Effect of gestational age on in-vitro responses of pregnant rat aorta*

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The hypothesis that the changes in vascular reactivity seen during pregnancy are determined by the gestational age was examined. Experiments were designed to investigate changes in vascular responses with progression of pregnancy. The contractile responses to potassium and phenylephrine (in the presence and absence of NO-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor) and the relaxant responses to acetylcholine and sodium nitroprusside were measured in isolated aortic rings from pregnant rats at various stages of gestation and from non-pregnant female rats. Potassium-evoked contractile response was higher early in pregnancy and was decreased at term (P < 0.05). The contractile response to phenylephrine was decreased and the relaxant response to acetylcholine was increased in early pregnancy (P < 0.05). Inhibition of nitric oxide synthase caused an increase in the contractile response to phenylephrine in all the groups, but the attenuation of the response in early pregnancy was maintained (P < 0.05). There was a small decrease in the maximal relaxant response to sodium nitroprusside at term (P < 0.05). It was concluded that the effects of pregnancy on the responses of rat aorta in vitro vary at different stages of gestation. Vascular resistance may be lowered by changes in vascular reactivity in early gestation and by a decrease in the contractile potential of the vasculature during the later stages.

Key words: endothelium/nitric oxide/pregnancy/rat aorta/vascular smooth muscle

Introduction

Pre-eclampsia is a major cause of maternal and perinatal morbidity and mortality (Roberts, 1994). Various theories have been proposed for its causation but the exact aetiology remains unknown (Roberts, 1994). Failure to achieve a better insight into this clinical syndrome stems in part from an incomplete understanding of the mechanisms responsible for the vascular adaptations occurring during a normal pregnancy.

Pregnancy is associated with an increase in the circulating blood volume, haemodilution, an increase in cardiac output, a decrease in peripheral resistance and a decrease in the mean arterial pressure (Gant et al., 1987; Magness and Gant, 1994). The decrease in peripheral resistance, which is a key phenomenon, is thought to result from a decrease in the sensitivity of the vasculature to pressor agents and an increase in the production of vasodilator agents. Various studies carried out in pregnant women, rats or guinea pigs support the existence of these mechanisms, whereas others have failed to identify the presence of these adaptive phenomena during pregnancy.

There is general agreement that the responses to angiotensin II are blunted during pregnancy (Gant et al., 1973), but no consensus has been reached on the decrease in responsiveness to α-adrenergic agonists (Gant et al., 1987; Molnar and Hertelendy, 1992; McCarthy et al., 1994). Vascular endothelium is known to be important in cardiovascular homeostasis and disease (Rubanyi, 1993). There is disagreement regarding the role of vascular endothelium and nitric oxide in the refractoriness to pressor agents seen during pregnancy. Some studies support the hypotheses that endothelium-mediated relaxation and nitric oxide production are increased during pregnancy and are responsible for blunting of responses to pressor agents (Weiner et al., 1991; Conrad et al., 1993; Nathan et al., 1995). Other studies have reported results to the contrary (Umans et al., 1990; Kim et al., 1994; Pascoal and Umans, 1996). These variations could be explained by differences in the species under investigation, methods used, vascular bed studied and so on. Differences in gestational age may also be a possible source of disagreement between the various studies.

It may be incorrect to assume that the mechanisms responsible for the cardiovascular adaptations remain the same throughout pregnancy. Various endocrine changes occur as pregnancy advances (Yen, 1994). It is possible that in this continuously changing milieu, the systems controlling vascular reactivity may be shifting from one predominant mechanism to another throughout gestation. In-vitro studies of human vessels have generally been limited to tissue taken from women at term. These studies may have overlooked some of the changes occurring during other periods of pregnancy. Even in experimental animals, most of the studies have been carried out at one particular stage of gestation or another or in non-pregnant animals treated with steroids to simulate pregnancy. Thus depending on the gestational duration or the hormonal environment, different results may have been obtained.


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We have previously demonstrated that there is a progressive decrease in the endothelium-derived relaxing factor production and the sensitivity of vascular smooth muscle to endothelium-derived relaxing factor in human umbilical artery throughout gestation (Izumi et al., 1994). Also, we have shown that the relaxant responses of the rat aorta to corticotrophin-releasing factor are gestationally regulated (Jain et al., 1997). To our knowledge, no study to date has examined in-vitro responses of the maternal vasculature longitudinally at different stages of pregnancy. The objective of this study was to investigate whether changes in the contractile and endothelium-dependent and endothelium-independent relaxant responses occur in isolated rat aorta during early and late pregnancy and at term.

Materials and methods

Animals

Sprague–Dawley mature cycling virgin female rats (200–250 g) and age matched timed pregnant rats were obtained from Charles River Laboratories. They were housed separately in temperature and humidity controlled quarters with constant light:dark cycles of 12 h:12 h and were provided with food and water ad libitum. The pregnant rats used in these studies have a gestational period of 22 days, day 1 being the day the sperm plug was observed. The animals were killed by CO₂ inhalation. All procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch. All the animals in the same group were not studied at the same time, due to technical limitation. A system of organ chambers was used with eight organ baths. Since two aortic rings (see below) were studied from each rat, the rats were studied in subgroups of four.

In-vitro experiments

The vascular responses were studied in non-pregnant rats and in pregnant rats on day 8 (early gestation), day 16 (late gestation) and day 22 (term). The thoracic aorta was dissected out through a midline thoraco–abdominal incision and was placed in cold physiological saline solution (PSS). The aorta was separated from the connective tissue, taking care not to damage the endothelium or intima. Two rings ~2 mm wide were prepared from a short supradiaphragmatic segment of the aorta from each rat. Preliminary experiments failed to demonstrate any significant differences in the responses of the two rings from the same rat. The rings were mounted on stainless steel hooks in 10 ml organ chambers containing PSS. One hook was fixed to a tissue support and the other was connected to a force-displacement transducer (FT03, Grass Instruments, Quincy, MA, USA). The transducers were connected to a Grass model 7D polygraph recorder (Grass Instruments). The changes in isometric force were recorded using Windaq/200 data acquisition software and analysed using Windaq playback software (Dataq Instruments Inc., Akron, OH, USA). The bathing solution was maintained at 37°C and was continuously aerated with a mixture of 95% O₂ and 5% CO₂. The rings were equilibrated in PSS for 1 h under a preload of 1.5 g. This preload was found to be optimal in the preliminary experiments. Then two successive stimulations of 15 min duration were given with high-K⁺ PSS (50 mM), separated by a 15 min equilibration in PSS. The presence or absence of endothelium in the rings was confirmed by contracting them with phenylephrine (3×10⁻⁷ M) and eliciting relaxation with acetylcholine (10⁻⁶ M). Failure to relax was used as an indicator of the absence of endothelium. To quantify the contractile potential of the rat aorta in the non-pregnant state and at different stages of pregnancy, the contractile responses to depolarizing solutions with increasing K⁺ concentration (replacing Na⁺ iso-osmotically) were studied. To test the hypothesis that the responses to pressor agents change during pregnancy, the contractile responses to cumulative concentrations of phenylephrine (10⁻⁷–10⁻⁵ M) were studied. The responses were normalized to the potassium-evoked contraction (50 mM KCl) to differentiate between the changes in the contractile potential and sensitivity to α-adrenoceptor agonists. To assess the production of nitric oxide by the endothelium, the responses to phenylephrine were also studied in the presence of an inhibitor of nitric oxide synthase, Nω-nitro-L-arginine methyl ester (L-NAME). The rings were preincubated with L-NAME (10⁻⁴ M) for 15 min. To test for differences in endothelium-dependent relaxation, responses to cumulative concentrations of acetylcholine (10⁻⁹–10⁻⁵ M) were studied in rings precontracted with phenylephrine (10⁻⁵ M). To investigate any differences in the intrinsic responsiveness of the vascular smooth muscle to nitric oxide, the relaxant responses to cumulative concentrations of sodium nitroprusside (10⁻⁹–10⁻⁵ M) were studied. For studying responses to sodium nitroprusside, the rings were preincubated with L-NAME (10⁻⁴ M) for 15 min in order to prevent interference by the endogenous nitric oxide.

Statistics

The concentration producing 50% of the response (ED₅₀) and area under the curve were calculated for the cumulative concentration–response curves. All data are expressed as mean ± SEM, n represents the number of animals used in each experiment. One-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparisons test was used to determine statistical significance of differences between the groups (P < 0.05).

Results

The contractile responses to depolarizing solutions with increasing K⁺ concentration were significantly increased in early pregnancy and decreased at term as compared to non-pregnant rats (Figure 1). The responses in late pregnancy were not different from non-pregnant rats. All together, these results show a progressive decrease in the responses as the pregnancy advances.

The phenylephrine responses in early pregnancy were significantly decreased compared to those of non-pregnant rats and rats in late pregnancy and at term (Figure 2, panel A). The differences in the ED₅₀ were not significant but the areas under the concentration–response curves were significantly different (Table 1). In the presence of L-NAME, the responses to phenylephrine were increased in all the groups as compared to those in the absence of L-NAME (Figure 2, panel B). Even after treatment with L-NAME, however, the attenuated response in early pregnancy compared to later gestation was maintained. Although the ED₅₀ was not different, the area under the concentration–response curve was significantly decreased in
The relaxant responses to sodium nitroprusside were not different in early and late pregnancy as compared to the non-pregnant state (Figure 4). The maximal relaxant response to sodium nitroprusside was decreased at term. The ED50 and the areas under the concentration–response curves were not significantly different (Table IV).

### Discussion

Pregnancy is a state of altered haemodynamics associated with an increase in cardiac output and a decrease in peripheral vascular resistance (Gant et al., 1987; Magness and Gant, 1994). Various studies have been carried out in vivo as well as in vitro investigating changes in the vascular reactivity during pregnancy. Despite these efforts, considerable controversy still exists as to the causes of lowered peripheral vascular resistance during pregnancy. We postulated that differences in the vascular reactivity at different times during pregnancy may be a possible source of these inconsistencies. Our results demonstrate that in-vitro responses of isolated rat aorta to contractile and relaxant agents are different during pregnancy as compared to the non-pregnant state, and these differences are dependent on the gestational age. We have shown that the contractile potential of rat aorta is increased early in pregnancy and then progressively decreases with advancing gestation. In addition, early pregnancy is associated with a decreased responsiveness to phenylephrine and increased relaxation to acetylcholine. Also, maximal relaxation by sodium nitroprusside is decreased at the term of pregnancy.

Ezimokhai et al. (1994) showed that there is a decrease in the potassium-evoked contraction in the rat aorta from near-term pregnant rats. Our results are in agreement with this finding, and additionally demonstrate an initial increase in the potassium-evoked contraction during early pregnancy which then progressively decreases as the pregnancy advances, being the same as the non-pregnant state during late pregnancy and the lowest at term. The increase in the extracellular K+ causes depolarization of the vascular smooth muscle causing entry of Ca2+ through voltage-dependent calcium-channels (Knot et al., 1996). Potassium-evoked contractions are therefore a useful tool to study the coupling of membrane depolarization and Ca2+ entry and the contractile potential of the vascular smooth muscle. The decrease in the potassium-evoked contraction may be due to a decrease in the smooth muscle cells or contractile proteins in the vascular wall, decreased depolarization induced Ca2+ entry or increased efficiency of Ca2+ sequestration in the vascular smooth muscle. A similar decrease in the contractile force has been reported in the rat mesenteric artery during pregnancy (McLaughlin and Keve, 1986). Hence a progressive decrease in the contractile potential with advancing gestation could be a possible mechanism to decrease peripheral vascular resistance during pregnancy.

Various investigators have demonstrated that, in rats as well as guinea pigs, pregnancy is associated with decreased vascular responses to pressor agents (Weiner et al., 1991; Molnar and...
Hertelendy, 1992). This change has been seen to persist late into pregnancy. Our results are in partial agreement with these studies. Although we did see a decrease in pressor responses to phenylephrine, it occurred only in early gestation; the responses during late gestation or at term were not different from those of the non-pregnant state. Similar results were obtained in another study using perfusion of the rat tail artery where the decrease in the pressor responses to noradrenaline

![Figure 2](image2.png)

**Figure 2.** Phenylephrine (PE) responses in aortic rings with intact endothelium from non-pregnant and pregnant (days 8, 16 and 22) female rats. A, Responses in absence of L-NAME, B, responses in presence of L-NAME (10^{-4} M), C, difference in the response in the absence and presence of L-NAME. Each point represents mean ± SEM, n = 15, 11, 11 and 12 for the non-pregnant, day 8, day 16 and day 22 groups respectively. One-way ANOVA followed by Newman–Keuls multiple comparisons test was used, *P < 0.05, day 8 versus non-pregnant and day 22, **P < 0.05, day 8 versus non-pregnant and day 16, ***P < 0.05, day 8 versus non-pregnant, day 16 and day 22. ● Non-pregnant, ▲ day 8, ▲ day 16 and ▼ day 22.

![Figure 3](image3.png)

**Figure 3.** Responses to acetylcholine (ACh) in aortic rings with intact endothelium from non-pregnant and pregnant (days 8, 16 and 22) female rats. The rings were precontracted with phenylephrine (PE, 10^{-5} M). Each point represents mean ± SEM, n = 9, 7, 8 and 7 for the non-pregnant, day 8, day 16 and day 22 groups respectively. One-way ANOVA followed by Newman–Keuls multiple comparisons test was used, *P < 0.05, day 8 versus non-pregnant and day 22, **P < 0.05, day 8 versus non-pregnant and day 16, ***P < 0.05, day 8 versus non-pregnant, day 16 and day 22. ● Non-pregnant, ▲ day 8, ▲ day 16 and ▼ day 22.

**Table III.** Acetylcholine responses

<table>
<thead>
<tr>
<th>Rats</th>
<th>ED_{50} (log M)</th>
<th>Area under the curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>−6.97 ± 0.07 ^a</td>
<td>293.54 ± 18.26 ^a</td>
</tr>
<tr>
<td>Day 8</td>
<td>−7.35 ± 0.1 ^b</td>
<td>386.13 ± 29.32 ^b</td>
</tr>
<tr>
<td>Day 16</td>
<td>−7.21 ± 0.08 ^a</td>
<td>299.94 ± 26.47 ^a</td>
</tr>
<tr>
<td>Day 22</td>
<td>−7.14 ± 0.03 ^a</td>
<td>301.05 ± 9.38 ^a</td>
</tr>
</tbody>
</table>

Areas under the curves are expressed in arbitrary units. Values with different superscripts vary significantly; one-way ANOVA followed by Newman–Keuls multiple comparisons test was used, $P < 0.05$.

![Table III](image4.png)
was found to be greatest early in pregnancy (Dogterome and De Jong, 1974). In a study of pressor responses to angiotensin II in human pregnancy, resistance to the pressor responses was found to be maximum early in pregnancy and this resistance returned to normal in late gestation (Gant et al., 1973). In addition, studies investigating in-vitro responses of the rat mesenteric as well as human subcutaneous arteries near term failed to document a decrease in the pressor responses to phenylephrine (McCarthy et al., 1994; Pascoal et al., 1995). These studies support our results that the decrease in responses to phenylephrine occurs in early gestation and is reverted back to that of the non-pregnant stage later in pregnancy.

L-NAME, a nitric oxide synthase blocker, is known to increase the contraction of rat aorta in response to α-adrenergic agonists by inhibiting nitric oxide generation by the endothelium (Carrier and White, 1985). We confirmed this in aorta from pregnant as well as non-pregnant female rats. L-NAME, however, failed to counter the attenuation of response to phenylephrine seen in early pregnancy. In addition, the increase in response to phenylephrine in the presence of L-NAME was not significantly different between the various groups. This would suggest that nitric oxide is not responsible for the decrease in responses to α-adrenergic agonists seen during pregnancy in the rat aorta. The presented data also show that the endothelium-dependent relaxation in response to acetylcholine is increased in early pregnancy and reverts back to that of the non-pregnant state in late pregnancy and at term. Various studies have shown that, in rats and guinea pigs, nitric oxide production is increased during pregnancy and that this change occurs early in pregnancy (Ahokas et al., 1991; Conrad et al., 1993; Weiner et al., 1994). In contrast to our results, Weiner et al. (1991), as well as Molnar and Hertelendy (1992), have shown that increased production of nitric oxide is responsible for the decreased responses to adrenergic agonists. However, other studies have demonstrated that in rats the decreased adrenergic contractile responses are not dependent on endothelial nitric oxide production (Parent et al., 1990; Davidge and McLaughlin, 1992). Also, Pascoal et al. (1995) have shown that, in rat mesenteric microvessels, pregnancy does not alter basal nitric oxide synthesis but does enhance acetylcholine-induced nitric oxide release. Hence, the basal nitric oxide production may remain unchanged during pregnancy, whereas the induced nitric oxide production may be increased during early gestation. In addition, mechanisms other than nitric oxide production may be responsible for the decrease in responses to α-adrenergic agonists during early gestation (discussed below). Thus, decreased responses to pressor agents and increased endothelium-dependent relaxation (and nitric oxide production) may be more important in modulating vascular reactivity in early pregnancy whereas later in gestation other mechanisms, such as the contractile potential of the vasculature, may gain predominance.

The responses to sodium nitroprusside were not changed during pregnancy, but were slightly decreased at term. The decrease was significant only at very high concentrations of sodium nitroprusside, and may not be physiologically relevant. Pohl and Busse (1987) have reported a decrease in the relaxant responses of the vasculature to nitric oxide donors during pregnancy and have suggested that this decrease may be due to increased production of nitric oxide during pregnancy.

The maximal response to phenylephrine was decreased in early pregnancy, in the absence as well as presence of L-NAME, but the ED50 was not significantly altered. These results suggest that there is a change in the post-receptor mechanisms in the vascular smooth muscle, i.e. Ca2+ entry through receptor-operated calcium channels and Ca2+ release from the intracellular stores, although a down-regulation of the α-adrenergic receptors may also account for the decreased responsiveness. The studies on α-adrenergic receptors have been somewhat contradictory, some investigators have found a decrease in the receptors, while others noted an increase or no change (Smiley and Finster, 1996). In addition to differences in the species and the vessels studied, variation in the stage of gestation may account for these deviations. The increased acetylcholine-induced endothelium-dependent relaxation during early pregnancy was characterized by an increase in the maximal response as well as a decrease in the ED50. Also, there was no increase in the responses to sodium nitroprusside

Table IV. Sodium nitroprusside responses

<table>
<thead>
<tr>
<th>Rats</th>
<th>ED50 (log M)</th>
<th>Area under the curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>-7.63 ± 0.05</td>
<td>513.90 ± 15.1</td>
</tr>
<tr>
<td>Day 8</td>
<td>-7.63 ± 0.06</td>
<td>516.74 ± 14.28</td>
</tr>
<tr>
<td>Day 16</td>
<td>-7.69 ± 0.13</td>
<td>515.43 ± 23.52</td>
</tr>
<tr>
<td>Day 22</td>
<td>-7.47 ± 0.04</td>
<td>457.73 ± 13.33</td>
</tr>
</tbody>
</table>

Areas under the curves are expressed in arbitrary units.

One-way ANOVA followed by Newman–Keuls multiple comparisons test was used; no significant differences were found.

Figure 4. Responses to sodium nitroprusside (SNP) in aortic rings with intact endothelium from non-pregnant and pregnant (days 8, 16 and 22) female rats. The rings were preincubated with L-NAME (10^-5 M) and contracted with phenylephrine (PE, 10^-5 M). Each point represents mean ± SEM, n = 15, 11, 11 and 12 for the non-pregnant, day 8, day 16 and day 22 groups respectively. One-way ANOVA followed by Newman–Keuls multiple comparisons test was used. *P < 0.05, day 22 versus non-pregnant, day 8 and day 16. ● Non-pregnant, ■ day 8, ▲ day 16 and ▼ day 22.
at this stage of pregnancy. This may be due to an increase in the endothelial muscarinic receptor sensitivity as well as an upregulation of the postreceptor mechanisms like Ca\(^{2+}\) entry or production of endothelial relaxing factors (e.g. nitric oxide, endothelium-derived hyperpolarizing factor, prostacyclins) by the vascular endothelium in early pregnancy.

Pregnancy in rats and other species is associated with an increase in the oestrogens and progestins whereas a withdrawal of progesterone and reversal of progesterone:oestrogen ratio occurs at term (Puri and Garfield, 1982; White et al., 1995). Steroid hormones are known to influence reactivity of the vascular smooth muscle as well as production of the various endothelial factors (White et al., 1995). The changes in the sex steroids may be the underlying mechanism responsible for the changes in vascular reactivity observed early in pregnancy and at term.

This study shows that, in reference to vascular reactivity, pregnancy is a dynamic state. Different vasoregulatory mechanisms may be operatic at different stages of gestation, i.e. alteration of the vascular sensitivity versus change in the vascular contractile potential. Decreased responses to pressor agents and increased production of endothelial relaxing factors may play a significant role in early gestation, while a decrease in the contractile potential of the vasculature may be more important later in pregnancy. In addition, caution needs to be exercised when evaluating studies of vascular reactivity carried out at a specific stage of gestation. Interpretations and generalizations must take into consideration the gestational duration. Our results may also explain the inability of studies carried out on human vessels to elicit some of the changes seen in the vasculature of experimental animals, since most of the human tissues are obtained from patients at term, who were either in labour or close to labour. It is conceivable that vascular responses in the peripartum period may not reflect those seen prior to the onset of labour.

Our study was carried out on the rat aorta. Although aorta is a conduit vessel and has very little role in the regulation of peripheral vascular resistance, it may be a better and more consistent representative of the general vascular responses, since there is a wide variability in the responses of different regional vascular beds. However, it would be important to carry out similar longitudinal studies in resistance vessels to confirm the presence or absence of these vaso-adaptive phenomena in the regional vascular beds.

References


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