Effects of calcitonin gene-related peptide on vascular resistance in rats: role of sex steroids

MICHELLE GREWAL,1 JANIS CUEVAS,1 GAUTAM CHAUDHURI,1,2 AND LAUREN NATHAN1

1Department of Obstetrics and Gynecology and 2Department of Molecular and Medical Pharmacology, University of California, Los Angeles, California 90095

Grewal, Michelle, J anis Cuevas, Gautam Chaudhuri, and Lauren Nathan. Effects of calcitonin gene-related peptide on vascular resistance in rats: role of sex steroids. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H2063–H2068, 1999.—It has been demonstrated in reflex-intact animals that the sensitivity to calcitonin gene-related peptide (CGRP) is increased during pregnancy and that this action is mediated by sex steroids but not by nitric oxide (NO). We assessed the effects of CGRP in the following groups of anesthetized ganglion-blocked rats: 1) pregnant, 2) ovariectomized, and 3) ovariectomized and treated with estradiol and progesterone. Changes in mean arterial pressure (MAP) were assessed after the administration of varying doses of CGRP. Decreases in MAP after CGRP administration were significantly greater in pregnant rats and ovariectomized rats administered sex steroids than in ovariectomized controls. The CGRP antagonist CGRP8–37 produced a pressor response of similar magnitude in both pregnant and ovariectomized rats. We also assessed the effects of CGRP and the modulating role of NO in the isolated uterine vascular bed preparation. CGRP reduced perfusion pressure to a greater degree in ovariectomized animals treated with sex steroids than in ovariectomized animals. This response was attenuated by pretreatment with an NO synthesis inhibitor. CGRP8–37 produced a similar increase in perfusion pressure in both groups. We conclude that 1) the increased vascular sensitivity observed during pregnancy or after treatment with sex steroids is in part mediated by NO, and 2) CGRP8–37 has a vasoconstrictor action of its own.

nitric oxide; uterine vascular bed; estradiol; progesterone

PREGNANCY is associated with an increase in cardiac output and an increase in plasma volume. However, the systemic arterial pressure is not increased due to a decrease in systemic vascular resistance (23). The mechanism by which the decrease in systemic vascular resistance occurs is not known, although various hypotheses have been put forward to explain this phenomenon. It has been suggested that sex steroids may be involved in the regulation of vascular tone during gestation (16). Sex steroids may mediate this action by regulating the production of vasoactive substances, including nitric oxide (NO) (15, 21, 27), prostaglandins (19), and calcitonin gene-related peptide (CGRP) (4, 7, 8, 36).

CGRP is a 37-amino acid neuropeptide (25) and is the most potent endogenous vasodilator known (3, 10, 17, 30). Circulating immunoreactive CGRP (iCGRP) levels increase up to the time of delivery and then decline sharply in the postpartum period (28). Administration of a CGRP antagonist to N-nitro-L-arginine methyl ester (L-NAME)-treated pregnant rats in late gestation led to an increase in mean arterial pressure (MAP), and this has been taken as further indirect evidence to indicate that there is an increased CGRP level during pregnancy (6). CGRP may modulate this decrease in vascular resistance, because, in addition to increased circulating levels of CGRP, the sensitivity of the vasculature to CGRP is increased during pregnancy (4, 6–8, 36). This increased sensitivity is mediated by both estradiol and progesterone (8) and is thought to be NO independent, because CGRP was able to produce a decrease in MAP in animals rendered hypertensive by treatment with an inhibitor of NO synthesis (7).

CGRP is produced in the dorsal root ganglia, which contain the cell bodies of the sensory nerves that terminate peripherally on blood vessels (3). It has also been shown that circulating CGRP is largely derived from the perivascular sensory nerve terminals (9, 26). Almost all studies related to CGRP have been performed on fully awake, reflex-intact animals (4, 6–8, 36). The present study was therefore undertaken to assess the effects of CGRP and a CGRP antagonist (CGRP8–37) on MAP in ganglion-blocked pregnant and nonpregnant animals. We also assessed the direct effects of CGRP and CGRP8–37 on resistance vessels by utilizing the in situ perfused uterine vascular bed preparation. The pregnant rat was chosen as the animal model because some of the cardiovascular changes this species undergoes during pregnancy, i.e., decrease in MAP and attenuation of pressor responsiveness to exogenous vasoconstrictors, are similar to those of women (20, 23).

MATERIALS AND METHODS
Animal Preparation

Experiments were conducted on female Sprague-Dawley rats (200–350 g) (Harlan, Indianapolis, IN). Animals were housed under conditions of controlled temperature and light cycle and were provided free access to food pellets and water. Animals were divided into the following groups: 1) pregnant rats in late gestation (day 17 or 18), 2) ovariectomized rats, and 3) ovariectomized rats receiving a 12-day regimen of a combination of estradiol benzoate (100 µg/kg) for 12 days and progesterone hexanoate dissolved in corn oil (15 mg/kg), which was initiated on the 7th day and injected subcutaneously daily for 6 days. These doses have previously been shown to produce effects similar to those found during pregnancy (2). Ovariectomy was performed under pentobarbital sodium anesthesia (40 mg/kg ip) 3–5 days before the experiments. The day that sperm were first seen in the vaginal lavage was considered day 1 of pregnancy.
Pressor Responses to CGRP

After the establishment of anesthesia, polyethylene catheters (PE-50) were inserted into the external jugular vein and the common carotid artery for drug infusion and blood pressure recording, respectively. Blood pressure was measured by a Statham transducer and recorded on a Hewlett-Packard polygraph. Animals were ventilated on a rodent respirator (1 ml/100 g, 75 strokes/min). To eliminate all autonomic reflexes, animals were injected with the ganglion blocker pentolinium (5 mg/kg iv) 10 min before the experiments. After cannulation of the carotid artery, phenylephrine was infused at a concentration of 2 µg/ml at a rate of 0.2 ml/min to maintain a baseline blood pressure that ranged from 80 to 110 mmHg so that absolute changes in MAP after drug infusion could be recorded and compared between groups.

In Situ Uterine Vascular Bed Perfusion Technique

After the establishment of anesthesia with urethan (1.25 g/kg ip), a laparotomy was performed. The right internal iliac artery was cannulated with a polyethylene catheter (PE-50) through which Krebs bicarbonate containing phenylephrine blocker pentolinium (5 mg/kg iv) 10 min before the experiments. After cannulation of the carotid artery, phenylephrine was infused at a concentration of 2 µg/ml at a rate of 0.2 ml/min to maintain a baseline blood pressure that ranged from 80 to 110 mmHg so that absolute changes in MAP after drug infusion could be recorded and compared between groups.

Experimental Protocols

Study 1: Pressor responses to CGRP. Animals in each of the three groups received three graded doses of CGRP (0.01, 0.03, and 0.1 µg iv) or vehicle as a bolus injection in a volume of 0.3 ml. MAP from baseline was measured after each dose when the peak rise in MAP occurred, within 1–2 min. Blood pressure was allowed to return to baseline and stabilize before each subsequent dose was administered.

Study 2: Effect of CGRP on uterine vascular resistance and role of NO. Experiments were performed on ovariec-tomized animals and ovariectomized animals supplemented with estradiol and progesterone. Varying concentrations of acetylcholine (ACh; 10⁻⁶–10⁻⁵ M) were infused initially to confirm the expected fall in perfusion pressure. Perfusion pressure was continuously recorded, and any change in perfusion pressure was measured as the percent change from baseline before administration of the drug. Varying concentrations of CGRP (0.01 and 0.03 µg) were administered as a bolus through the cannula perfusing the uterine vascular bed, and the percent change in perfusion pressure was measured. To evaluate whether responses to CGRP were dependent on NO, the responses to ACh and CGRP were compared before and 30 min after constant perfusion of an inhibitor of NO synthesis, l-NAME (3 x 10⁻⁴ M).

Study 3: Role of CGRP antagonist. To indirectly assess whether there were increased circulating levels of CGRP in pregnant animals, compared with levels in ovariec-tomized animals, the CGRP antagonist CGRP₈₋₃₇ (6) was injected as a bolus (100 µg) and the change in MAP assessed. The direct effects of this antagonist on uterine resistance vessels, if any, were assessed in the in situ rat uterine perfused vascular bed preparation.

Drugs

ACh, phenylephrine hydrochloride, urethan, pentolinium ditartrate, l-NAME, CGRP, the CGRP antagonist CGRP₈₋₃₇, estradiol benzoate, and progesterone hexanoate were purchased from Sigma Chemical (St. Louis, MO). All drugs were dissolved in 4% bovine serum albumin (BSA) in 0.9% saline.

Statistics

All data are expressed as means ± SE. Differences between treatment group means were compared using two-way ANOVA. Differences were considered to be significant at P < 0.05. The mean values were obtained from at least five animals in each of the groups.

RESULTS

Study 1: Pressor Responses to CGRP

In all groups of animals, there was a dose-related decrease in MAP after CGRP. The magnitude of fall in MAP after CGRP was greater in pregnant animals and in ovariectomized animals treated with estradiol and progesterone compared with that in ovariec-tomized animals receiving vehicle (Fig. 1). The percent fall in MAP (± SE) in the three groups of animals is shown in Fig. 2, where it can be seen that the effects of CGRP were significantly greater in pregnant rats and ovariectomized rats treated with estradiol and progesterone than in ovariectomized animals receiving vehicle alone.
Study 2: Effect of CGRP on Uterine Vascular Resistance and Role of NO

ACh produced a fall in uterine perfusion pressure in both groups of animals. CGRP (0.01 and 0.03 µg) produced a concentration-dependent decrease in uterine perfusion pressure in ovariectomized animals. After treatment with L-NAME, the effects of both ACh and CGRP were significantly attenuated (Fig. 3). Figure 4 shows the mean percent decrease (±SE) in uterine perfusion pressure after ACh and CGRP both before and after pretreatment with L-NAME. The effect of the lowest concentration of CGRP (0.01 µg) was significantly greater in ovariectomized animals treated with estradiol and progesterone compared with the response observed in ovariectomized animals receiving vehicle. The responses to ACh and CGRP in both groups were significantly attenuated after pretreatment with L-NAME.

Study 3: Role of CGRP Antagonist

CGRP, a 37-amino acid neuropeptide (25), is present in the circulation (10, 34, 35) and in a wide variety of human and rat tissues, including uterine tissues (31, 32). Its receptors are distributed widely in the cardiovascular tissues (33). During pregnancy, circulating iCGRP levels increase up to the time of delivery and then decline sharply in the postpartum period (28). The sensitivity of the uterine arteries to circulating endogenous CGRP seems to be higher in comparison with that of nonpregnant uterine arteries (22). On this basis it has been suggested that CGRP is involved in regulating the uteroplacental blood supply (30), in regulating the vascular adaptations that occur during normal pregnancy, and perhaps in the pathophysiology of preeclampsia (30). However, the exact mechanism by which CGRP regulates the vascular adaptations that occur during normal pregnancy is not known. In some arterial beds, it has been shown (1, 12, 13, 29) that endothelium is essential for the vasodilatory actions of CGRP. On the other hand, it has been reported that the vasodilatory actions of CGRP in the human uterine artery are independent of the endothelium (22) and are not mediated through the release of endothelium-dependent vasodilators such as NO and vasodilator prostanoids (22, 36) but that they may be mediated by specific CGRP receptors (18, 36) on the vascular smooth muscle.

It has now been unequivocally demonstrated that the blood pressure-lowering effects of CGRP are substantial during pregnancy compared with those during labor and the postpartum period (36). On this basis it has been hypothesized that the sensitivity to CGRP is elevated during pregnancy and that this elevation may be responsible for the uterine vascular relaxation and increased blood flow to the uterus (36). It has been suggested that the increased vascular sensitivity to CGRP during pregnancy is unlikely to be mediated by...
NO, because the response to CGRP was still observed in L-NAME-treated rats rendered hypertensive (6, 36). The increased sensitivity to CGRP is now thought to be mediated by estrogen and/or progesterone (7, 8). The CGRP antagonist GCRP 8–37 has been shown by one group of investigators (6) to raise the MAP in pregnant, but not in nonpregnant, animals, thereby further strengthening the hypothesis that increased circulating levels of CGRP during pregnancy may play an important role in the hemodynamic changes that occur in this condition. In all these studies, fully awake, reflex-intact animals were utilized; therefore, any changes in blood pressure response to CGRP being mediated by reflex activity cannot be completely ruled out.

The primary objective of our study, therefore, was to assess in ganglion-blocked animals the blood pressure response to CGRP in ovariectomized rats, ovariectomized rats treated with estradiol and progesterone, and rats in late pregnancy (days 17–19). Anesthetized, ganglion-blocked animals in all three groups received constant phenylephrine infusion to increase the MAP to comparable levels to ensure that responses to CGRP were assessed in animals with comparable baseline MAP. In ovariectomized animals, we observed a concentration-related decrease in MAP. This effect of CGRP at the two lower concentrations (0.01 and 0.03 µg) was significantly potentiated in ovariectomized animals treated with estradiol and progesterone and in pregnant animals compared with that in ovariectomized animals receiving vehicle alone. The effect of the higher concentration of CGRP (0.1 µg) was also potentiated, but the values were not statistically significant due to the wide variation in responses from one animal to another observed at this concentration in all three groups. These results indicate that the effects of CGRP on blood pressure are not due to any changes in reflex activity and most likely represent changes in the sensitivity of resistance vessels to CGRP during pregnancy as suggested by other investigators (6–8). This change in sensitivity during pregnancy is most likely mediated by estradiol and progesterone and not due to any modulation by fetal products, because the increased sensitivity to CGRP during pregnancy was similar to that observed in ovariectomized animals treated with estradiol and progesterone. In this regard our results are similar to those of other workers (7, 8) who have demonstrated that estradiol and/or progesterone can increase the sensitivity to CGRP. However, our results and those of other investigators (7, 8) do not definitively indicate that this increased sensitivity in vivo during pregnancy to CGRP is mediated at the level of resistance vessels, because the possibility that the clearance of CGRP is decreased during pregnancy, or after treatment with sex steroids, has not been ruled out.

We therefore elected to use an in situ uterine vascular bed perfusion technique. This technique allowed us to study the effects of CGRP directly on the resistance vessels of the uterine vascular bed without any confounding influence of clearance of CGRP or any effects of CGRP on cardiac output that might have modulated the response on blood pressure during pregnancy or after treatment with sex steroids. The uterine vascular bed was chosen because it has been suggested by other
investigators (30) that CGRP may modulate uteroplacental blood flow. In this preparation the perfusion flow was maintained at a constant rate, and therefore any change in perfusion pressure would reflect changes in vascular resistance, indicating dilation of resistance vessels. Our observation that ACh, an endothelium-dependent vasodilator (5), led to a decrease in perfusion pressure indicates that the endothelium was intact and functional in this vascular bed (5). In the uterine vascular bed of ovariectomized animals, CGRP produced a concentration-dependent decrease in perfusion pressure. This response to CGRP was significantly potentiated at the lower concentration (0.01 µg) in ovariectomized animals treated with sex steroids and reached the peak decrease in perfusion pressure. At higher concentrations, no significant differences were observed because of wide variation in the responses from one animal to another. After pretreatment with L-NAME, the responses to ACh were significantly attenuated compared with pre-L-NAME values, indicating that part of the Ach response in this vascular bed is mediated by NO. Similarly, the responses of the resistance vessels to CGRP at the two lower concentrations (0.01 and 0.03 µg) tested were also significantly attenuated, indicating that the vasodilator response to CGRP in the uterine vascular bed is at least in part endothelium independent and mediated by release of NO.

There were no differences in the responsiveness of the vascular bed to CGRP after L-NAME pretreatment between ovariectomized animals and animals treated with estradiol and progesterone, indicating that the differences observed between the two groups before pretreatment with L-NAME were NO mediated. This is consistent with our previous reports (15) that estradiol increases NO production. The differences in results between our studies and those of other investigators (22) who have observed that the relaxant effects of CGRP on human uterine arteries are endothelium independent could be explained on the basis that we assessed the effects of CGRP on resistance vessels, whereas the other investigators used conduit vessels. Our results therefore indicate that the increase in vasodepressor response observed in pregnant animals or in ovariectomized animals treated with estradiol and progesterone may in part be explained by increased sensitivity of the uterine vascular bed to CGRP, an effect that is partially modulated by NO.

Some investigators have utilized the CGRP antagonist CGRP<sub>8-37</sub> to demonstrate the presence of circulating CGRP (6). Those investigators observed that, after administration of the antagonist, an increase in MAP was observed only in pregnant animals compared with nonpregnant controls, and this increase in MAP lasted for a very short duration. This was taken to indicate indirectly that circulatory levels of CGRP were higher in pregnant animals. By contrast, in our study using ganglion-blocked animals, we observed a rise in MAP with the CGRP antagonist of a similar magnitude in both ovariectomized animals as well as in pregnant animals. Further studies utilizing the uterine vascular perfused bed from both ovariectomized animals and those treated with estradiol and progesterone revealed that this antagonist caused an increase in perfusion pressure again of a similar magnitude in both the groups. Because the perfusion medium in these experiments was Krebs bicarbonate, in which there was no CGRP, the rise in perfusion pressure must be due to a vasoconstrictor action of this antagonist. These results therefore suggest that this antagonist should be used with caution to study the role of endogenous CGRP, because, at some doses, this antagonist can have its own vasoconstrictor action.

In conclusion, our results confirm those reported by other investigators (7, 8) that the increased vasodilator response observed with CGRP during pregnancy is mediated by sex steroids via an action on the vascular wall. Our results, however, indicate that the action of CGRP on the uterine vascular bed is partially mediated through increased release of NO, and in this respect they differ from results of other investigators (22), who have suggested that the effect of CGRP on the vasculature is endothelium independent. CGRP<sub>8-37</sub> acts on the CGRP<sub>1</sub> receptor and can competitively displace CGRP (11, 14, 24). However, our studies indicate that this antagonist may have some vasconstrictor activity, at least in some vascular beds.

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Address for reprint requests and other correspondence: L. Nathan, Dept. of Obstetrics and Gynecology, UCLA School of Medicine, Los Angeles, CA 90095-1740.

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