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Demonstration of the existence of nitric oxide-independent as well as nitric oxide-dependent vasodilator mechanisms in the *in situ* renal circulation in near term pregnant rats

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1 We have investigated the role of endogenous nitric oxide on renal vascular reactivity in late pregnancy in *in situ* blood perfused kidneys of α-chloralose anaesthetized Wistar-Kyoto rats. Nitric oxide synthesis inhibition was achieved by intravenous administration of N^G-nitro-L-arginine or N^G-nitro-L-arginine methyl ester.

2 Intra-arterial mean blood pressure was lower in pregnancy compared with nonpregnant controls. Following nitric oxide synthesis inhibition mean blood pressure increased in both pregnant and nonpregnant groups, but remained lower in pregnant animals.

3 Basal renal perfusion pressure was similar in pregnant and nonpregnant groups. Intravenous administration of N^G-nitro-L-arginine resulted in dose-dependent increases in renal perfusion pressure but responses were substantially depressed in pregnancy.

4 Renal vasoconstrictor responses to regional angiotensin II (AII) were decreased in pregnancy, whereas those to noradrenaline (NA) did not differ from nonpregnant controls. N^G-nitro-L-arginine (5 mg kg^{-1}) potentiated renal responses to regional AII and NA in both groups, but AII responses remained lower in pregnancy. Blunted renal AII responses in pregnancy were still evident following large doses of N^G-nitro-L-arginine methyl ester (100 mg kg^{-1}).

5 The results demonstrate that nitric oxide synthesis inhibition increases renal perfusion pressure to a lesser extent in pregnant compared with nonpregnant rats, and that reduced renal pressor responses to AII are still evident in pregnancy after nitric oxide synthesis inhibition.

6 These results suggest that although endogenous nitric oxide synthesis modulates renal vasoconstrictor responses in both pregnant and nonpregnant animals, this mechanism does not fully account for the blunted renal vasoconstrictor responses to regional AII or nitric oxide inhibitors in near term pregnant rats. The nature of this important physiological vasodilator mechanism in pregnancy remains to be elucidated.

Keywords: Vascular reactivity; nitric oxide; kidney; pregnancy

Introduction

Pregnancy is associated with generalized maternal vasodilatation by mechanisms which have not been fully elucidated. It has been claimed that endogenous NO is the critical mediator of this phenomenon (Deng *et al*., 1996). Evidence that increased endogenous nitric oxide (NO) synthesis contributes to the vasodilatation of pregnancy comes from findings of increased urinary excretion and plasma concentration of NO metabolites in pregnant rats (Conrad *et al*., 1993), increased NO synthase activity in pregnant guinea-pigs (Weiner *et al*., 1994). Also studies in conscious pregnant rats have shown that systemic administration of a potent NO synthase inhibitor restored the lower mean blood pressure (MBP) and blunted systemic pressor responses to various vasoconstrictor agonists to nonpregnant values (Molnar & Hertelendy, 1992). However, there is also evidence suggesting that NO synthesis alone cannot account for pregnancy-induced vasodilatation. For example, late pregnancy did not alter the pressor effect of N-methylarginine, another NO synthase specific inhibitor in conscious rats (Umans *et al*., 1990). In isolated perfused mesenteric vessels endothelium-dependent relaxation to acetylcholine was unchanged in late pregnant rats and reduced vasoconstrictor responses to electrical field stimulation (ES), vasopressin and endothelin could not be restored to nonpregnant levels by NO synthesis inhibition with N^G-nitro-L-arginine methyl ester (L-NAME) (Ralevic & Burnstock, 1996). Moreover, our previous studies with an *in situ* blood perfused mesentery of the rat showed that lower MBP and blunted responses to ES and angiotensin II (AII) in pregnancy were still evident following NO synthesis inhibition by N^G-nitro-L-arginine (L-NOARG), while only the decreased responses to noradrenaline (NA) were restored to those of L-NOARG-treated nonpregnant animals (Chu & Beilin, 1993). These previous investigations suggest that enhanced endogenous NO synthesis may only be partly responsible for the reduced vasoconstrictor reactivity in pregnancy and that NO-independent vasodilator mechanisms may also be involved in different vascular beds. Furthermore, prostaglandins are unlikely to be involved as cyclo-oxygenase inhibition failed to reverse either the decreased systemic presser responses (Conrad & Colpoys, 1986) or blunted mesenteric vascular responses in pregnant rats (Chu & Beilin, 1993).

As the kidney plays a key role in blood pressure regulation (Bachmann & Mundel, 1994), and disturbances of renal function occur early in pre-eclampsia (Whitworth & Brown, 1993; Gaber *et al*., 1994), it is important to understand factors normally controlling renal vascular tone in pregnancy. Danielson & Conrad, (1995) showed that the increases of renal blood flow and glomerular filtration rate (GFR) reach their peak during late in the first or early in the second trimester in pregnant rats and are restored by NO synthesis inhibition, while renal blood flow returns to nonpregnant levels late term (Conrad, 1984). Control of the renal circulation in late pregnancy in the rats is of particular interest, as this is the time at which the maximum blood pressure fall occurs in this species

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(Recklhoff *et al*., 1992) and it is also the time in man when pre-eclampsia is most likely to manifest. In the present study we have used an *in situ* blood perfused renal preparation to determine whether the characteristics of the depressed vasoconstrictor reactivity in response to AII and NA previously seen in the mesenteric circulation (Chu & Beilin, 1993) are shared by the kidney, and to assess the extent to which renal vascular responses in pregnancy are dependent on changes in endogenous NO synthesis.

Methods

Pregnant rat models

Eighteen to 20 day pregnant and age matched (12–14 week old) nonpregnant female Wistar-Kyoto (WKY) rats, weighing 180–220 g before mating, were used. Pregnancy was produced by mating oestrous phase female rats with a normal male rat. The day on which the plug was found was labelled day 1 of pregnancy. Gestation was further confirmed by examination of the uterus during experimentation.

The in situ *blood perfused renal preparation*

The *in situ* blood perfused renal preparation (Figure 1) involves cannulation of the renal artery via the mesenteric artery. This aspect of the procedure was originally devised by Schurek *et al*. (1975) to avoid renal ischaemia when studying isolated perfused kidneys. Rats were anaesthetized with α-chloralose (150 mg kg^{-1}) administered subcutaneously. After a tracheal cannula had been inserted and the left carotid artery had been isolated, the left femoral artery and vein were cannulated for MBP measurement and continuous supplementation of 0.9% NaCl (0.1 ml min^{-1}), respectively. Then an abdominal midline incision was made, and the superior mesenteric artery and right renal artery were isolated. Complete haemostasis was achieved by using electrical cautery before administration of heparin (1500 units kg^{-1}, i.v.). After the left carotid artery had been cannulated and the perfusion line filled with blood, the distal end of the superior mesenteric artery was ligated and the proximal side of the superior mesenteric artery was cannulated with the cannula pointing to aorta. Blood perfusion was begun from the left carotid artery to the aorta by a roller pump at a constant flow. After around 5 minutes had been allowed to ensure establishment of the blood perfusion line the mesenteric arterial cannula was fed through the aorta into the right renal artery. The right renal artery was then tied proximal to the site of insertion of the cannula so that blood only entered the renal artery from the roller pump. A small stainless steel cannula (23 Guage) was used for the cannulation so that aortic blood flow was not significantly influenced. Finally, the mesenteric vein was ligated to prevent release of vasoactive substances into the systemic circulation from the now ischaemic mesentery. The abdomen was covered with moistened gauze and the body temperature was maintained at 37°C.

Renal blood flow was not interrupted during the above surgical procedure. Renal perfusion pressure was measured by a side arm connecting to a pressure transducer. Both MBP and renal perfusion pressure were monitored and recorded with a Grass Model 7B Polygraph. Changes in perfusion pressure indicated alterations in renal vascular resistance in the constant flow perfusion system. The preparation was allowed to equilibrate for 30 min before examination of renal vascular reactivity. In this preparation, MBP and basal renal perfusion pressure were stable, and contractile responses to regional NA (1–30 ng) and AII (0.3–3 ng) were reproducible for up to 2 h. The administration of NA and AII into the renal circulation at the doses used in the present study had no significant effects on systemic blood pressure. At the end of the experiment the perfused kidney was removed, blotted dry and weighed.

The effects of NO synthesis inhibition by L-NOARG (5 mg kg^{-1}) on dose-dependent renal responses to NA (3–30 ng) and AII (0.3–3 ng) were initially examined at a renal flow rate of 2 ml min^{-1}, corresponding to around 3 ml min^{-1} g^{-1} kidney. This flow rate resulted in basal perfusion pressures of around 50 mmHg in nonpregnant rats (Table 2). However, as these pressures are below the autoregulatory range and as renal blood flow is normally around 7.5 ml min^{-1} g^{-1} kidney weight in anaesthetised rats (Deng *et al*., 1995), we performed a further series of experiments at flow rates of 5 ml min^{-1} (corresponding to around 7.2 ml min^{-1} g^{-1} kidney weight) which produced renal perfusion pressures of 80–90 mmHg.

In the first set of experiments a dose of L-NOARG of 5 mg kg^{-1} was used to enable a direct comparison to be made with previous experiments on the mesenteric circulation in pregnancy (Chu & Beilin, 1993). In separate experiments larger doses of L-NOARG (up to 100 mg kg^{-1}) or nitro-L-arginine methyl ester (L-NAME 100 mg kg^{-1}), another NO synthesis

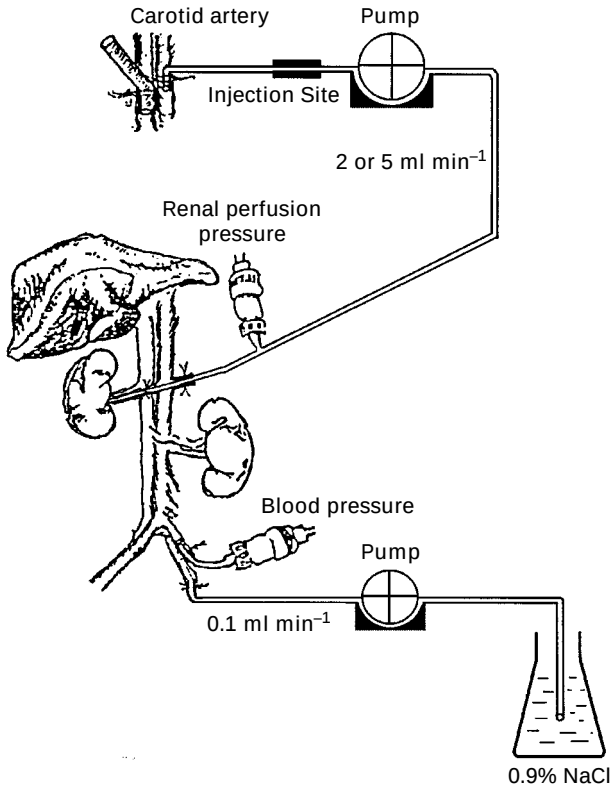

Figure 1 An *in situ* blood perfused renal preparation in an α-chloralose anaesthetized rat. Blood was pumped from left carotid to right renal artery by a roller pump at a constant flow rate of 2 or 5 ml min^{-1}. The renal cannula was a small stainless steel tube which passed through the abdominal aorta by way of the superior mesenteric artery. The renal perfusion pressure was measured by connection of a side arm to a pressure transducer. The left femoral artery was cannulated for mean blood pressure measurement. Mean blood pressure recorded from left femoral artery was not changed after insertion of renal cannula through the abdominal aorta indicating that blood supply to the uterus was not influenced by the renal cannula. The left femoral vein cannula was for continuous fluid supplementation.

Table 1 Effect of NO synthesis inhibition on mean blood pressure (MBP) (mmHg) in pregnant and nonpregnant rats

	L-NOARG		*L-NAME*	
	Before	*After*	*Before*	*After*
Pregnant	93.1±4.9*	131.3±4.8*#	96.5±4.5*	141.5±2.6*#
Nonpreg	112.9±3.5	145.4±3.8#	119.2±3.0	150.3±2.8#

*$P<0.05$ vs nonpregnant group. #$P<0.05$ vs before L-NOARG (5 mg kg^{-1}) or L-NAME (100 mg kg^{-1}).

inhibitor that is more water soluble, were administered to ensure that the results observed were not due to inadequate inhibition of NO synthesis. Both L-NOARG and L-NAME have been shown to induce maximal systemic pressor responses in the rat at doses of 100 mg kg^{-1} (i.v.) (Rees *et al*., 1990), suggesting that maximum inhibition of NO synthesis has occurred at this dose.

Table 2 Effect of NO synthesis inhibition on basal renal perfusion pressure (BRPP) (mmHg) in pregnant and nonpregnant rats

	L-NOARG		*L-NAME*	
	Before	*After*	*Before*	*After*
Pregnant	46.4±2.1	107.2±9.5*#	48.0±1.4	92.33±9.6*#
Nonpreg	50.9±2.1	140.5±11.1#	47.0±0.9	153.5±10.0#

*$P<0.05$ vs nonpregnant group. #$P<0.05$ vs L-NOARG or L-NAME.

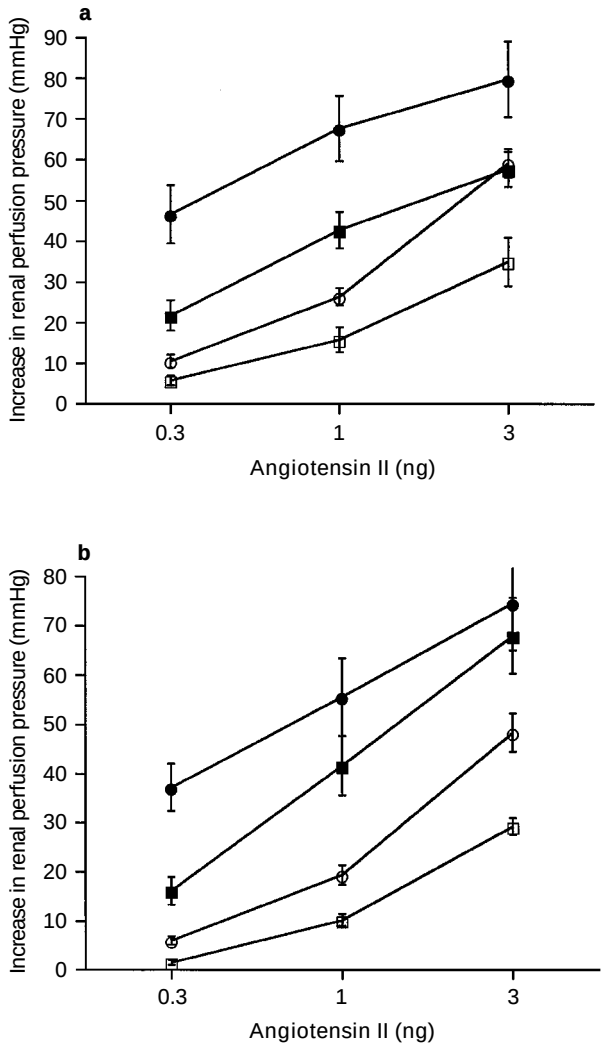

Figure 2 (a) Renal vasoconstrictor responses to local administration of angiotensin II (AII) were decreased in pregnant (□, $n=9$) compared with nonpregnant (○, $n=11$, $P<0.05$) rats. L-NOARG (5 mg kg^{-1}, i.v.) significantly enhanced the AII responses in both pregnant (■, $n=9$, $P<0.05$) and nonpregnant (●, $n=12$, $P<0.05$) groups. The AII responses were still depressed in pregnant (■, $n=9$) compared with nonpregnant (●, $n=12$, $P<0.05$) groups even after L-NOARG. (b) Renal vasoconstrictor responses to local administration of AII were decreased in pregnant (□, $n=9$) compared with nonpregnant (○, $n=11$, $P<0.05$) rats. L-NAME (100 mg kg^{-1}, i.v.) significantly potentiated the AII responses in both pregnant (■, $n=9$, $P<0.05$) and nonpregnant (●, $n=11$, $P<0.05$) groups, but did not make the responses equal between the groups ($P<0.05$). Vertical lines show s.e.mean.

Renal vascular reactivity with flow rates of 2 ml min^{-1}

Renal vasoconstrictor responses to AII Vasoconstrictor responses to AII (0.3–3 ng) were obtained by (0.01 ml) local bolus injections into the renal perfusion line at 10 min intervals. Those injections of AII were repeated commencing 30 min after L-NOARG or (5 mg kg^{-1}, i.v.) or L-NAME (100 mg kg^{-1}, i.v.).

Renal vasoconstrictor reactivity to NA Vasoconstrictor reactivity to NA (1–30 ng) was examined by (0.01 ml) local bolus injections into the renal perfusion line at 3 min intervals. Injections of NA were again repeated commencing 30 min after L-NOARG (5 mg kg^{-1}, i.v.). Larger doses of NO inhibitors were not used here as renal responses to NA were unchanged in pregnancy.

Renal vasoconstrictor responses to systemic NO blockade To examine possible differences in the threshold dose of L-NOARG and the maximal renal responses to systemic NO synthesis inhibition, dose-dependent renal vasoconstrictor responses to intravenous bolus injection of L-NOARG (0.3, 0.7, 2, 7 mg kg^{-1}, i.v.; corresponding to cumulative doses of 0.3, 1, 3, 10 mg kg^{-1}, respectively) were constructed at 30 min intervals. Renal vasoconstrictor responses to larger doses of L-NOARG (cumulative dose up to 30–100 mg kg^{-1}) were tested in different groups of animals by use of a similar protocol, except L-NOARG solution was administered by continual intravenous infusion to avoid problems related to the poor water solubility of L-NOARG.

Renal vascular reactivity with flow rates of 5 ml min^{-1}

Renal responses to NA (10 ng) and AII (1 ng) were examined before and 15 min after each of three doses of L-NOARG (0.5, 1.0. 3.5 mg kg^{-1}, i.v., corresponding to cumulative doses 0.5 mg kg^{-1}, 1.5 mg kg^{-1} and 5 mg kg^{-1}, respectively).

Drugs and solutions

NA, AII, L-NOARG, L-NAME and α-chloralose were purchased from Sigma chemicals. Heparin sodium was purchased

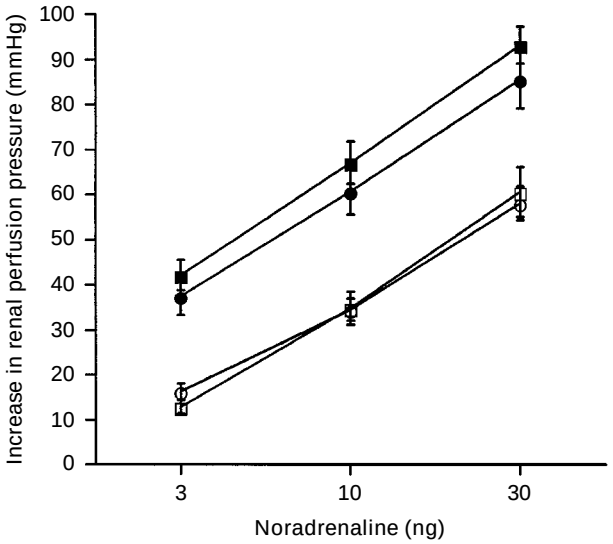

Figure 3 Renal vasoconstrictor reactivity to regional noradrenaline (NA) was similar in pregnant (□, $n=12$) and nonpregnant (○, $n=12$) rats. L-NOARG (5 mg kg^{-1}, i.v.) significantly potentiated renal NA responses in both pregnant (■, $n=12$, $P<0.05$) and nonpregnant (●, $n=12$, $P<0.05$) groups. Vertical lines show s.e.mean.

from Delta West, Western Australia. Chemicals were dissolved in 0.9% NaCl and solutions were made up daily.

Statistical analysis

Results are expressed as means ± s.e.mean with n representing the number of animals. To test the statistical differences in dose-dependent renal responses to various agonists the area under individual curves was first mathematically calculated as described previously (Matthew *et al*., 1990) and the means of the areas under the curves were used for comparisons. MBP, basal renal perfusion pressure, and dose-dependent renal responses before and after NO inhibition in pregnant and nonpregnant groups were analysed with one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. A P value of less than 0.05 was considered significant. Renal responses to a standard dose of NA or AII were compared between pregnant and nonpregnant rats with Student's unpaired t test.

Results

Body weight, kidney weight and blood pressures

Body weight was greater in pregnant (274.5 ± 4.3 g, $n=12$) than nonpregnant animals (202.6 ± 3.5 g, $n=12$, $P<0.01$). Kidney weights were similar between groups (pregnant: 0.740 ± 0.031 g; nonpregnant: 0.737 ± 0.032 g, $n=12$). MBP was significantly lower in pregnant than in nonpregnant rats

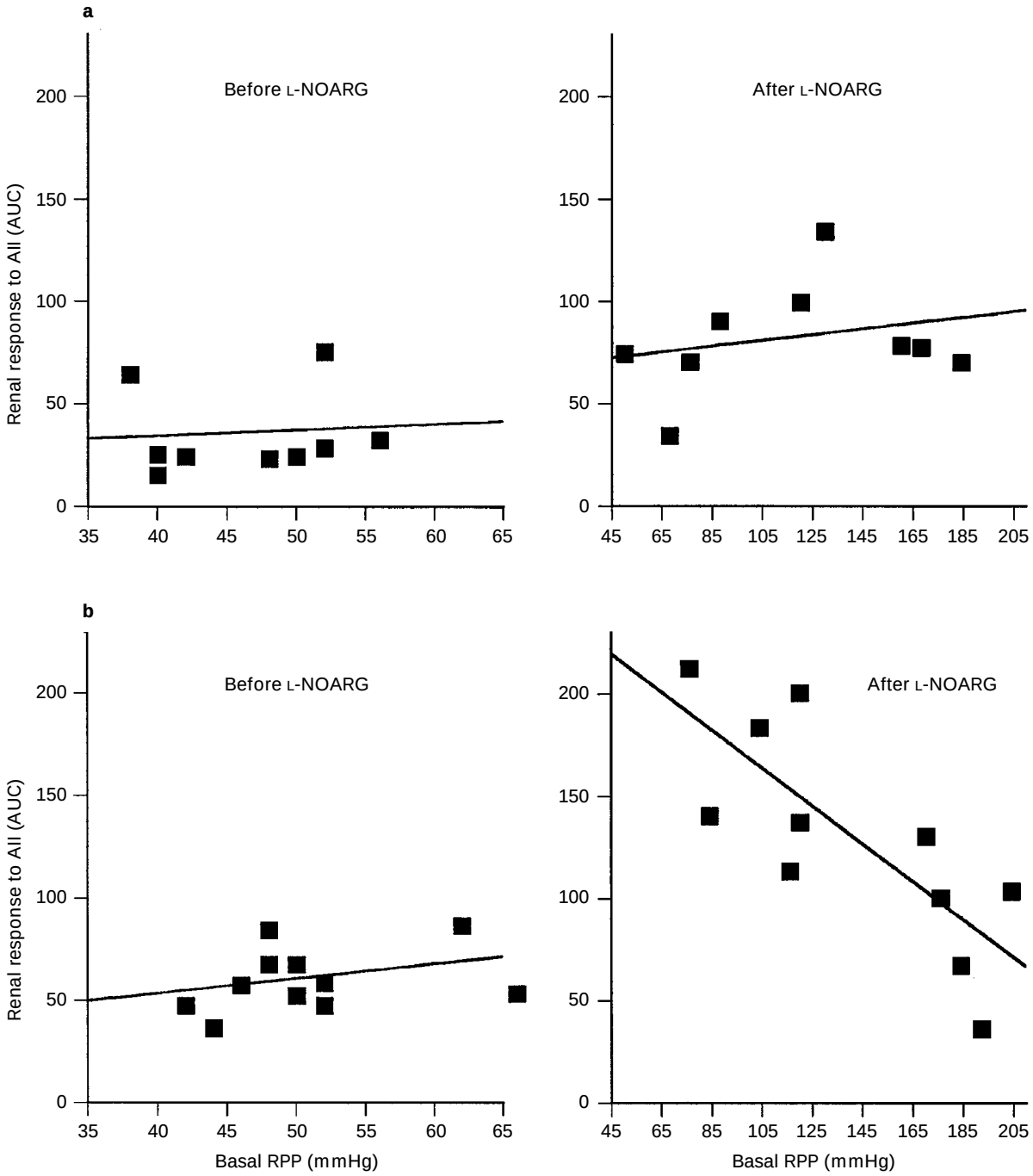

Figure 4 There was no correlation between basal renal perfusion pressure (BRPP) and renal vasoconstrictor responses to angiotensin II (AII) (area under the curve, AUC) in pregnant rats before or after NO inhibition (a). However, there was a negative correlation between BRPP and AII responses (AUC) in the nonpregnant group after ($n=11$, $r=-0.78$, $P<0.01$), but not before, NO synthesis inhibition by L-NOARG (5 mg kg^{-1}) (b).

(Table 1). Both L-NOARG (5 mg kg^{-1}, i.v.) and L-NAME (100 mg kg^{-1}, i.v.) increased MBP in pregnant and nonpregnant groups, but MBP was still significantly lower in pregnant than nonpregnant animals after NO synthesis inhibition.

Renal vascular reactivity with renal perfusion flow of 2 ml min^{-1}

Effects of NO synthesis inhibition on responses to AII Before inhibition of NO synthesis basal renal perfusion pressure was not significantly reduced in pregnant compared with nonpregnant controls. After NO synthesis inhibition by L-NOARG (5 mg kg^{-1}, i.v.) or L-NAME (100 mg kg^{-1}, i.v.) basal renal perfusion pressure increased in both pregnant ($P<0.01$) and nonpregnant ($P<0.01$) animals (Table 2), but the increases were substantially less in pregnancy ($P<0.01$).

Renal responses to AII were reduced in pregnant (mean of the areas under the curves $=36.4\pm6.5$, $n=9$) compared with nonpregnant rats (mean of the areas under the curves $=61.5\pm4.4$, $n=11$, $P<0.05$) (Figure 2). L-NOARG (5 mg kg^{-1}, i.v.) enhanced renal AII responses in both pregnant (mean of the areas under the curves $=82.7\pm8.4$, $P<0.01$) and nonpregnant (mean of the areas under the curves $=131.2\pm15.6$, $P<0.01$) groups, but did not restore the blunted responses to AII in pregnancy ($P<0.05$) (Figure 2a). L-NAME (100 mg kg^{-1}, i.v.) also enhanced renal AII response

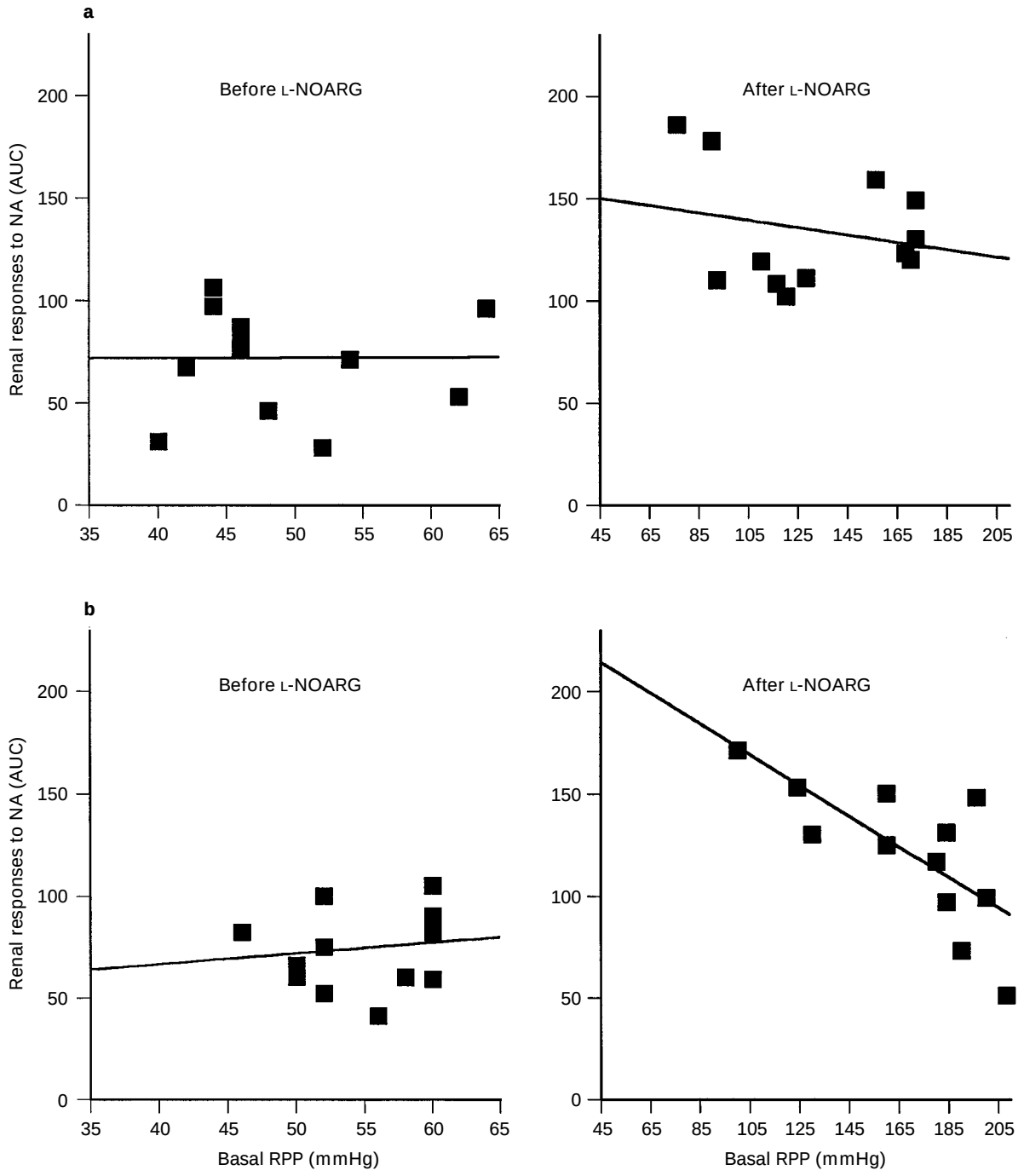

Figure 5 There was no correlation between basal renal perfusion pressure (BRPP) and renal vasoconstrictor responses to noradrenaline (NA) (area under the curve, AUC) in pregnant rats before or after NO inhibition (a). In contrast, a negative correlation between BRPP and responses to NA (AUC) was seen in the nonpregnant group after ($n=12$, $r=-0.72$, $P<0.05$), but not before, NO inhibition by L-NOARG (5 mg kg^{-1}) (b).

in both pregnant and nonpregnant groups and again did not restore the blunted responses to AII in pregnancy (Figure 2b).

NO synthesis inhibition and renal responses to NA Before inhibition of NO synthesis basal renal perfusion pressure was not significantly reduced in pregnant (49.0 ± 2.1 mmHg) compared with nonpregnant (54.7 ± 1.4 mmHg, $n=12$) controls. After NO synthesis inhibition by L-NOARG (5 mg kg^{-1}, i.v.) basal renal perfusion pressures showed less of an increase in pregnant (130.8 ± 9.8 mmHg, $n=12$) compared with nonpregnant (168.0 ± 9.4 mmHg, $n=12$) animals ($P<0.01$).

Renal reactivity to NA was similar in pregnant (mean of the areas under the curves $= 72.4 \pm 7.2$, $n=12$) and nonpregnant (mean of the areas under the curves $= 74.7 \pm 5.4$, $n=12$) rats (Figure 3). Following L-NOARG (5 mg kg^{-1}, i.v.) renal NA responses were similarly enhanced in both pregnant (mean of the areas under the curves $= 134.9 \pm 7.8$, $P<0.01$) and nonpregnant (mean of the areas under the curves $= 122.4 \pm 9.7$, $P<0.01$) rats.

Relation between basal renal perfusion pressure before and after inhibition of NO synthesis and subsequent renal vasoconstrictor responses To exclude the possibility that decreased renal vasoconstrictor responses to AII in pregnancy were secondary to lower basal renal perfusion pressure in pregnant compared with nonpregnant groups, the correlation between basal renal perfusion pressure and renal vasoconstrictor responses to AII (Figure 4) and NA (Figure 5) were examined. Before NO inhibition there was no significant correlation between basal renal perfusion pressure and vasoconstrictor responses to either AII (Figure 4a) or NA (Figure 5a). Following NO inhibition (5 mg kg^{-1}, i.v.) renal vasoconstrictor responses to AII (Figure 4b) or to NA (Figure 5b) were negatively correlated with basal renal perfusion pressure in nonpregnant, but not in pregnant animals. This suggests that blunted renal responses to AII in pregnancy after NO inhibition are not due to differences in renal perfusion pressure after NO inhibition.

Renal dose-dependent responses to systemic L-NOARG Renal vasoconstrictor responses to L-NOARG (0.3, 0.7, 2, 7 mg kg^{-1}, i.v.; corresponding to cumulative doses of 0.3, 1, 3 and 10 mg kg^{-1}, respectively) were substantially reduced in pregnant (mean of the areas under the curves $= 118.3 \pm 9.6$, $n=7$) compared with nonpregnant (mean of the areas under the curves $= 275.5 \pm 30.0$, $n=7$, $P<0.01$) rats at all dose levels (Figure 6a). Renal responses to larger doses of L-NOARG (cumulative doses: 30–100 mg kg^{-1}, i.v. infusion) were also lower in pregnant (mean of the areas under the curves $= 61.2 \pm 10.6$, $n=7$) compared with nonpregnant (mean of the areas under the curves $= 139.4 \pm 4.0$, $n=7$, $P<0.01$) groups (Figure 6b).

Renal vascular reactivity with a renal flow rate of 5 ml min^{-1}

Basal renal perfusion pressure was similar in pregnant (82 ± 4 mmHg) and nonpregnant (90 ± 3 mmHg, $n=8$) groups. Bolus injection of L-NOARG (0.5, 1, 3.5 mg kg^{-1}, i.v.; corresponding to cumulative doses: 0.5, 1.5, 5 mg kg^{-1}, respectively) increased renal perfusion pressure in a dose-dependent manner, but the effects were significantly less in pregnant (mean of the areas under the curves $= 110.8 \pm 16.4$) than in nonpregnant animals (mean of the areas under the curves $= 223.5 \pm 14.3$, $P<0.01$, $n=8$) (Figure 7a).

Renal responses to AII (1 ng) were blunted in pregnancy (Figure 7b). The lowest dose of L-NOARG (0.5 mg kg^{-1}) enhanced renal AII responses in both pregnant and nonpregnant groups. This effect was obscured by the profound renal vasoconstriction after larger doses of L-NOARG (cumulative doses: 1.5, 5 mg kg^{-1}) in nonpregnant rats, which led to depressed renal responses to AII.

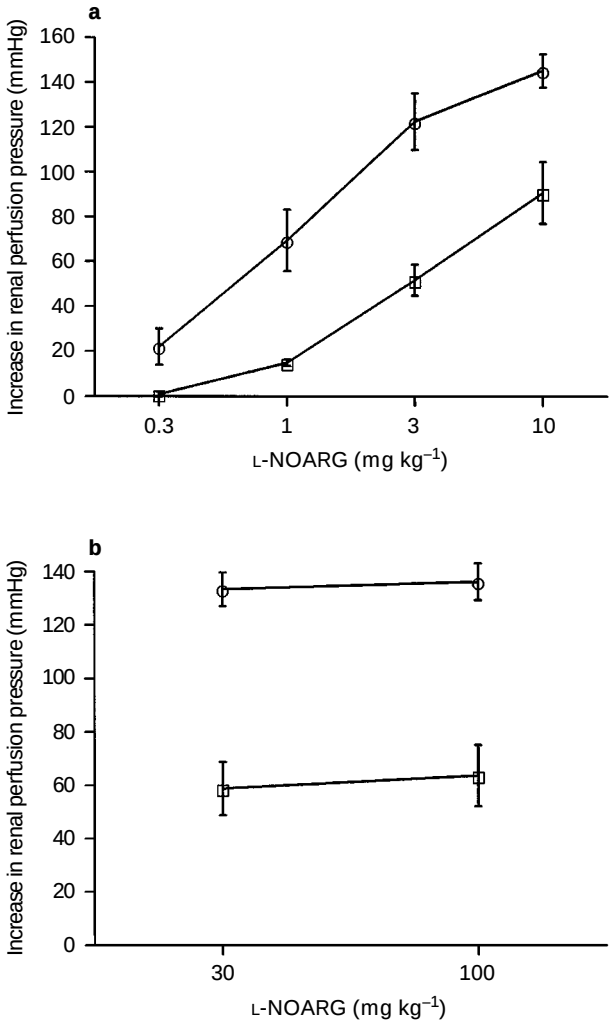

Figure 6 (a) Renal vasoconstrictor reactivity to intravenous injection of L-NOARG (0.3, 0.7, 2, 7 mg kg^{-1}; corresponding to cumulative doses: 0.3, 1, 3 and 10 mg kg^{-1}, respectively) was decreased in pregnant (□, $n=7$) compared with nonpregnant (○, $n=7$, $P<0.01$) rats. (b) Renal vasoconstrictor responses to intravenous infusion of L-NOARG (up to 30–100 mg kg^{-1}) were depressed in pregnant (□, $n=7$) compared with nonpregnant (○, $n=7$, $P<0.01$) rats.

There were no significant differences in NA responses between pregnant and nonpregnant groups before or after L-NOARG (cumulative doses: 0.5–1.5 mg kg^{-1}) (Figure 7c). L-NOARG dose-dependently enhanced NA responses but following larger dose of L-NOARG (cumulative dose: 5 mg kg^{-1}) the effect was again obscured by profound renal vasoconstriction in nonpregnant rats.

After inhibition of NO synthesis, renal perfusion pressure was much greater in nonpregnant than pregnant rats. As renal perfusion pressures were negatively correlated with renal responses to AII we have presented the absolute renal perfusion pressures in response to AII (Figure 7d). This shows the lower absolute renal pressure achieved following AII in pregnant rats.

Discussion

The present study presents two lines of evidence indicating the existence of renal vasodilator mechanisms which are independent of NO synthesis in late pregnancy. Firstly, renal vascular responses to AII were decreased compared to nonpregnant controls, and remained so after NO synthesis inhibition. Secondly, renal pressor responses to inhibitors of NO synthesis

were substantially lower in pregnant animals. The diminished renal responsiveness to AII was seen at both maximal and submaximal doses of inhibitors of NO synthesis and at renal perfusion pressures both within and below the autoregulatory range.

We considered the possibility that the persistence of reduced responses to AII after NO inhibition in pregnancy might be due to the lower basal perfusion pressures prevailing. However, following inhibition of NO synthesis, basal renal perfusion pressure was inversely correlated with vasoconstrictor responses to AII in non-pregnant animals only, an effect which would, if anything, tend to mask reductions in responsiveness to AII in pregnancy.

The lack of reduction in renal sensitivity to NA in late pregnancy contrasts with the changes we found in the mesenteric circulation (Chu & Beilin, 1993) in which vasoconstrictor responses to both AII and NA were impaired and the NA responses were restored to normal by NO synthesis inhibition. This indicates that there are important regional differences in the changes in vascular reactivity in pregnancy which could influence the ultimate pattern and timing of any changes in systemic arterial pressures. Interestingly in this respect the renal circulation of the rat apparently behaves more like the human systemic circulation in pregnancy (Gant *et al*., 1973).

The present findings that renal AII, but not NA responses were blunted in pregnant rats suggests changes in AII receptors or in post receptor signalling. Although it has been suggested that AII receptor down-regulation may play a role (Conrad *et al*., 1989) due to activation of renin-angiotensin system in pregnancy, other mechanisms may be operating. For example Carroll *et al*. (1996) demonstrated that AII stimulated the release of vasodilator arachidonic acid metabolites from the cytochrome P-450 pathway in rat isolated perfused kidneys. Some of these metabolites may function as endothelial hyperpolarizing factors (Campbell *et al*., 1996), increased synthesis of which could counterbalance the vasoconstrictor effects of AII. Furthermore, in the uterine circulation in pregnancy, AII causes vasodilatation, which is thought to be related to phospholipase-mediated prostaglandin E_2 (PGE_2) and prostacyclin synthesis (Schramek *et al*., 1995). Increased activation of these pathways in renal vasculature in pregnancy could account for impaired vasoconstrictor responses to AII.

The observation that renal pressor responses to systemic NO synthesis inhibitors were substantially reduced in pregnancy further indicates the operation of a NO-independent vasodilator mechanism(s) in the renal circulation in pregnant WKY. This finding is largely consistent with those of Losonczy *et al*. (1996) in anaesthetized late-pregnant rabbits. They showed that although NO synthesis inhibition increased MBP more in pregnancy, the percentage increases in total peripheral vascular resistance were depressed. Decreases in renal blood flow and increases in renal vascular resistance index in re-

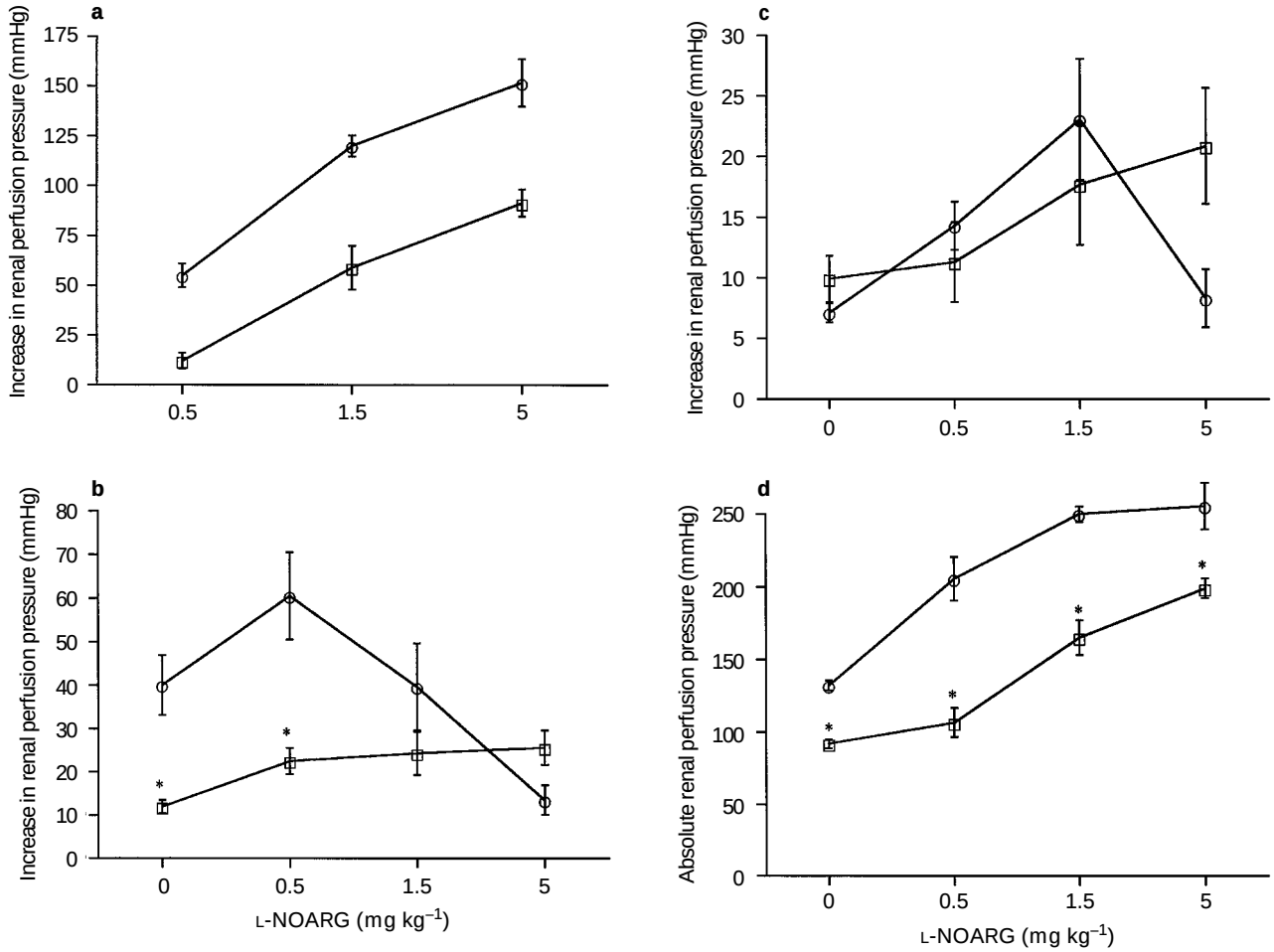

Figure 7 Results from the preparations with renal perfusion flow of 5 ml min^{-1}. (a) Renal perfusion pressure was significantly decreased after L-NOARG (0.5, 1, 3.5 mg kg^{-1}, i.v.; corresponding to cumulative doses: 0.5, 1.5 and 5 mg kg^{-1}, respectively) in pregnant (□, $n=8$) compared with nonpregnant (○, $n=8$) groups ($P<0.01$). (b) Renal responses to local bolus injection of AII (1 ng) were blunted in pregnant (□, $n=8$) compared with controls (○, $n=8$) before and after L-NOARG (0.5 mg kg^{-1}) (*$P<0.01$). (c) There was no significant difference in renal NA (10 ng) responses between pregnant (□, $n=8$) and nonpregnant (○, $n=8$) groups. (d) Renal vasoconstrictor responses to AII expressed as absolute value (background renal perfusion pressure plus increase in renal perfusion pressure in response to AII) were significantly depressed in pregnant (□, $n=8$) compared with nonpregnant (○, $n=8$) rats (*$P<0.01$).

sponse to L-NAME also tended to be less in pregnancy. Their results suggest that, as in our late-pregnant rats, endogenous NO synthesis has less impact on vascular tone in late-pregnant rabbits and that an as yet unidentified mechanism is involved in pregnancy-induced maternal vasodilatation.

Our findings and those of Losonczy *et al*. (1996) differ in some respects from those of Danielson & Conrad (1995) for they used chronically instrumented, conscious 12–14 day pregnant rats and showed that baseline glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were significantly increased, and effective renal vascular resistance was decreased by 30–40% compared with nonpregnant controls. Moreover, during acute infusion of L-NAME (2–50 μg min^{-1}) or N^{G}-monomethyl-L-arginine (L-NMMA, 100 μg min^{-1}), effective renal vascular resistance, GFR and ERPF were equalized in pregnant and nonpregnant rats (the only exception being GFR during the 20 μg min^{-1} L-NAME infusion), because pregnant rats showed a greater decline in GFR, ERPF and elevation in effective renal vascular resistance at each timepoint during the infusion of the NO synthesis inhibitor. They, therefore, concluded that NO mediates the reduced renal vascular resistance and hyperfiltration during mid-pregnancy in conscious rats. The factors that contribute to the differences between our results and those of Danielson and Conrad (1995) may not only include the use of anaesthesia and surgery which may amplify some non-NO mediated vasodilator mechanism, but gestational age. Thus, by using micropuncture Baylis & Reckelhoff (1991) demonstrated that renal blood flow in pregnant rats was maximal at around 12 days gestation and then returned to nonpregnant levels until term. Since intravascular flow is a substantial stimulus for endogenous NO synthesis (Miller & Vanhoutte, 1988) this may enhance the contribution of endogenous NO to renal vasodilatation in mid pregnancy. In contrast, in our experiments and those of Losonczy *et al*. (1996), which were conducted close to term at 18 to 20 days gestation, renal blood flow and flow-induced changes in NO synthesis may have returned towards normal, leaving non-NO mediated mechanisms to predominate. Further investigations with animals at different gestational stages may help to clarify this issue.

Deng *et al*. (1996) have shown that the increased NO production that occurs in normal pregnancy was relatively resistant to inhibition. However, this is unlikely to account for the blunted renal vasoconstrictor responses to NO blockade in our experiments, as the difference between pregnant and nonpregnant animals persisted in the presence of very high doses of L-NAME (100 mg kg^{-1}, i.v.) or L-NOARG (infusion up to 100 mg kg^{-1}). Rather, we suggest that the non-NO-mediated mechanism that accounts for the decreased responses to AII may also be responsible for the decreased effect of the NO inhibitors in pregnancy.

In summary, renal vasoconstrictor responses to regional AII and systemic NO blockade, but not to regional NA, are decreased in late-pregnant anaesthetized rats. Systemic NO synthesis inhibition increased mean blood pressure and renal perfusion pressure as well as potentiating renal vasoconstrictor responses to regional AII and NA in both pregnant and nonpregnant rats, but did not completely restore the decreased MBP or reverse the blunted renal responses to AII in pregnancy. The present results suggest that, although endogenous NO plays a role in the modulation of renal vascular tone in both pregnant and nonpregnant rats, it is unlikely to be responsible for the blunted renal AII responses in late pregnancy. Coupled with the diminished renal vasoconstrictor responses to systemic NO blockade in pregnant rats, the results suggest that an important, alternative renal vasodilator mechanism is active in near term pregnancy. The precise nature of this remains to be identified, but it is likely to represent an important mechanism for controlling regional blood flow and arterial pressure in both the pregnant and non pregnant state. The ontogeny of vasodilator mechanisms in late- compared with mid-pregnancy may be paralleled in man and may be a further clue as to why renal and other manifestations of pre-eclampsia are most likely to develop in the third trimester.

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