Pregnancy and steroid hormones enhance the vasodilation responses to CGRP in rats

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Gangula, P. R. R., H. Zhao, S. Supowit, S. Wimalawansa, D. DiPette, and C. Yallampalli. Pregnancy and steroid hormones enhance the vasodilation responses to CGRP in rats. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H284–288, 1999.—We recently reported that calcitonin gene-related peptide (CGRP) reversed the hypertension induced by nitric oxide inhibition in pregnant rats and that this effect appeared to be progesterone dependent. In the present study, we examined whether the vasodilator responses to CGRP are increased during pregnancy and whether these responses are steroid hormone dependent. Three groups of ovariectomized (Ovx) rats (n = 4–8 rats/group) were studied 3 days after daily treatment (subcutaneous injection) with progesterone (P; 2 mg/injection, twice daily for 3 days, in 0.2 ml of sesame oil), 17β-estradiol (E; 2.5 µg/injection, twice daily for 3 days, in 0.2 ml of sesame oil), or vehicle (sesame oil). A fourth group (n = 6 rats) of pregnant rats was studied on day 19 of gestation. A fifth group of adult, nonpregnant rats (n = 6 rats), regardless of stage of estrous cycle, was also used in this study. Mean arterial blood pressure (MAP) was continuously monitored in fully awake and free-moving instrumented rats. MAP was measured before and after administration of either saline or varying bolus doses of CGRP (9–360 pmol/kg body wt). CGRP produced a dose-dependent decrease in MAP in all rats with a significant (P < 0.05) reduction in MAP beginning with a CGRP dose of 90 pmol/kg and with maximal effects observed at 360 pmol/kg. Decreases in MAP in response to CGRP were significantly (P < 0.05) greater in pregnant compared with nonpregnant rats. Similarly to pregnant rats, Ovx rats given both E and P treatments produced greater decreases in MAP in response to CGRP at 90, 180, and 360 pmol/kg doses compared with both ovary-intact and Ovx nonpregnant rats, which were not different from each other. In summary, these data show that 1) the hypotensive effects of CGRP are dose dependent and 2) the hypotensive effects of CGRP are enhanced during pregnancy and in Ovx rats treated with either E or P. Therefore, we suggest that the decrease in vascular tone that is seen during pregnancy may be mediated, at least in part, by a sex steroid hormone-induced increase in the vascular sensitivity to the vasodilator effects of CGRP.

blood pressure; calcitonin gene-related peptide; estrogen; progesterone; gestation

NORMAL PREGNANCY INVOLVES pronounced vascular adaptations that include increases in blood volume, cardiac output, and heart rate, as well as decreases in mean arterial blood pressure (MAP) and vascular resistance. Abnormal alterations of these cardiovascular adaptations to pregnancy may lead to impaired fetal growth and pregnancy-associated hypertension. The mechanism responsible for maintaining lower vascular reactivity during pregnancy is still unknown. However, sex steroid hormones have been implicated in the regulation of vascular tone in gestation (18). These hormonal influences appear to be mediated through several locally produced vasoactive agents, including nitric oxide (NO) (30), prostaglandins (20), and calcitonin gene-related peptide (CGRP) (8, 12, 13, 36).

CGRP, a 37-amino acid neuropeptide (28), is the most potent endogenous vasodilator known (7, 35). CGRP elicits its vasodilator effects by relaxing smooth muscle via a cAMP-dependent pathway. CGRP is a 1,000-fold more potent vasodilator than other known vasodilators, such as ACh and substance P, and 50-fold more potent than isoprenaline (2, 9). Systemic administration of CGRP decreases blood pressure (BP) in a dose-dependent manner (5, 14) and also produces significant positive chronotropic and inotropic effects (9, 14). This peptide is distributed throughout the central and peripheral nervous systems and is located in areas known to be involved in cardiovascular function (24, 29). A prominent site of CGRP production is the dorsal root ganglion, which contains the cell bodies of the sensory nerves that terminate peripherally on blood vessels and other tissues innervated by the sensory nervous system and centrally in luminae I or II of the dorsal horn of the spinal cord (6). Indeed, it has been reported that circulating CGRP is largely derived from the perivascular sensory nerve terminals (15, 29).

Our previous studies showed that during pregnancy CGRP administration reverses the BP response and fetal mortality (36) induced by chronic administration of the NO inhibitor N-nitro-L-arginine methyl ester (L-NAME). We later reported that the vasodilator effects of CGRP are modulated by progesterone (13), leading to the conclusion that vasoactive effects of this peptide depend on the elevated levels of steroid hormones that are seen in pregnancy. Other studies (12) indicate that CGRP plays a compensatory vasodilator role to attenuate the elevated blood pressure in L-NAME-treated pregnant rats. In support of these findings, it has been reported that during pregnancy in humans, circulating immunoreactive CGRP levels increase until the time of delivery, with a sharp reduction in the postpartum period (31). Also, it has been demonstrated that the sensitivity of human uterine arteries to the vasodilator effects of endogenous CGRP is higher in

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pregnancy compared with the nonpregnant state (25). Furthermore, uterine CGRP receptors are elevated during pregnancy compared with the term, postpartum, or nonpregnant state (8). Together these studies suggest that the sensitivity of vascular tissues and of the uterus to CGRP increases during pregnancy and that this may be dependent on the presence of sex steroid hormones.

Until now, many of the previous studies on the role of CGRP in the regulation of systemic and regional hemodynamics have been limited to males (5, 6). Furthermore, there are no reports available on the role of CGRP in the regulation of cardiovascular function during pregnancy or the participation of female sex hormones in this process. Therefore, the purpose of this study was to determine whether CGRP-induced systemic vasodilation is increased during pregnancy and, if so, whether these changes are female steroid hormone dependent. Our findings show that exogenous CGRP elicits a dose-dependent hypotensive effect in female rats and that this effect is substantially enhanced during pregnancy, possibly through a sex steroid hormone-mediated mechanism.

MATERIALS AND METHODS

Animals. Time-mated pregnant rats on day 14 of gestation (day 1 is the day of positive sperm smear; 300–320 g body wt) and adult nonpregnant (170–200 g body wt) animals were purchased from Harlan Sprague Dawley (Indianapolis, IN). Before the hemodynamic studies were begun, all animals were housed in a climate-controlled room with a 12:12-h light-dark schedule and were fed standard rat chow with water to drink ad libitum. All procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch (Galveston, TX).

Treatments. Nonpregnant animals were bilaterally ovariectomized (Ovx) under halothane (Halocarbon, River Edge, NJ) anesthesia. One week after ovariectomy, groups of Ovx animals were injected subcutaneously with progesterone (P; 2 mg/injection, twice daily for 3 days, in 0.2 ml sesame oil), 17β-estradiol (E; 2.5 µg/injection, twice daily for 3 days, in 0.2 ml of sesame oil), or vehicle (sesame oil). Pregnant rats on day 19 of gestation were also used for measuring the vasodilator responses to CGRP. Both hormones were purchased from Sigma (St. Louis, MO). Adult nonpregnant rats regardless of stage of estrous cycle were also utilized to examine whether endogenous ovarian hormones modulate the vasodilator responses to CGRP.

MAP measurements. Human CGRP was synthesized by S. J. Wimalawansa using standard solid-phase t-butoxycarbonyl chemistry; purified; and characterized by mass spectrometry, amino acid analysis, and sequencing. On the day of the acute measurements, both pregnant (day 19 of gestation) and nonpregnant Ovx animals treated with steroid hormones were anesthetized with ketamine (45 mg/kg body wt; Fort Dodge Laboratory, Fort Dodge, IA) and xylazine (5 mg/kg body wt; Burns Veterinary Supply, New York, NY). Catheters (PE-50, Clay Adams, Parsippany, NJ) were inserted into the left carotid artery to continuously measure MAP using a DBP001 direct BP system (Kent Scientific, Litchfield, CT), and into the right jugular vein to administer either vehicle (saline) or CGRP.

Three to four hours after surgery, with the animals fully awake and in a free moving state, MAP measurements were determined immediately before and 1 min after bolus injections of either the vehicle or varying doses of CGRP (9–360 pmol/kg body wt). Groups of 4–8 pregnant rats, adult nonpregnant rats, or Ovx rats were treated with P, E, or vehicle and used for measuring changes in MAP in response to each dose of CGRP.

Changes in MAP at 1 min after administration of either saline or varying doses of CGRP from the baseline MAP before the injections are assessed. In all rats MAP was continuously monitored for at least 5 min after each dose of CGRP or saline, and we observed maximal responses, which occurred within 1 min. Therefore, we calculated the MAP at 1 min after CGRP injection in order to compare the different groups. The changes in MAP in response to each dose of CGRP are expressed as means ± SE for each group. One-way ANOVA followed by the Bonferroni t-test and Student's t-test were used for statistical analysis. The acceptable level of significance was P < 0.05.

RESULTS

MAP was significantly (P < 0.05; n = 4–8 rats/group) decreased on day 19 in pregnant rats (95 ± 2 mmHg) vs. nonpregnant Ovx rats (106 ± 4 mmHg) (Table 1). However, no differences in MAP were observed between nonpregnant ovari-intact rats (93 ± 1.3 mmHg) and pregnant rats. Furthermore, MAP in Ovx rats was significantly higher compared with that of ovari-intact nonpregnant rats. Moreover, both E (90 ± 3 mmHg) and P (91 ± 2 mmHg) significantly (P < 0.05; n = 20) reduced the baseline MAP in the ovarian hormone-depleted (Ovx) rats (Table 1).

Figure 1 illustrates the effect of exogenous CGRP on MAP in pregnant and nonpregnant Ovx rats. No changes in MAP were observed after treatment with either saline (vehicle) or the lowest dose (9 pmol/kg body wt) of CGRP in either group. However, administration of CGRP produced a significant (P < 0.05) dose-dependent decrease in MAP in both groups beginning with 90 pmol/kg body wt of the peptide (Fig. 1). The decrease in MAP (changes from baseline MAP) in response to varying doses of CGRP was significantly greater in pregnant rats (90 pmol, −10.3 ± 4.9 mmHg; 180 pmol, −15.0 ± 3.0 mmHg; 360 pmol, −24.0 ± 3.7 mmHg) compared with Ovx rats (90 pmol, −3.0 ± 0.6 mmHg; 180 pmol, −8.9 ± 1.4 mmHg; 360 pmol, −14.3 ± 2.9 mmHg).

In addition, we used intact nonpregnant rats, without regard to stage of estrous cycle, to examine whether

Table 1. Effect of pregnancy and steroid hormones on mean arterial blood pressure in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
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<tbody>
<tr>
<td>Intact nonpregnant</td>
<td>93 ± 1.3*</td>
</tr>
<tr>
<td>Ovx + vehicle</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>Ovx + 17β-estradiol</td>
<td>90 ± 2*</td>
</tr>
<tr>
<td>Ovx + progesterone</td>
<td>91 ± 2*</td>
</tr>
<tr>
<td>Day 19 of pregnancy</td>
<td>95 ± 2*</td>
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Values are means ± SE; n = 4–8 rats/group. Ovariectomized adult (Ovx) rats were treated with 17β-estradiol (2.5 µg/injection sc, twice daily for 3 days, in 0.2 ml of sesame oil), progesterone (2 mg/injection sc, twice daily for 3 days, in 0.2 ml of sesame oil), or with sesame oil only (vehicle). MAP, mean arterial blood pressure. *P < 0.05 vs. Ovx + vehicle (by ANOVA).
endogenous steroid hormones decrease MAP in response to CGRP. Administration of CGRP decreased MAP dose dependently in pregnant rats. Although the decreases in MAP in response to CGRP in intact pregnant rats (90 pmol, $-6.0 \pm 0.6$ mmHg; 180 pmol, $-11.0 \pm 2.5$ mmHg; 360 pmol, $-16.0 \pm 2.7$ mmHg) were less than those in pregnant rats, these differences were not significant. Similarly, no significant differences between ovary-intact and Ovx animals were observed in their MAP responses to CGRP (Fig. 1).

We next examined the effects of E and P on the ability of CGRP to lower MAP in Ovx rats. As shown in Fig. 2, neither the saline nor the 9-pmol CGRP injection significantly altered MAP in Ovx rats treated with either vehicle or the female sex hormones. However, CGRP significantly ($P < 0.05$) decreased the MAP in a dose-dependent manner beginning at 90 pmol/kg body wt with the maximal effects achieved at 360 pmol/kg body wt in Ovx and in E- and P-treated rats. Decreases induced by varying doses of CGRP in the MAP were significantly greater ($P < 0.05$) in E-treated (90 pmol, $-10 \pm 3$ mmHg; 180 pmol, $-15 \pm 2$ mmHg; 360 pmol, $-24 \pm 2$ mmHg) rats compared with the Ovx group (Fig. 2). Similarly, decreases in MAP in response to varying doses of CGRP were significantly greater in P-treated (90 pmol, $-10 \pm 2$ mmHg; 180 pmol, $-15 \pm 2$ mmHg; 360 pmol, $-22 \pm 3$ mmHg) animals compared with Ovx rats (Fig. 2).

DISCUSSION

The present study demonstrates that MAP was lower in pregnant, ovary-intact nonpregnant, and steroid hormone-treated Ovx rats compared with Ovx animals. Systemic administration of CGRP decreased MAP in a dose-dependent manner in all the groups studied. The hypotensive effects of CGRP were much greater in pregnant and in steroid hormone-treated rats compared with the untreated adult Ovx and ovary-intact virgin rats. This suggests that the vasodilator effects of CGRP are markedly elevated at day 19 of pregnancy and after treatment with sex steroid hormones. These differences in BP responses appear to be due to changes in vasodilation rather than in cardiac output, because CGRP does not alter the cardiac output (Ref. 6 and P. R. R. Gangula and C. Yallampalli, unpublished observation). These findings are consistent with our previous report (13) that demonstrated that CGRP lowers the BP in L-NAME-induced hypertensive pregnant rats but not in L-NAME-treated nonpregnant steroid hormone-depleted (Ovx) rats. Together these observations suggest that the vascular responsiveness to the depressor effects of CGRP is increased during pregnancy and that this effect may be steroid hormone dependent.

In several animal species including humans, BP decreases with pregnancy (10, 21). This decrease in BP appears to be progressive with maximal decreases occurring during the last week of gestation in unanesthetized normotensive rats (33). It has been suggested that the fall in BP, which is maximal during the last week of pregnancy in the rat, may be mediated through a decreased sensitivity of the vasculature to vasoconstrictor agents (3). Other investigators (1) have postulated that the magnitude of the decrease in BP during the last week of pregnancy was directly related to the increased number of fetuses and their size in spontaneously hypertensive rats. These data suggest that the fetal-placental unit may play an important role in the regulation of maternal systemic vascular tone and BP during pregnancy, at least in genetically hypertensive animals.

The mechanisms involved in the decreased responsiveness to vasoconstrictor agents during pregnancy are not completely understood. Increased production of NO or prostacyclin, with a resultant dominance of the vasodilator effects of prostacyclin over thromboxane A2
Calcitonin gene-related peptide (CGRP) is a potent vasodilator that plays a role in lowering blood pressure (BP) during pregnancy. Several studies suggest a role for estrogens in the regulation of vascular tone and BP homeostasis (4, 17, 23). However, the mechanisms by which estrogens elicit these effects are not well understood. One of the proposed mechanisms includes induction of basal NO (17, 26, 27) in the endothelial cells, thus regulating the vascular smooth muscle tone. Our data show that estrogens significantly enhance the BP-lowering effect of CGRP. One possible explanation for this is that estrogens may stimulate other vasodilator factors, such as NO (30) or prostacyclin (22), and these agents in turn may increase the hypotensive actions of CGRP. Several in vitro studies using aortic rings suggest that the vasodilator effects of CGRP are mediated through NO (16, 32). It has also been reported that the depressor effects of CGRP were mediated through its receptors coupled to different second-messenger systems, such as cAMP and cGMP, and/or the release of endothelium-derived relaxing factors (19). Additional studies are required to understand the mechanism that is responsible for the CGRP-mediated vasodilation in the presence of estrogens. We speculate that increased concentrations of CGRP receptors in blood vessels may be involved in regulating vascular adaptations during pregnancy and that both estrogen and progesterone may increase vascular CGRP receptors.

In summary, pregnancy increased the sensitivity to CGRP in reducing BP compared with that of nonpregnant Ovx rats. Supplementation to nonpregnant Ovx rats of progesterone or estrogen at the levels that occur during pregnancy enhanced the vasodilator effects of CGRP. We suggest that a decrease in vascular reactivity during pregnancy in rats could be mediated by an increased sensitivity of the vasculature to CGRP. Therefore, our studies provide evidence that CGRP may play an important role in reducing vascular tone during pregnancy, and this may be mediated by sex steroid hormones.

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