gestation was chosen because other studies have shown that decreased pressor sensitivity to infused ANG II is present at this time [15,16] in the rat. We wanted to see whether this was reversed by NO synthase inhibition.

The dose of L-NAME (5 mg·kg⁻¹·day⁻¹) was chosen following pilot studies with pregnant SHRs. In other studies in the literature, a dose of 10 mg·kg⁻¹·day⁻¹ L-NAME was used [2,6,7,21]. These experiments complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 1992 [22], and were approved by the Animal Ethics Committee of the University of New South Wales.

Metabolic clearance studies
On the day of the experiment, the rats were anaesthetized using gaseous anaesthesia: halothane (2.5%) in nitrous oxide (1 litre·min⁻¹) and oxygen (0.5 litre·min⁻¹). Tapered polyethylene cannulae (internal diameter 0.75 mm; external diameter 1.45 mm; Dural Plastics, Auburn, NSW, Australia) were inserted into the right carotid artery and left jugular vein. The carotid cannula was used for blood pressure measurement and blood sampling. Blood pressure was measured using a Bell and Howell pressure transducer and a Neomedix chart recorder. After a 1 h rest equilibration period, during which vehicle alone (Haemaccel; Hoechst Australia Ltd, Sydney, NSW, Australia) was infused at 0.017 ml·min⁻¹ via the jugular catheter, arterial blood was sampled to determine the basal ANG II concentration. ANG II (Auspep, Melbourne, Australia) was then infused via the jugular catheter at 20 pmol·kg⁻¹·min⁻¹ for 60 min. As the aim of the study was to evaluate ANG II metabolism rather than pressor responsiveness per se, only a single infusion dose was studied. Arterial blood was then sampled to determine the mean steady-state concentration of ANG II.

The ANG II concentration in the plasma and infusate was measured by RIA (see below). The MCR and theoretical secretion rate were calculated by the method of Tait et al. [23].

ANG II receptor studies
On day 14–17 of gestation, the SHRs were anaesthetized as described above. Arterial blood was collected to determine plasma levels of ANG II. Kidneys, uterus and
aorta were harvested following blood sampling and were immediately snap-frozen in liquid nitrogen.

**Radioligand preparation**

[Sar¹,Ile⁶]ANG II (Auspep, Melbourne, Australia) was iodinated by the lactoperoxidase technique, with initial purification on a C-18 Sep-Pak cartridge (Waters Corp., Milford, MA, U.S.A.). Further purification was performed by gradient reverse-phase HPLC using a C-18 Selectosil column (Phenomenex, Torrance, CA, U.S.A.) as described previously [24]. The specific radioactivity of the ligand was determined at 2-week intervals by self-displacement in an RIA [24]. The specific radioactivity averaged 1380 Ci/mmol and remained unchanged over an 8-week period, indicating balanced decay of the ligand.

**Aortic and uterine plasma membrane preparation**

Each tissue was thawed in ice-cold 20 mM NaHCO₃. For the uterus, the surrounding connective tissue was removed and the endometrium was carefully separated from the uterine horns. Each tissue was divided into 2 mm squares and placed in 3 ml of 20 mM NaHCO₃. After three 15 s homogenizations at 25000 rev./min using an OMNI 2000 homogenizer, NaHCO₃ (20 mM) was added to a final volume of 10 ml. The homogenate was then maintained at 4 °C and centrifuged at 180,000 g and centrifuged at 100,000 g for 10 min (4 °C), the plasma was applied to and customers were thawed at 4 °C, glomeruli were isolated by differential sieving and washed with fresh cold PBS [25]. The glomeruli were then centrifuged (4 °C) at 180,000 g for 10 min, and the glomerular pellet was resuspended in a 10 mM sodium phosphate buffer (pH 7.4) containing 10 mM sodium phosphate buffer (pH 7.4) containing 10 mM MgCl₂, 1 mM EGTA and 0.2 % BSA, pH 7.4).

**Glomerular isolation**

After being thawed at 4 °C, glomeruli were isolated by differential sieving and washed with fresh cold PBS [25]. The glomeruli were then centrifuged (4 °C) at 180,000 g for 10 min, and the glomerular pellet was resuspended in a 10 mM sodium phosphate buffer (pH 7.4) containing 5 mM EDTA, 1 mM PMSF, 0.02 % sodium azide, 0.2 % BSA and 1 mg/ml bacitracin. Microscopic examination of the glomerular suspension showed that the preparations were > 95 % pure.

**Receptor binding assay**

Aliquots of 200 μl of tissue suspension were incubated with various concentrations of ¹²⁵I-[Sar¹,Ile⁶]ANG II: 0.4–4.7 nM for uterus and aorta, and 0.1–100 nM for glomeruli. The higher concentration was required in the glomerular binding assay to achieve saturation. Non-specific binding was determined by incubation in the presence of an excess of unlabelled [Sar¹,Ile⁶]ANG II (1.5 μM) at each radioligand concentration. After incubation for 50 min at room temperature, bound and free ligand were separated by rapid filtration [23]. The protein content of each membrane or glomerular preparation was determined by the method of Lowry et al. [26].

**Plasma ANG II assay**

For determination of the plasma ANG II concentration, arterial blood was collected into pre-cooled syringes containing 0.5 ml of EDTA (0.3 M) and 0.3 ml of 2,3-dimercapto-1-propanol (0.2 M). After centrifugation at 180 g for 10 min (4 °C), the plasma was applied to and extracted on a Sep-Pak C-18 cartridge using 2 ml of acetonitrile/distilled water/acetic acid (74:24:4, by vol.). Specimens were then blown down to dryness under nitrogen and stored at −20 °C until assay. Samples were reconstituted with barbitone buffer and assayed by RIA as described previously [24]. The polyclonal antibody used in this assay cross-reacts with ANG III, but has negligible cross-reactivity with ANG I under assay conditions. Addition of ANG I to plasma aliquots before Sep-Pak extraction in amounts ranging from 160 to 2500 pmol·l⁻¹ displaced the same amount of labelled ANG II as did 1 % or less of the equivalent concentration of ANG II. In contrast, addition of ANG III to plasma in amounts ranging from 50 to 500 pmol·l⁻¹ displaced the same amount of labelled ANG II as 97 % of the equivalent concentration of ANG II. Recovery of ANG II added to plasma before extraction exceeded 97 %, and no correction for recovery was therefore made. The intra-assay coefficient of variation for this assay was 5 %, and the interassay coefficient of variation was 6 %.

**Calculations and statistical analysis**

Receptor parameters (dissociation constant and binding site density) were determined using the iterative computer program LIGAND [27]. Binding site density was expressed in units of fmol of ANG II bound per mg of protein. Comparisons between groups for each parameter were made by ANOVA. When significant differences were found, individual comparisons were carried out using an LSD test for planned comparisons (CSS; Statistica). P values of < 0.05 were considered significant.

**RESULTS**

**Blood pressure and renal function**

In pilot studies, an initial dose of L-NAME of 10 mg·kg⁻¹·day⁻¹ was used. At this dose of L-NAME, we found that blood pressure rose to approx. 220 mmHg and that an eclamptic syndrome involving grand mal seizures and death occurred in the pregnant SHRs.

There was no significant difference in body weight between SHRs treated with L-NAME (5 mg·kg⁻¹·day⁻¹) and their respective controls (Table 1). Both pregnant groups showed a similar increase in weight. Treatment with L-NAME caused similar increases in blood pressure in both pregnant and non-pregnant SHRs (Table 1). There was an increase in urine volume in both groups of pregnant SHRs compared with their non-pregnant counterparts (controls, P < 0.05; L-NAME, P < 0.001; pregnant compared with non-pregnant). Treatment with
Table 1  Effects of NO synthase inhibition by l-NAME (5 mg·kg⁻¹·day⁻¹) on body weight, blood pressure, urine volume and renal function

Values are means ± S.E.M. for n = 6 in each group. Significance of differences: *P < 0.05, **P < 0.01, ***P < 0.001 for pregnant compared with non-pregnant rats within each treatment group; †P < 0.05, ††P < 0.001 for l-NAME-treated rats compared with respective untreated control group.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Body weight (g)</th>
<th>Systolic pressure (mmHg)</th>
<th>Urine volume (ml·24h⁻¹)</th>
<th>Creatinine clearance (ml·min⁻¹·100 g⁻¹)</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
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<tr>
<td>Non-pregnant</td>
<td>193.41 ± 5.37</td>
<td>179 ± 1</td>
<td>9.9 ± 1.7</td>
<td>0.51 ± 0.04</td>
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<tr>
<td>Pregnant</td>
<td>218.30 ± 1.50</td>
<td>178 ± 2</td>
<td>15.3 ± 1.7†</td>
<td>0.63 ± 0.03*</td>
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<tr>
<td>l-NAME</td>
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<tr>
<td>Non-pregnant</td>
<td>186.60 ± 4.16</td>
<td>207 ± 5†††</td>
<td>13.1 ± 0.9†</td>
<td>0.44 ± 0.04</td>
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<tr>
<td>Pregnant</td>
<td>218.10 ± 5.20</td>
<td>205 ± 4†††</td>
<td>21.3 ± 1.8***†</td>
<td>0.50 ± 0.03*</td>
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Table 2  Effects of treatment with l-NAME (5 mg·kg⁻¹·day⁻¹) on plasma and urine electrolytes and protein

Values are means ± S.E.M. for n = 6 in each group. Significance of differences: *P < 0.05, **P < 0.01, ***P < 0.001 for pregnant compared with non-pregnant rats within each treatment group; †P < 0.05, †††P < 0.001 for l-NAME-treated rats compared with the respective untreated control group.

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<tr>
<th>Rats</th>
<th>Plasma</th>
<th>Urine</th>
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<tr>
<td></td>
<td>Na (mmol·l⁻¹)</td>
<td>Albumin (g·l⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<tr>
<td>Non-pregnant</td>
<td>130.4 ± 0.1</td>
<td>36.1 ± 1.1</td>
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<tr>
<td>Pregnant</td>
<td>137.2 ± 0.3*</td>
<td>37.5 ± 0.6</td>
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<tr>
<td>l-NAME</td>
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<tr>
<td>Non-pregnant</td>
<td>139.7 ± 0.5</td>
<td>40.4 ± 0.2†</td>
</tr>
<tr>
<td>Pregnant</td>
<td>135.2 ± 0.5**</td>
<td>40.0 ± 1.0†</td>
</tr>
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l-NAME also resulted in significant increases in urine volume compared with the respective untreated control groups (non-pregnant, P < 0.05; pregnant, P < 0.05; l-NAME compared with control). Endogenous creatinine clearance increased in both pregnant groups compared with their non-pregnant counterparts (Table 1; controls, P < 0.05; l-NAME, P < 0.05; pregnant compared with non-pregnant). However, treatment with l-NAME caused a decrease in creatinine clearance compared with the respective group of control SHRs (P < 0.05).

**Plasma and urine biochemistry**

Plasma sodium decreased in both groups of pregnant SHRs compared with their non-pregnant counterparts (control, P < 0.05; l-NAME, P < 0.01; Table 2). However, this fall was significantly greater in the l-NAME-treated SHRs (P < 0.01). Plasma albumin did not differ between pregnant and non-pregnant SHRs in each treatment group, although treatment with l-NAME resulted in increases in plasma albumin in both non-pregnant (P < 0.05) and pregnant (P < 0.05) SHRs (see Table 2).

Urinary sodium excretion was unchanged by pregnancy in the controls. In the l-NAME-treated SHRs, there was a significant increase in urinary sodium excretion in the pregnant compared with the non-pregnant animals (P < 0.05). Urinary sodium excretion in the pregnant l-NAME-treated SHRs was also significantly increased compared with that in pregnant control SHRs (P < 0.05). Urinary protein excretion did not differ significantly between pregnant and non-pregnant controls. In contrast, with the l-NAME-treated SHRs there was a significant increase in urine protein in pregnant compared with non-pregnant SHRs (P < 0.001). Urinary protein excretion in pregnant SHRs treated with l-NAME was also significantly greater than that in pregnant controls (P < 0.001).

**Metabolic clearance studies**

Mean arterial pressure in the pregnant control SHRs was not significantly lower than in the non-pregnant SHRs (pregnant, 116 ± 3 mmHg; non-pregnant, 121 ± 3 mmHg). A similar lack of effect of pregnancy on mean arterial pressure was observed for the l-NAME-treated SHRs (pregnant, 140 ± 3 mmHg; non-pregnant, 141 ± 7 mmHg). Following infusion of ANG II for 60 min, mean arterial pressure increased significantly in
Figure 1  Plasma ANG II concentrations in control and L-NAME-treated SHRs
The open bars represent data from non-pregnant SHRs, and the shaded bars depict data for pregnant SHRs. Values are means ± S.E.M. for n = 8 SHRs per group. P values on the graph represent the significance of differences between the groups indicated by the lines.

Figure 2  MCR for ANG II corrected for body weight (upper panel) and for endogenous creatinine clearance (lower panel) in control and L-NAME-treated SHRs
Data for non-pregnant SHRs are depicted by the open bars, and those for pregnant SHRs by the hatched bars. Values are means ± S.E.M. for n = 8 SHRs per group. Significance of differences between non-pregnant and pregnant SHRs: upper panel, *P < 0.05; lower panel: **P < 0.01. P values on the graphs represent the significance of differences between the groups indicated by the lines.

Figure 3  ANG II receptor numbers (upper panel) and dissociation constants (lower panel) in the glomeruli for control and L-NAME-treated SHRs
Data for non-pregnant SHRs are depicted by the open bars, and those for pregnant SHRs by the hatched bars. Values are means ± S.E.M. for n = 8 SHRs per group. Significance of differences between non-pregnant and pregnant SHRs: upper panel, **P < 0.01; lower panel, ****P < 0.0005. P values on the graphs represent the significance of differences between the groups indicated by the lines.

all groups of SHRs, and the increment in mean arterial pressure was similar in all groups (non-pregnant control, 9±2 mmHg; pregnant control, 10±2 mmHg; non-pregnant L-NAME-treated, 9±1 mmHg; pregnant L-NAME-treated, 8±1 mmHg).

For the control SHRs, the plasma ANG II concentration before starting the infusion did not differ significantly between the pregnant and non-pregnant groups (Figure 1). Plasma ANG II was similarly unchanged by pregnancy in the L-NAME-treated SHRs (Figure 1). However, treatment with L-NAME increased the basal ANG II concentration in both non-pregnant (P < 0.0005) and pregnant (P < 0.005) SHRs.

The MCR for ANG II was decreased in pregnant controls compared with non-pregnant controls (P < 0.005; Figure 2, upper panel). In contrast, in the L-NAME-treated SHRs, pregnancy resulted in a significant increase in the MCR for ANG II (P < 0.05). Thus treatment with L-NAME significantly decreased the MCR for ANG II in non-pregnant SHRs (P < 0.05),
but increased the MCR for ANG II in pregnant SHRs ($P < 0.01$).

As ANG II is cleared from the circulation predominantly by filtration at the glomerulus and subsequent metabolism on the brush border of the proximal tubule [28], and since the glomerular filtration rate (as shown by endogenous creatinine clearance) was affected by both pregnancy and treatment with L-NAME, the MCR was also assessed after correction for the glomerular filtration rate (Figure 2, lower panel). For the control SHRs, the MCR corrected for glomerular filtration rate decreased from $122.6 \pm 9 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}$ in the non-pregnant group to $51.7 \pm 2.6 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}$ in the pregnant group ($P < 0.005$). In contrast, for the L-NAME-treated SHRs, MCR corrected for glomerular filtration rate increased from $68.5 \pm 6 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}$ in the non-pregnant SHRs to $93.4 \pm 8 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}$ in the pregnant SHRs ($P < 0.005$). Thus treatment with L-NAME significantly decreased the MCR for ANG II in non-pregnant SHRs ($P < 0.05$), but increased the MCR for ANG II (corrected for glomerular filtration rate) in pregnant SHRs ($P < 0.01$).

**Figure 4** ANG II receptor numbers (upper panel) and dissociation constants (lower panel) in the aorta for control and L-NAME-treated SHRs

Data for non-pregnant SHRs are depicted by the open bars, and those for pregnant SHRs by the hatched bars. Values are means ± S.E.M. for $n = 8$ SHRs in each group. Significance of difference between non-pregnant and pregnant SHRs: *$P < 0.05$. $P$ values on the graphs represent the significance of differences between the groups indicated by the lines.

**Figure 5** ANG II receptor numbers (upper panel) and dissociation constants (lower panel) in the uterus in control and L-NAME-treated rats

Data for non-pregnant SHRs are depicted by the open bars, and those for pregnant SHRs by the hatched bars. Values are means ± S.E.M. for $n = 8$ SHRs per group. Significance of differences between non-pregnant and pregnant SHRs: upper panel, ******$P < 0.0001$; lower panel, ****$P < 0.001$, ******$P < 0.0001$. $P$ values on the graphs represent the significance of differences between the groups indicated by the lines.

**ANG II receptors**

In control SHRs, pregnancy significantly increased ANG II receptor numbers in the glomerulus ($P < 0.01$; Figure 3, upper panel), while the ANG II receptor number tended to increase in the aorta (Figure 4, upper panel) and to decrease in the myometrium (Figure 5, upper panel), although these changes were not statistically significant. In the L-NAME-treated SHRs, ANG II receptor numbers increased non-significantly in the glomerulus, while there were significant decreases in ANG II receptor numbers in both the aorta ($P < 0.05$) and the myometrium ($P < 0.0001$) with pregnancy.

In the non-pregnant SHRs, treatment with L-NAME decreased the ANG II receptor number in the glomerulus ($P < 0.01$), but increased the ANG II receptor number in
the aorta (P < 0.0005) and myometrium (P < 0.0005). In the pregnant SHRs, glomerular and myometrial ANG II receptor numbers were decreased (P < 0.01 and P < 0.01 respectively), while in the aorta the ANG II receptor number increased (P < 0.05), following treatment with L-NAME.

In all three tissues, the ANG II receptor affinity was decreased or the dissociation constant was increased in pregnant compared with non-pregnant SHRs (Figures 3, 4 and 5, lower panels). However, this increase in dissociation constant only achieved statistical significance for the myometrial (P < 0.0001) and the glomerular (P < 0.0005) receptors.

Similarly, in the L-NAME-treated SHRs, the dissociation constant for the myometrial ANG II receptors was increased in pregnant compared with non-pregnant SHRs (P < 0.001). In the pregnant SHRs, treatment with L-NAME decreased the dissociation constant for the myometrial ANG II receptors (P < 0.001).

**DISCUSSION**

Treatment with L-NAME increased the systolic blood pressure in both non-pregnant and pregnant SHRs, and the observed changes in the levels of ANG II and its receptor in both groups would be consistent with a contributory role in this elevation of blood pressure. In both groups, L-NAME increased the circulating concentration of ANG II and increased the expression of ANG II receptors in the vasculature. Both of these changes would be expected to result in increased vasoconstriction, and thereby to contribute to an elevation of blood pressure. That these changes in ANG II and ANG II receptors contribute, at least in part, to the increase in blood pressure is further supported by other studies, which have demonstrated that the increase in blood pressure that occurs on NO synthase inhibition can be prevented by treatment with angiotensin receptor blockers or inhibitors of angiotensin-converting enzyme [29,30].

Although there was an increase in plasma ANG II levels in both pregnant and non-pregnant SHRs treated with L-NAME when compared with their respective controls, this appears to have been achieved by different mechanisms. In the pregnant control rats, ANG II levels were increased (albeit non-significantly) compared with the non-pregnant controls. This increase occurred in association with a decrease in the MCR for ANG II in the pregnant controls, suggesting that ANG II levels had increased as a result of a decrease in catabolism. Similarly, in the L-NAME-treated non-pregnant SHRs, the increase in ANG II levels compared with those in non-pregnant controls was associated with a decrease in the rate of clearance of ANG II from the circulation. This again suggests that a decrease in ANG II catabolism may underlie the increase in plasma ANG II levels. In contrast, in the pregnant rats treated with L-NAME, the increase in plasma ANG II levels was associated with an increase in the rate of removal of ANG II from the circulation. This increase in MCR compared with that in the pregnant controls would be expected to decrease ANG II levels, suggesting that, in the L-NAME-treated SHRs, ANG II synthesis and/or secretion had increased.

In the pregnant SHRs, in addition to an elevation in blood pressure, other features of the pre-eclamptic syndrome, such as an increase in urinary protein excretion, a decrease in renal function and the occurrence of grand mal seizures (at higher doses of L-NAME), were also present. These features in addition to the increase in blood pressure were not present in non-pregnant rats treated with L-NAME, and may be regarded as specific effects of reduced NO production in pregnancy associated with hypertension. Further, the decrease in renal function and the grand mal seizures may be specific to L-NAME-treated pregnant SHRs, as they have not been reported in normotensive pregnant rats treated with L-NAME [21].

We were unable to demonstrate the increase in pressor sensitivity to infused ANG II reported by other workers [2,5,6,31]. This may reflect the lower dose of L-NAME employed in our study (5 mg·kg⁻¹·day⁻¹) compared with other reports (10 mg·kg⁻¹·day⁻¹). Alternatively, this lack of pressor responsiveness may reflect strain differences as, in the SHR, pregnancy itself did not result in a decrease in pressor sensitivity to infused ANG II, which also contrasts with reports in other rat strains [15–17]. Similar increases in mean arterial pressure in response to infused ANG II were observed in both the pregnant and non-pregnant control groups. This absence of pressor refractoriness in the untreated control groups may reflect the combined effects of a decreased rate of clearance of the infused ANG II from the circulation in association with increased expression of ANG II receptors in the vasculature. However, in pregnant Wistar Kyoto (WKY) rats, pressor refractoriness was preserved while similar changes in vascular ANG II receptor expression and ANG II metabolism were observed [13]. Thus it seems unlikely that these changes underlie the absence of refractoriness to the pressor effects of infused ANG II. The conclusion that changes in circulating ligand clearance and/or receptor expression do not underlie variations in pressor sensitivity to infused ANG II is further supported by the data from the L-NAME-treated SHRs. In the non-pregnant SHRs treated with L-NAME, there was no increase in pressor sensitivity to infused ANG II. This was the case despite a decrease in the rate of clearance of ANG II from the circulation and an increase in ANG II receptor expression in the vasculature, both changes which might be predicted to result in an increase in pressor sensitivity. Similarly, in the pregnant SHRs, treatment with L-NAME did not elicit the increase in pressor sensitivity reported by other
workers [2,5,6,31]. In these rats, the rate of clearance of ANG II from the circulation was accelerated rather than decreased, and there was an increase in ANG II receptor numbers in the vasculature compared with untreated pregnant SHRs, both of which might be predicted to decrease pressor sensitivity.

We conclude that changes in circulating ANG II levels and in the expression of ANG II receptors in the vasculature may participate in the elevation of blood pressure that occurs during treatment with NO synthase inhibitors. However, it appears that changes in these parameters are not involved in alternations in pressor responsiveness to infused ANG II. Further, treatment of pregnant SHRs with 1-NAME decreased the glomerular filtration rate, increased urine protein excretion, and caused hypertension and grand mal seizures, which are all features of the pre-eclamptic syndrome.

REFERENCES