Nitric oxide-mediated changes in vascular reactivity in pregnancy in spontaneously hypertensive rats

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1 To examine the mechanisms which may account for pregnancy-induced vasodilatation in spontaneously hypertensive rats (SHR), we have investigated the changes in vascular reactivity and the effects of endothelial nitric oxide (NO) inhibition in the *in situ* blood-perfused, mesenteric resistance vessels of 18–20 day pregnant SHR. The effects of N^G-nitro-L-arginine (L-NOARG) were compared in pregnant and nonpregnant SHR and gestation matched normotensive Wistar-Kyoto (WKY) rats.

2 Intra-arterial mean blood pressures (MBP) were similar in pregnant and nonpregnant SHR. Basal perfusion pressures (BPP) were decreased in pregnant compared with nonpregnant SHR. Pregnant WKY had lower MBP and BPP than either pregnant or nonpregnant SHR.

3 Vasoconstrictor responses to electrical stimulation (ES) and intra-arterial noradrenaline (NA) were decreased in pregnant compared with nonpregnant SHR. These responses were still greater in pregnant SHR when compared with pregnant WKY. Vascular reactivity to angiotensin II (AII) in pregnant SHR was reduced to a similar level to that in pregnant WKY.

4 L-NOARG (5 mg kg⁻¹, i.v.), an inhibitor of nitric oxide synthase, increased MBP and BPP in all groups. After L-NOARG, BPP were equalized between pregnant and nonpregnant SHR. Pregnant WKY still showed lower MBP and BPP than SHR groups.

5 L-NOARG potentiated vascular responses to ES, NA and AII in all groups. The blunted vascular responses to NA and ES were normalized and the reactivity to AII was only partially reversed in pregnant SHR compared with nonpregnant SHR. Pregnant WKY still had much lower vascular responses to ES and NA than either pregnant or nonpregnant SHR. L-NOARG enhanced vascular responses to AII to a greater extent in pregnant SHR than in pregnant WKY.

6 These results demonstrate that blunted responses to NA and ES were NO-dependent, while diminished reactivity to AII was only partially dependent on NO in the *in situ* blood perfused mesenteric resistance vessels of pregnant SHR.

7 The present results in pregnant SHR differ from our previous finding with pregnant normotensive WKY, in which blunted responses to NA, but not to ES, were equalized by L-NOARG. Pregnancy-induced vasodilatation in hypertensive rats appears to be more dependent on endothelial NO than in normotensive WKY. A defect of the endothelial NO generating pathway which promotes vasodilatation in pregnancy may contribute to the predisposition of women with essential hypertension to develop pre-eclampsia.

Keywords: Vasoconstrictor response; N^G-nitro-L-arginine, nitric oxide; blood perfused mesenteric resistance vessels; pregnant spontaneously hypertensive rat

Introduction

Late pregnancy in normotensive rats is characterized with lower systemic arterial mean blood pressure (MBP), widespread vasodilatation and decreased pressor responses to various vasoconstrictor agents (Paller, 1984; Molnar & Hertelend, 1992; Chu & Beilin, 1993). These changes appear to be, at least in part, related to the increase in or the sensitivity to endogenous endothelial nitric oxide (NO). In studies with conscious pregnant rats, decreased MBP and reduced systemic pressor responses to intravenous administration of noradrenaline (NA), arginine vasopressin (AVP) and angiotensin II (AII) have been reported to be restored by N^G-nitro-L-arginine (L-NOARG), a potent nitric oxide synthase inhibitor (Molnar & Hertelendy, 1992). However, in our previous study with an *in situ* blood-perfused mesenteric bed of pregnant WKY, we found that L-NOARG completely reversed the blunted vascular reactivity to NA, but did not normalize the lower MBP and the diminished mesenteric vascular responses to electrical stimulation of sympathetic nerves (ES) and AII (Chu & Beilin, 1993). These findings suggested that endothelial NO, generated from L-arginine,

contributes to pregnancy-induced vasodilatation by modulating the effect of circulating NA, but that other, as yet unidentified mechanisms, are also involved in modulating the effects of sympathetic nerve activity and circulating AII in pregnant normotensive rats.

Pre-existing essential hypertension predisposes to pre-eclampsia in human pregnancy (Butler & Alberman, 1958). The mechanisms underlying this predisposition are unclear. The spontaneously hypertensive rat (SHR) mimics some aspects of human essential hypertension, with higher systemic blood pressure, increased vascular reactivity and altered endothelial function (Mulvany & Halpern, 1977; Luscher & Vanhoutte 1986). Furthermore, pregnancy-induced renal vasodilatation is defective in midterm gestational SHR as compared with normotensive pregnant WKY rats (Baylis, 1989). In view of the differences in systemic blood pressure and the differing changes in renal haemodynamics between pregnant SHR and WKY, we postulated that mesenteric resistance vessels of SHR might show different characteristics from WKY during pregnancy, with less blunting of vascular reactivity, and possibly different effects with regard to different agonists. In this study, we have examined the changes in vascular reactivity and the effects of endothelial

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NO inhibition by L-NOARG in the *in situ* blood perfused mesentery of 18–20 day pregnant SHR as compared with age-matched nonpregnant SHR, and age- and gestation-matched, pregnant WKY. A nonpregnant WKY group was not included in the present study as we used pregnant and nonpregnant WKY in our previous work (Chu & Beilin, 1993). Some of this work has been presented previously in abstract form (Chu & Beilin, 1992a).

Methods

Pregnant rat models

Eighteen to 20 day pregnant SHR (14 week old), age-matched virgin SHR and age- and gestation-matched pregnant WKY rats were used. Pregnancy was produced by mating an oestrous phase female with a normal male rat. The day when a plug was found was labelled as day 1 of pregnancy. Pregnancy was further confirmed by examining the pups during the experiment. Fifteen animals were initially designated to each group. Results from unsuccessful pregnant animals (no pups, dead pups and/or reabsorbed pups) were discarded.

Vascular reactivity

The *in situ* blood perfused mesenteric preparations and the methods for obtaining dose-effect curves have been described elsewhere (Chu & Beilin, 1993), and are briefly outlined here. The preparation is based on the method of Jackson & Campbell (1980). Animals were anaesthetized with α -chloralose (150 mg kg^{-1} , s.c.). The left femoral artery was cannulated for intra-arterial mean blood pressure measurement with a pressure transducer connecting to a Grass model 7B polygraph. The left femoral vein was also cannulated for continued 0.9% NaCl supplementation (0.1 ml min^{-1}) and intravenous drug administration. After complete haemostasis had been achieved by use of electrical cautery, heparin was administered ($1500 \text{ units kg}^{-1}$, i.v.). Blood was perfused from left carotid to superior mesenteric artery by a roller pump at a constant flow rate of 2 ml min^{-1} . Vascular reactivity was indicated by the changes of perfusion pressure in this constant flow system. In order to abolish central nervous influences on the preparation, the mesenteric artery proximal to the cannulation point and the surrounding tissues were severed. Bipolar platinum electrodes were then placed around superior mesenteric artery for sympathetic nerve stimulation. Finally the abdomen was covered with moistened gauze and the body temperature was maintained at 37°C by a lamp above the animal.

Vascular responses to noradrenaline and electrical stimulation After 30 min equilibration, dose-dependent responses to NA ($100\text{--}560 \text{ ng } 0.05 \text{ ml}^{-1}$) were constructed by bolus injection at 3 min intervals. The preparation was allowed to wash out for a 20 min period and frequency-dependent responses to ES (3–9 Hz, 15 V, 1 ms, until maximal response) were obtained at 3 min intervals. The animal was then treated with L-NOARG (5 mg kg^{-1} , i.v.) and after 30 min the response curves to NA and ES were repeated.

Contractile reactivity to angiotensin Responses to AII were studied in separate groups of animals. Dose-response curves to AII ($10\text{--}300 \text{ ng } 0.05 \text{ ml}^{-1}$) were constructed at 10 min intervals starting at 30 min equilibration. The dose-response curves were then repeated after pretreatment with L-NOARG (5 mg kg^{-1} , i.v.) for 30 min.

Drugs

Noradrenaline bitartrate, AII and L-NOARG were purchased from Sigma chemicals. Heparin sodium was obtained

from Delta West, Western Australia. α -Chloralose was from BDH chemicals.

All drugs were dissolved in 0.9% NaCl and solutions were made up freshly each day.

Statistical analysis

Data are expressed as mean $\pm$ s.e.mean with n = number of rats. Differences in blood pressures or basal perfusion were first analysed by one way analysis of variance (ANOVA). If a significant group effect was found, the differences between the three groups were further tested by an unpaired two tail t test.

To compare dose-dependent responses to NA and AII or frequency-dependent responses to ES, areas under individual curves were calculated mathematically as described previously (Matthews *et al.*, 1990; Chu & Beilin, 1993). Then the means of the areas of the three groups were analysed by ANOVA. The significance of differences between groups was further examined by unpaired two tailed t test. A P value < 0.05 was considered to be significant.

Results

Blood pressures

MBP were similar between pregnant and nonpregnant SHR (Table 1). Pregnant WKY had lower MBP than either pregnant or nonpregnant SHR. L-NOARG significantly increased MBP in all three groups.

Vascular reactivity

Basal perfusion pressure (BPP) was decreased in pregnant compared with nonpregnant SHR (Table 1). Pregnant WKY had lower BPP than either pregnant or nonpregnant SHR groups. L-NOARG increased BPP in all three groups and abolished the difference between pregnant and nonpregnant SHR.

Mesenteric vascular responses to NA were reduced in pregnant (area = 208 ± 9 , $n = 13$) compared with nonpregnant (area = 275 ± 9 , $n = 15$, $P < 0.01$) SHR (Figure 1a). Pregnant WKY showed decreased responses to NA (area = 129 ± 7 , $n = 14$) as compared with either pregnant ($P < 0.01$) or nonpregnant ($P < 0.01$) SHR. In the preparations pretreated with L-NOARG, responses were enhanced in all three groups and the difference between pregnant (area = 373 ± 31 , $n = 13$) and nonpregnant (area = 405 ± 16 , $n = 15$) SHR was abolished. After L-NOARG, pregnant WKY still showed lower responses (area = 168 ± 7 , $n = 14$, $P < 0.01$) than SHR groups (Figure 1b).

Reactivity to ES was also blunted in pregnant (area = 170 ± 14 , $n = 13$) compared with nonpregnant (area = 241 ± 27 , $n = 15$, $P < 0.01$) SHR (Figure 2a). Pregnant WKY had lower responses (area = 34 ± 5 , $n = 14$) than either pregnant ($P < 0.01$) or nonpregnant ($P < 0.01$) SHR. After pretreating the animals with L-NOARG the reactivity was increased in all three groups and was equalized between pregnant (area = 413 ± 22 , $n = 13$) and nonpregnant (area = 413 ± 28 , $n = 15$) SHR groups. Pregnant WKY still had diminished reactivity (area = 94 ± 11 , $n = 14$, $P < 0.01$) compared with SHR groups (Figure 2b).

AII-induced contractile responses were attenuated to a similar level between pregnant SHR (area = 102 ± 9 , $n = 12$) and pregnant WKY (area = 88 ± 6 , $n = 11$) when compared with nonpregnant SHR (area = 184 ± 17 , $n = 10$, $P < 0.01$) (Figure 3a). L-NOARG potentiated the contractile responses in all three groups but the differences between pregnant (area = 199 ± 15 , $n = 12$) and nonpregnant (area = 275 ± 26 , $n = 10$, $P < 0.05$) SHR were still evident and the responses in pregnant SHR became greater than in pregnant WKY (area = 132 ± 9 , $n = 11$, $P < 0.05$). In order to examine

Table 1 Effects of N^G-nitro-L-arginine (L-NOARG, 5 mg kg⁻¹) on intra-arterial mean blood pressures (MBP) and mesenteric basal perfusion pressure (BPP) in pregnant rats

	MBP (mmHg)		BPP (mmHg)	
	L-NOARG (-)	L-NOARG (+)	L-NOARG (-)	L-NOARG (+)
Nonpregnant SHR	147 ± 5 (n = 14)	204 ± 4 (n = 14)	54.1 ± 1.2 (n = 15)	63.6 ± 3.2 (n = 15)
Pregnant SHR	146 ± 5 (n = 13)	205 ± 3 (n = 13)	48.3 ± 1.1* (n = 13)	63.2 ± 3.8 (n = 13)
Pregnant WKY	95 ± 2† (n = 12)	150 ± 4† (n = 12)	30.6 ± 1† (n = 14)	39 ± 2† (n = 14)

P* < 0.01 when compared with nonpregnant SHR group.†*P* < 0.01 when compared with either pregnant or nonpregnant SHR groups.L-NOARG significantly increased MPB and BPP in all groups (*P* < 0.01). Figure 1** (a) Responses to noradrenaline (NA) were blunted in pregnant SHR (□, *n* = 13) compared with nonpregnant SHR (○, *n* = 15, *P* < 0.01). Pregnant WKY (Δ, *n* = 14) had reduced responses when compared with either pregnant SHR (*P* < 0.01) or nonpregnant SHR (*P* < 0.01) groups. (b) N^G-nitro-L-arginine (L-NOARG) potentiated vascular responses in all three groups and abolished the difference between pregnant SHR (■, *n* = 13) and nonpregnant SHR (●, *n* = 15). After L-NOARG, pregnant WKY (▲, *n* = 14) still had much lower responses than either pregnant SHR (*P* < 0.01) or nonpregnant SHR (*P* < 0.01). **Figure 2** (a) Reactivity to electrical stimulation (ES) was diminished in pregnant SHR (□, *n* = 13) compared with nonpregnant SHR (○, *n* = 15, *P* < 0.01). Pregnant WKY (Δ, *n* = 14) showed obviously reduced responses when compared with either pregnant SHR (*P* < 0.01) or nonpregnant SHR (*P* < 0.01). (b) N^G-nitro-L-arginine (L-NOARG) enhanced the reactivity in all groups and abolished the differences between pregnant SHR (■, *n* = 13) and nonpregnant SHR (●, *n* = 15) groups. After L-NOARG the responses were still decreased in pregnant WKY (▲, *n* = 14) compared with SHR groups (*P* < 0.01).

Figure 3 (a) Responses to angiotensin II (AII) in pregnant SHR (□, $n = 12$) were attenuated to a similar level as in pregnant WKY (Δ, $n = 11$) when compared with nonpregnant SHR (○, $n = 10$, $P < 0.01$). (b) In preparations pretreated with L-NOARG, the responses were elevated in all groups. The blunted responses were partly reversed in the pregnant SHR group, but the differences between pregnant SHR (■, $n = 12$) and nonpregnant SHR (●, $n = 10$, $P < 0.05$) were still evident. The responses in pregnant WKY (▲, $n = 11$) were lower than either pregnant SHR ($P < 0.05$) or nonpregnant SHR ($P < 0.01$).

whether the blunted responses to AII are partially reversed by L-NOARG in pregnant SHR, we have made a further comparison between the changes in AII responses after L-NOARG (calculated as percentage of initial response prior to L-NOARG), and found that the responsiveness to AII was increased to a greater extent in pregnant ($94.3 \pm 12.0\%$) than in nonpregnant ($54.6 \pm 13.7\%$, $P < 0.05$) SHR.

Discussion

In near-term pregnant SHR, reduced tail-cuff systolic blood pressures have been well-documented in previous studies (Lorenz *et al.*, 1984; Yong *et al.*, 1992), while intra-arterial blood pressures varied according to whether an anaesthetic is used. In awake, late pregnant SHR, MBP have been reported to be decreased (Lundgren *et al.*, 1979). In the present study

we failed to detect a decrease in MBP in α -chloralose-anaesthetized, pregnant SHR, which is consistent with findings in previous studies with Nembutal plus Brietal sodium anaesthetized pregnant SHR (Yong *et al.*, 1992). L-NOARG substantially increased MBP and BPP in all groups in the present and in our previous studies (Chu & Beilin, 1983). These observations demonstrate that basal endogenous NO plays an important role in the regulation of blood pressure in both pregnant and nonpregnant rats of either normotensive or hypertensive breeds.

Our previous study with *in situ* blood-perfused mesenteric resistance vessels (Chu & Beilin, 1993) and the study by others with conscious animals (Molnar & Hertelend, 1992) have demonstrated that endogenous NO, at least in part, contributes to the blunted vascular reactivity in pregnant normotensive rats. Less information is available whether NO also plays a role in pregnancy-induced vasodilatation in SHR. In the present study, we found that L-NOARG equalized the blunted mesenteric vascular responses to NA and ES, but only partially reversed the reduced responses to AII in pregnant SHR. These results demonstrate that endogenous NO is responsible for the decreased mesenteric vascular responses to NA or ES, and partially involved in reduced reactivity to AII. Furthermore, the effects of L-NOARG on ES responses contrast to our previous findings with pregnant WKY (Chu & Beilin, 1993), in which the diminished responses to ES were not normalized by L-NOARG. These results indicate that an as yet unidentified NO-independent vasodilator mechanism(s) is responsible for the decreased mesenteric vascular reactivity to ES in pregnant WKY while in hypertensive rats, pregnancy-associated vasodilatation appears to be more dependent on NO.

The initial reactivity to AII prior to L-NOARG decreased to a similar level in pregnant SHR as compared with pregnant WKY, but L-NOARG enhanced the responses to a greater extent in pregnant SHR. The greater increases in the mesenteric vascular responses to AII after L-NOARG in pregnant SHR compared with pregnant WKY also suggests that the blunted reactivity to AII is likely to be more dependent on NO in pregnant SHR than pregnant WKY.

What NO-independent mechanism(s) accounts for the attenuated responses to ES seen in pregnant WKY is unclear. It has been well documented that endogenous NA released from sympathetic nerve endings mediates ES-induced vasoconstrictions in rat mesenteric resistance vessels (Jackson & Campbell, 1980; Chu & Beilin, 1993). The release of NA from mesenteric sympathetic nerves is unlikely to be impaired in pregnancy as ES-induced NA spillover was unchanged in the blood perfused mesenteric circulation of pregnant WKY (Chu & Beilin, 1992b). We suggested that an as yet unidentified vasodilator neurotransmitter, which is increased in pregnancy and released during ES, accounts for the blunted vascular responses to ES in pregnant normotensive rats, and may be defective in pregnant hypertensive rats. Calcitonin gene-related peptide (CGRP), may be a candidate for this as CGRP containing fibres in mesentery have been found to be decreased in adult SHR compared with WKY (Kawasaki *et al.*, 1990).

In the present study, basal perfusion pressure was slightly but significantly decreased and vascular reactivity to various vasoconstrictor stimuli was attenuated in the *in situ* blood-perfused mesenteric resistance vessels of 18–20 day pregnant SHR as compared with nonpregnant SHR. These results are consistent with our previous study with pregnant WKY in which a similar blood-perfused, mesenteric preparation was used (Chu & Beilin, 1993), but contrast with our previous findings in which a Krebs-perfused isolated mesenteric preparation was used (Yin *et al.*, 1992). Using the isolated preparation, we found that there were no differences in constrictor responses to noradrenaline, endothelium-dependent relaxations to acetylcholine (ACh) and endothelium-independent relaxations to sodium nitroprusside (SNP) between 12–13 day pregnant and nonpregnant rats.

Yong *et al.* (1992) using a similar isolated mesenteric preparation have also demonstrated that in pregnant SHR, responses to intraluminal noradrenaline were reduced 4 days before delivery but not 1 day before delivery and the reactivity to ES was unchanged on either occasion. In pregnant WKY the responses to intraluminal noradrenaline were unaltered 1 or 4 days before delivery, while the reactivity to ES was decreased only on day 4 before delivery.

The reasons why different results have been obtained from *in vivo* and *in vitro* experiments are not fully understood. Interestingly, vascular responses to prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂) also contrast depending on whether *in vivo* or *in vitro* mesenteric vascular preparations are used (Jackson & Campbell, 1980). Several factors may contribute to the differences. Firstly, in the present experiments much smaller doses of agonists or frequency of ES have been applied due to much higher sensitivity of the *in vivo* preparation. Secondly, the *in vivo* blood-perfused mesenteric vascular bed is under more physiological conditions with regard to circulating vasoactive substances, antioxidants and oxygen pressure; while the *in vitro* Krebs-perfused preparation is exposed to a very basic physiological environment. Finally, the effects of any circulating factors originating from the uteroplacental unit or other part of the body in pregnancy may be undetectable in isolated preparations, particularly if they have a short biological half life. Considering the differences between *in vivo* and *in vitro* studies in pregnancy, it is reasonable to speculate that vasodilator substances with

relatively short half life originating from foetal/placental unit are responsible for the decreased mesenteric vascular reactivity in pregnant rats.

In summary, the present study demonstrate that blunted mesenteric vascular responses to NA and ES are NO-dependent and may reflect increased vascular NO synthesis in pregnant SHR. The diminished reactivity to AII is only partially dependent on NO in the *in situ* blood perfused mesentery of pregnant SHR and may be partially due to down regulation of AII receptors in response to increased circulating AII levels in pregnancy (Broughton Pipkin *et al.*, 1978; Brown & Venuto, 1986).

These observations taken in conjunction with the lack of pregnancy-induced renal vasodilatation described in hypertensive rats (Baylis, 1989; Baylis & Rechelhoff, 1991) provide one possible mechanism by which women with essential hypertension may be predisposed to pre-eclampsia, as they may be more dependent on NO-mediated pregnancy-induced vasodilatation and may have a defective capacity for endothelial NO synthesis sufficiently to allow adequate systemic, renal and uterine vasodilatation in pregnancy.

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