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The role of nitric oxide in the altered vascular reactivity of pregnancy in the rat

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1 Pregnancy is characterized by a decrease in systemic vascular resistance and a blunting of the angiotensin II (AII) pressor response. We studied the role of nitric oxide (NO) and prostanoids in these vascular changes of pregnancy in anaesthesized, ganglion blocked non-pregnant and pregnant rats.

2 Inhibition of NO synthesis with N^G-nitro-L-arginine methyl ester (L-NAME) led to an increase in mean arterial pressure (MAP) which was of a significantly greater magnitude in pregnant rats in late gestation than in non-pregnant rats, or rats in mid-gestation.

3 The pressor response to varying doses of AII was attenuated during late pregnancy, and this attenuation was partially reversed by L-NAME.

4 The pressor response to varying doses of a vasocontrictor, phenylephrine (PE), was also attenuated in late pregnancy. However, this attenuation was not reversed by L-NAME.

5 Inhibition of prostanoid biosynthesis with meclofenamate did not alter basal MAP, nor the pressor response to varying doses of AII or PE in pregnant and non-pregnant animals.

6 It is concluded that (a) increased NO synthesis occurs during late gestation and contributes both to the decrease in systemic vascular resistance, as well as the blunting of the pressor response to AII during pregnancy, and (b) prostaglandins are not important in the maintenance of basal vascular tone, or the blunting of the pressor response to AII during pregnancy.

Keywords: Pregnancy; nitric oxide; prostanoids; angiotensin II; vascular reactivity; pressor response; N^G-nitro-L-arginine methyl ester.

Introduction

Pregnancy is associated with a hyperdynamic state characterized by increased plasma volume and increased cardiac output. Systemic arterial pressure is not increased due to a decrease in systemic vascular resistance (Pan *et al.*, 1990). Concomitant with the decrease in systemic vascular resistance is a blunting of the pressor response to angiotensin II (AII) (Abdul Karim & Assali, 1961; Gant *et al.*, 1973; Pan *et al.*, 1990; Molnár & Hertelendy, 1992), plasma levels of which are increased during pregnancy (Weir *et al.*, 1975). The exact mechanism by which these vascular changes take place is not known.

Several investigators have proposed that the blunting of the AII pressor response and other vascular changes of pregnancy are due to release of vasodilator prostaglandins (Everett *et al.*, 1978; Gerber *et al.*, 1981), although this concept has been challenged by others (Conrad & Colpoys, 1986). More recently, it has been proposed that endothelium-derived relaxing factor (EDRF) (Furchgott & Zawadzki, 1980), now identified as nitric oxide (NO) (Palmer *et al.*, 1987; Ignarro *et al.*, 1987), is responsible for these vascular changes of pregnancy. Unlike PGI_2 (Conrad & Colpoys, 1986), NO has been unequivocally demonstrated to be responsible for maintaining basal vascular tone *in vivo* in all species studied (Rees *et al.*, 1989; Aisaka *et al.*, 1989; Collier & Vallance, 1989; Vargas *et al.*, 1991). NO is also released by various endothelium-dependent vasodilators (Furchgott, 1984) and probably by certain vasopressor agents such as AII (Yamaguchi & Nichimura, 1988). Findings by several investigators demonstrating increased endothelium-dependent vasodilatation of vessels obtained from pregnant animals suggest that NO may play an important role in modulating vascular tone during gestation (Weiner *et al.*, 1991).

We, therefore, decided to evaluate whether increased NO rather than prostaglandin formation in pregnant rats could account for both the fall in systemic vascular resistance as well as the blunting of the pressor response to AII observed during pregnancy. This study was performed on pregnant rats because this species offers many advantages. Some of the cardiovascular changes this species undergoes during pregnancy are similar to those in women. For example, the increase in uterine blood flow (Bruce, 1976), the decrease in mean arterial pressure (MAP) (Pan *et al.*, 1990; Molnár & Hertelendy, 1992) and attenuation of pressor responsiveness to exogenous vasoconstrictors (Pan *et al.*, 1990; Molnár & Hertenlendy, 1992) develop in the species similar to those seen in women. Rats are relatively inexpensive and their hormonal profiles during pregnancy are well defined.

Methods

Preparation of rats for blood pressure recording and drug injection

Experiments were conducted on urethane anaesthetized (1.25 g kg^{-1}, i.p.) Sprague-Dawley rats weighing 250-350 g obtained from Bantin and Kingman (Fremont, CA, U.S.A.). Rats were divided into three groups, (a) nonpregnant; (b) pregnant in mid-gestation (9-11 days pregnant); and (c) pregnant in late gestation (18-20 days pregnant). The day sperm were first seen in the vaginal lavage was considered day 1 of pregnancy. After establishment of anaesthesia, polyethelene catheters (PE50) were inserted into the right femoral and common carotid arteries for drug infusion and arterial blood pressure recordings, respectively. Blood pressure was measured by a Statham transducer and recorded on a Hewlett Packard polygraph. All drugs were dissolved in 0.9% saline and injected in a volume of 0.1 ml $100g^{-1}$ body weight. Animals were ventilated with a rodent respirator (1

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ml $100g^{-1}$, 75 strokes min^{-1}). In order to eliminate all autonomic reflexes and thereby evaluate responses at the vascular bed, animals were injected with the ganglion blocker, pentolinium (5 mg kg^{-1}, i.v.) 10 min prior to continuing the experiments. Each group contained 5–13 animals.

Role of NO in maintaining basal vascular tone during pregnancy

To assess the contribution of NO in the maintenance of basal vascular tone in pregnancy, each animal received 5 graded doses of the NO synthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) injected as a bolus (0.1, 0.3, 1.0, 3.0, 30 mg kg^{-1}, i.v.). The change in mean arterial pressure (MAP) from baseline was measured after each dose when the peak rise in MAP occurred, usually after 5 min. Blood pressure was allowed to stabilize before administering each subsequent dose. In some animals, after the peak responses to L-NAME were achieved, L- or D-arginine was injected as a bolus (5 fold concentration of L-NAME) and the pressor response observed.

Role of NO in modulating the pressor response to AII and phenylephrine during pregnancy

To assess the contribution of NO in the attenuation of the vasopressor response to AII during pregnancy, animals were injected with AII before and after administration of L-NAME. Catecholamine levels, (Lederman *et al.*, 1977), in contrast to AII (Weir *et al.*, 1975) are not increased in pregnancy. We therefore also decided to evaluate whether the pressor response to the α-adrenoceptor agonist, phenylephrine (PE), during pregnancy was modulated by NO to a similar magnitude as that observed with AII. We selected non-pregnant animals and animals in late pregnancy for this protocol as initial experiments demonstrated that the blunting of the AII pressor response was observed only in animals during late pregnancy. Animals were injected with 3–4 graded doses of AII (25, 50, 100, 200 ng kg^{-1}, i.v.) and PE (0.5, 1.0, 2.0 μg kg^{-1}, i.v.) in a random fashion and changes in MAP were recorded. Following completion of these dose responses, L-NAME (45 mg kg^{-1}, i.v.) was injected as a single bolus. Fifteen minutes later the dose responses to AII and PE were then repeated in a random fashion. As the baseline tone was increased following administration of L-NAME, we decided to express the results for this protocol as percentage change from baseline values.

Role of prostanoids in modulating basal vascular tone and pressor responses to AII and PE during pregnancy

To assess the role of prostanoids in the maintenance of basal vascular tone and the blunting of the pressor response to AII during pregnancy, animals were injected with graded doses of PE and AII, in the manner described above, both before and 30 min after a single bolus injection of meclofenamate (3 mg kg^{-1}, i.v.). Changes in MAP were then recorded. This dose of meclofenamate has been shown to inhibit prostaglandin synthesis in pregnant and non-pregnant rabbits (Chaudhuri *et al.*, 1982).

Drugs

AII acetate, PE hydrochloride, urethane, pentolinium ditartrate, L-NAME hydrochloride, L-arginine, D-arginine and meclofenamate sodium were purchased from Sigma Chemical Co. (St. Louis, MO U.S.A). All drugs were dissolved in 0.9% w/v saline.

Statistical analysis

All data are expressed as mean ± s.e.mean. Differences between teatment group means were compared by two and three-way ANOVA with repeated measurements. Differences were considered significant at the $P<0.05$ level.

Results

Role of NO in maintaining basal vascular tone during pregnancy

Basal MAP following pentolinium and prior to L-NAME was 50 ± 5 mmHg in non-pregnant animals, 63 ± 3 mmHg in animals in mid gestation and 65 ± 2 mmHg in animals in late gestation. L-NAME produced a dose-dependent rise in MAP in non-pregnant animals, as well as in animals in mid and late pregnancy. However, during late pregnancy, L-NAME produced a significantly greater rise in MAP compared to that observed in either non-pregnant animals or in animals in mid pregnancy (Figure 1). In separate experiments to assess the reversibility of L-NAME by L-arginine and D-arginine,MAP following L-NAME administration in non-pregnant animals was 76 ± 4 mmHg and in pregnant animals was 157 ± 10 mmHg. Administration of D-arginine after L-NAME did not change the MAP whereas 15 min after administration of L-arginine MAP in non-pregnant and pregnant animals was reduced to 47 ± 5 mmHg and 82 ± 13 mmHg, respectively.

Role of NO in modulating the pressor response to AII and PE during pregnancy

In non-pregnant animals, basal MAP following pentolinium was 50 ± 5 mmHg before and 70 ± 9 mmHg 15 min after L-NAME. In pregnant animals in late gestation, basal MAP was 65 ± 2 mmHg before and 95 ± 6 mmHg 15 min after L-NAME. AII caused a dose-dependent rise in MAP in all three groups. However, in late pregnancy, the pressor responses to AII were attenuated at all doses (Figure 2). PE also produced dose-dependent rises in MAP which were also

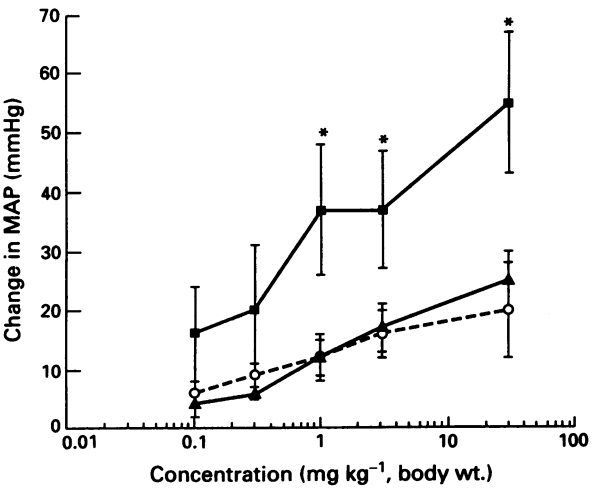

Figure 1 Pressor responses to N^G-nitro-L-arginine methyl ester (L-NAME) in anaesthetized, ganglion-blocked non-pregnant rats (▲), and pregnant rats in mid (○) and late (■) gestation. In late pregnancy, L-NAME produced a significantly greater rise in mean arterial pressure (MAP) compared to that observed in non-pregnant animals or animals in mid-gestation. MAP was measured when the peak rise in MAP occurred after each dose of L-NAME, usually after 5 min. Data are expressed as mean (±s.e.mean) rise in MAP of 9 animals in each group. $*P<0.05$ denotes significant difference from values obtained in non-pregnant animals.

attenuated in late pregnancy (Figure 2). However, the attenuation was of a lower magnitude in the PE group when compared to the AII group.

In non-pregnant animals, the pressor responses to different doses of AII were not altered by L-NAME (Figure 3). In contrast, the blunting of the pressor response to AII in late pregnancy was partially reversed by L-NAME as there was a significant potentiation of the pressor response to AII at the 25 and 50 ng kg^{-1} doses (Figure 3). The pressor response to PE was unchanged by L-NAME in non-pregnant and pregnant animals (Figure 4).

Role of prostanoids in modulating basal vascular tone and pressor responses to AII and PE during pregnancy

Inhibition of prostanoids with meclofenamate did not alter basal MAP. In non-pregnant animals basal MAP following pentolinium was 60 ± 4 mmHg before and 62 ± 4 mmHg 30 min after meclofenamate. In pregnant animals basal MAP was 64 ± 2 mmHg before and 66 ± 2 mmHg 30 min after meclofenamate. The pressor responses to AII (Figure 5) and PE (Figure 6) in non-pregnant animals were also unchanged by meclofenamate.

Discussion

The mechanisms involved in maintenance of vascular tone and altered vascular responses to pressor agents during pregnancy are not known. The role of NO in modulating these altered vascular responses has not been clearly delineated. However, there is evidence to suggest that increased NO synthesis occurs during pregnancy and that it may therefore play a role in modulating the altered vascular reactivity during pregnancy (Conrad & Vernier, 1989; Conrad *et al.*, 1993). Consistent with this concept, Conrad & Vernier (1989) and Conrad *et al.* (1993) have demonstrated an increase in endogenous NO production in pregnant rats. In addition, Weiner and colleagues (1994 a,b) have demonstrated that calcium-dependent NO synthase activity is increased in both vascular and non-vascular tissues during pregnancy.

The principle objective of this study was to elucidate whether the decrease in systemic vascular resistance, as well as the blunting of the pressor response to AII that occurs in pregnancy, can be explained on the basis of increased release of NO rather than increased release of prostanoids. This was achieved by studying the basal release of NO in non-pregnant rats and pregnant rats in mid and late gestation. We used ganglion-blocked animals because this would permit us to study the direct vascular effects of vasoactive substances, unmodified by reflex changes in vascular tone. The role of basal release of NO and prostanoids in the maintenance of vascular tone during pregnancy was studied indirectly by observing the pressor responses to L-NAME and meclofenamate. The role of NO and prostanoids in the blunting of the pressor response to AII was studied by observing the pressor responses to various doses of AII before and after the administration of L-NAME and mec-

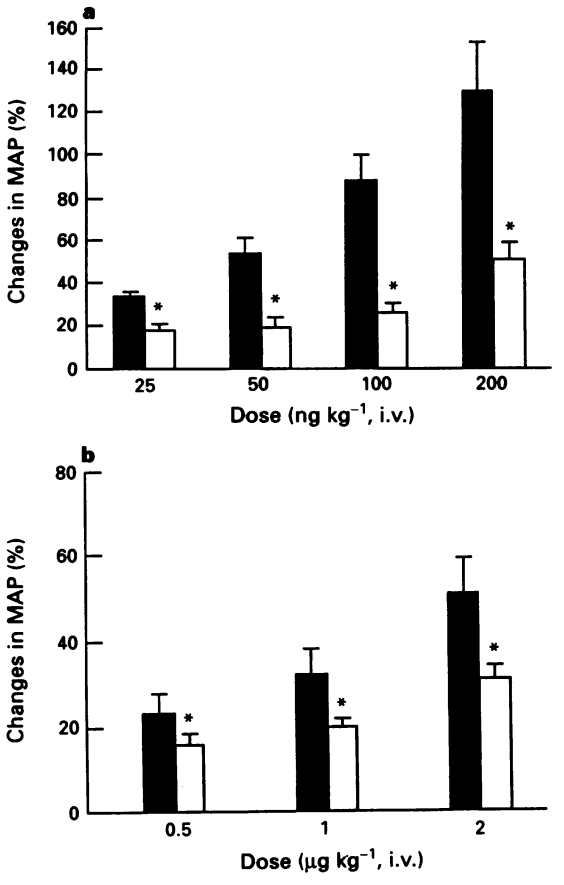

Figure 2 Pressor responses to (a) angiotensin II (AII) and (b) phenylephrine (PE) in anaesthetized, ganglion-blocked non-pregnant rats (solid columns), and pregnant rats in late gestation (open columns). A dose-dependent rise in mean arterial pressure (MAP) was seen in all groups. However, the pressor responses to both PE and AII in late pregnancy were attenuated. Data are expressed as mean (with s.e.mean) percentage changes in MAP of 7–13 animals in each group. *$P<0.05$ denotes significant difference from values obtained in non-pregnant animals.

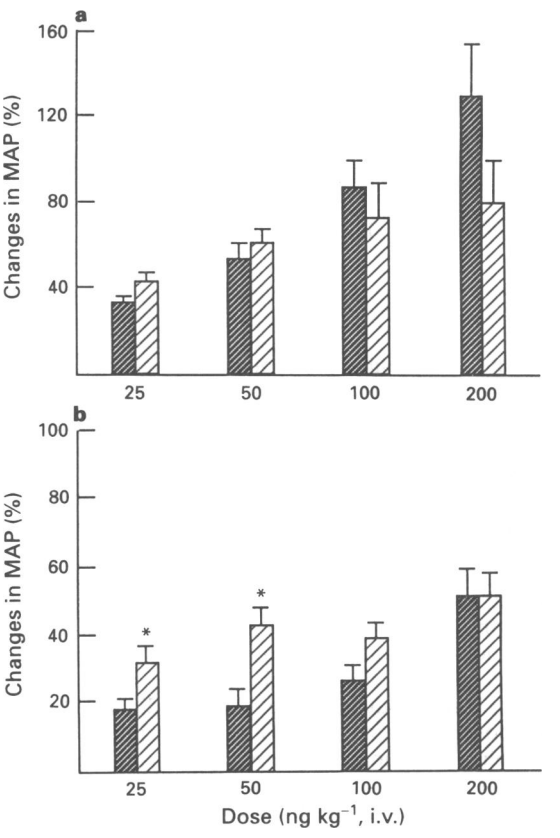

Figure 3 Pressor response to angiotensin II (AII) before (closely hatched columns) and after (widely hatched columns) N^G-nitro-L-arginine methyl ester (L-NAME) in anaesthetized, ganglion-blocked (a) non-pregnant rats and (b) pregnant rats in late gestation. In non-pregnant animals, the blunted pressor response to AII was unchanged after L-NAME administration, while in pregnant animals, the blunted pressor response to AII was partially reversed by L-NAME. Data are expressed as mean (with s.e.mean) percentage change in mean arterial pressure (MAP) of 7–11 animals in each group. *$P<0.05$ denotes significant difference from pre-L-NAME values.

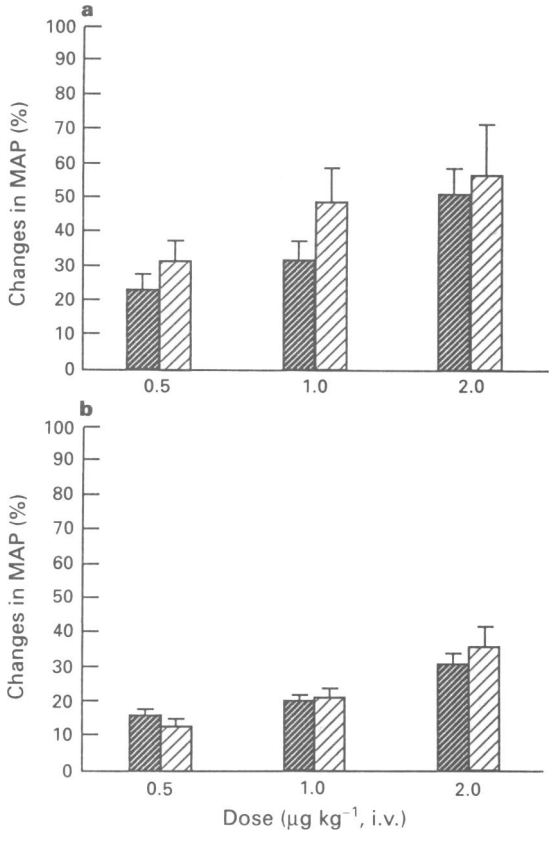

Figure 4 Pressor response to phenylephrine (PE) before (closely hatched columns) and after (widely hatched columns) N^G-nitro-L-arginine methyl ester (L-NAME) in anaesthetized, ganglion-blocked (a) non-pregnant rats and (b) pregnant rats in late gestation. The pressor response to PE was unchanged after administration of L-NAME in both pregnant and non-pregnant animals. Data are expressed as mean (with s.e.mean) percentage change in mean arterial pressure (MAP) of 8–13 animals in each group.

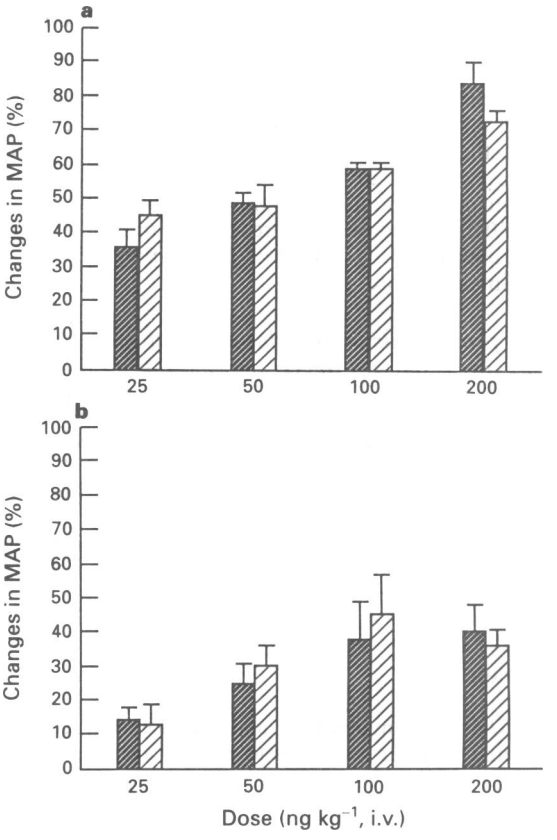

Figure 5 Pressor response to angiotensin II (AII) before (closely hatched columns) and after (widely hatched columns) meclofenamate in anaesthetized, ganglion-blocked (a) non-pregnant rats and (b) pregnant rats in late gestation. The pressor response to AII was unchanged after administration of meclofenamate in both non-pregnant and pregnant animals. Data are expressed as mean (with s.e.mean) percentage change in mean arterial pressure (MAP) of 5 animals in each group.

lofenamate. As plasma catecholamine levels are not altered during pregnancy, unlike levels of AII, we also evaluated whether the pressor responses to PE were modified by NO to a similar magnitude to that observed with AII.

Inhibition of NO synthesis by L-NAME led to an increase in MAP which was of a greater magnitude in pregnant rats in late gestation than that observed in either non-pregnant rats, or in pregnant rats in mid-gestation. This suggests that increased NO synthesis occurs during late pregnancy. Our results differ from those of Umans *et al.* (1990) who failed to observe any differences in the magnitude of rise in MAP between non-pregnant rats and rats in late pregnancy after they were given a single high dose of an NO synthesis inhibitor. Unfortunately, these workers did not perform a dose-response curve to the NO synthesis inhibitor, nor did they abolish autonomic reflexes with ganglion blockade. Thus, inhibition of NO synthesis could have stimulated autonomic reflex changes which would have helped maintain blood pressure at a steady state. A study by Molnár & Hertelendy (1992) also failed to reveal a difference in pressor response between non-pregnant and pregnant animals after administration of an inhibitor of NO synthesis. Again, these animals had intact autonomic reflexes which may have minimized any differences between the pregnant and non-pregnant groups. Using the spontaneously hypertensive rat model, Ahokas *et al.* (1991) saw significantly greater increases in MAP in the pregnant than non-pregnant rats after inhibition of NO synthesis, but they did not observe this difference in the normotensive rats. These investigators also used reflex intact animals which makes comparison to our study difficult.

One possible explanation for the accentuated rise in MAP after L-NAME in animals in late gestation may be the presence of other circulating pressor agents such as AII, the concentration of which is known to increase during pregnancy (Weir *et al.*, 1975). It is conceivable that during late gestation, in addition to increased basal release of NO, AII itself further stimulates NO release, thereby blunting its own pressor response. Therefore, in addition to removing the basal vasodilator mechanism, inhibition of NO synthesis during late gestation may have unmasked the pressor response to circulating AII and caused a significantly greater magnitude of rise in MAP in late pregnancy.

AII produces endothelium-dependent relaxation in the fowl aorta presumably by release of NO (Yamaguchi and Nishimura, 1988). We observed that responses to AII, as well as to PE, were blunted during late pregnancy, but that only the blunted AII response was partially reversed after inhibiting NO synthesis. One possible explanation for this observation is that in the pregnant state AII may stimulate greater release of NO than that observed with PE. However, this aspect needs direct confirmation by actually quantifying NO release *in vivo*. Our findings support the concept that increased NO synthesis plays at least a partial role in modulating vasoconstrictor tone during late pregnancy, but that other factors may be involved such as effects of dilution from increased plasma volume during pregnancy or changes in vasoconstrictor receptor density or affinity. Ahokas & Sibai (1992) found

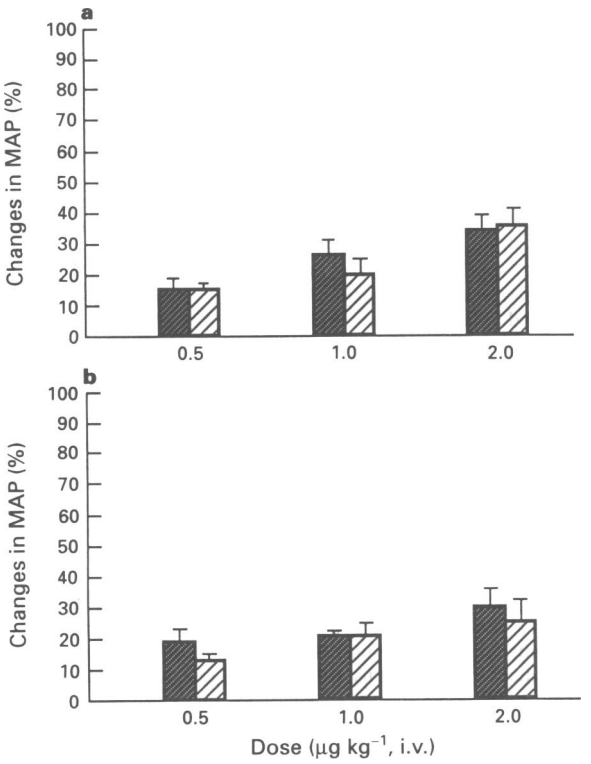

Figure 6 Pressor response to phenylephrine (PE) before (closely hatched columns) and after (widely hatched columns) meclofenamate in anaesthetized, ganglion-blocked (a) non-pregnant rats and (b) pregnant rats in late gestation. The pressor response to PE was unchanged after administration of meclofenamate in both non-pregnant and pregnant animals. Data are expressed as mean (with s.e.mean) percentage change in mean arterial pressure (MAP) of 5 animals in each group.

complete, rather than partial reversal of the blunted AII pressor response in the hindlimb preparation of a pregnant, spontaneously hypertensive rat after inhibition of NO synthesis. One explanation as to why our results may have differed in this aspect is that the AII pressor response in our experiments may have reached its peak value at the lower doses of AII.

The role of vasodilator prostanoids in modulating basal vascular tone during late pregnancy was also explored in this study as it has been shown that pregnancy results in increased prostaglandin production in women (Bay & Ferris, 1979; Goodman *et al.*, 1982) and in laboratory animals (Gerber *et al.*, 1981; Chaudhuri *et al.*, 1982; Paller *et al.*, 1989). Unlike NO, vasodilator prostanoids do not appear to play an important role in the maintenance of vascular tone in late pregnancy since meclofenamate did not change MAP in pregnant and non-pregnant animals. Our observations are similar to those of other investigators who also did not observe any change in MAP in pregnant guinea-pigs (Harrison & Moore, 1989) or any change in the perfusion pressure of the *in situ* blood-perfused mesentery of pregnant rats following inhibition of cyclo-oxygenase (Chu & Beilin, 1993).

In our study, inhibition of NO synthesis only partially restored the attenuated AII pressor response during pregnancy. We therefore investigated whether release of vasodilator protaglandins may at least be partially responsible for this attenuation. However, we were unable to demonstrate a role for prostanoids in mediating the altered response to AII during pregnancy since meclofenamate did not alter the pressor response to AII or PE. Our observations, consistent with those of other investigators (Conrad & Colpoys, 1986; Harrison & Moore, 1989; Chu and Beilin, 1993), do suggest that prostaglandins are not important in the maintenance of vascular tone during pregnancy. The differences that have been observed across studies regarding the relative contributions of NO and protaglandins to the AII pressor response during pregnancy may be explained on the basis of species variations.

We have demonstrated the importance of NO rather than vasodilator prostaglandins in the maintenance of vascular tone during pregnancy. The mechanism underlying this increased release of NO during pregnancy was not explored in this study. However, factors such as increased blood volume leading to increased shear stress, as well as increased oestradiol concentration (Weiner *et al.*, 1994a,b) may be involved. We have also not identified the vascular beds where NO may play an important role in modulating the vascular changes of pregnancy. Identification of these vascular beds would be important in order to understand fully the precise role of NO during pregnancy. Furthermore, the vasoconstrictors used in this study may have direct effects on the heart and venous circulation. Thus, the responses that we observed may not be entirely due to actions on resistance vessels.

Results of our studies have important clinical implications. It has been shown by others that the blunting of the AII response in pregnancy is reversed in patients with pre-eclampsia and in those destined to develop pre-eclampsia (Gant *et al.*, 1973). We have shown that the blunting of the AII response is at least partially modulated by NO. It is, therefore, possible that a disturbance in NO release due to endothelial dysfunction or injury may lead to a rise in systemic vascular resistance and contribute to the pathogenesis of pre-eclampsia.

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