Calcitonin gene-related peptide reverses the hypertension and significantly decreases the fetal mortality in pre-eclampsia rats induced by N^G^-nitro-L-arginine methyl ester

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We recently established that the chronic inhibition of nitric oxide production with N^G^-nitro-L-arginine methyl ester (L-NAME) increases blood pressure and fetal mortality in pregnant rats. Using this animal model, we have investigated whether calcitonin gene-related peptide (CGRP) can reverse the pre-eclampsia-like conditions produced by L-NAME. CGRP and L-NAME were chronically infused s.c. into pregnant rats separately or together starting on day 17 of gestation; a control group was given saline infusions. Systolic blood pressure was measured on gestational days 17, 18, 19 and 22 and post-partum days 1 and 2. The weight and mortality of the pups were recorded immediately after spontaneous delivery. Animals treated with L-NAME exhibited significant elevations of blood pressure on days 18, 19 and 22 of gestation and during post-partum, increased pup mortality (18.4 versus 0.0%) and decreased pup weights (5.14 ± 0.07 versus 6.20 ± 0.06 g). The co-administration of L-NAME and CGRP prevented the gestational (not the post-partum) L-NAME hypertension and decreased pup mortality to 6.4% but did not reverse the decreased fetal weight (5.31 ± 0.06 g). Our data indicate that CGRP (i) participates in regulation of the vascular adaptations that occur during normal pregnancy, (ii) has beneficial effects on the hypertension and increased mortality of experimental pre-eclampsia, and (iii) may exert differential effects on the systemic (i.e. maternal) and fetal components of utero-placental circulation. These findings may have important clinical implications.

Key words: CGRP/nitric oxide/pre-eclampsia/pregnancy/progesterone

Introduction

Pre-eclampsia is considered to be one of the most significant health problems in human pregnancy. The condition is generally recognized in the latter half of gestation and complicates 6–8% of gestations. It is the leading cause of fetal growth retardation and infant morbidity and mortality associated with premature delivery and maternal death. It is estimated that 20–25% of total perinatal mortality is caused by pregnancy-associated hypertensive disorders. Hypertension, decreased fetal growth and proteinuria are the hallmarks of pre-eclampsia. Because pre-eclampsia only occurs during pregnancy and mainly in conditions with a superabundance of placental tissue (i.e. gestational trophoblastic disease, diabetes mellitus and multiple gestation), it could be inferred that the causative factors of pre-eclampsia might originate in the placenta or with changes in the hormones associated with pregnancy. However, the occurrence of pre-eclampsia in incomplete molar pregnancies indicates that uterine and fetal factors are not required (Page, 1939).

Endothelium-derived relaxing factors (EDRF) are a group of labile substances that relax smooth muscle via a cascade of reactions which trigger the stimulation of soluble guanylate cyclase and the generation of guanosine cyclic monophosphate (cGMP; Furchgott, 1984). The chemical nature of the main EDRF has been identified as nitric oxide (NO; Moncada et al., 1988), but other molecules such as prostacyclin (PGI2) are also known to be released from endothelium and are responsible for vascular smooth-muscle relaxation. NO is generated from L-arginine by nitric oxide synthases which are inhibited by various analogues of L-arginine such as N^G^-nitro-L-arginine methyl ester (L-NAME; Rees et al., 1990). Recently we have shown that the NO–cGMP-generating system exists in the rat (Yallampalli et al., 1993, 1994b) and human (Buhimschi et al., 1995a) uterus and is up-regulated during pregnancy and down-regulated during labour (Yallampalli et al., 1994a).

We and others have shown previously that in pregnant rats the inhibition of NO synthesis during pregnancy causes hypertension, proteinuria and fetal growth retardation without affecting gestational length (Yallampalli and Garfield, 1993; Molnar et al., 1994; Buhimschi et al., 1995b). Other studies also suggest that NO plays a functional role in the blunted pressor responsiveness seen during pregnancy (Molnar and Hertelendy, 1992). In normotensive and spontaneously hypertensive non-pregnant rats, L-arginine produces a sustained and dose-dependent reduction in both systolic and diastolic blood pressure after the acute administration of an NO inhibitor (Tabrizchi and Triggle, 1992). These studies indicate that NO plays a major role in regulating the blood pressure, and alterations of this system may lead to hypertension and pre-eclampsia. We have utilized this animal model of pre-eclampsia to explore the interactions of several agents in the pathogenesis of pre-eclampsia, including the effects of steroid hormones on blood pressure, perinatal mortality and birth weight (Yallampalli and Garfield, 1993; Buhimschi et al., 1995b). In these studies we found that progesterone, but...
not oestrogen, regulates vascular adaptations during normal pregnancy (Buhimschi et al., 1995b).

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide (Rosenfeld et al., 1983) and the most potent endogenous vasodilator known (Tippins, 1986; DiPette and Wimalawansa, 1994). CGRP is present in circulation (Girgis et al., 1985; Wimalawansa and MacIntyre, 1988a; Wimalawansa et al., 1989) and in a variety of human and rat tissues including uterine tissues (Wimalawansa et al., 1987; Wimalawansa, 1993a). Its receptors are widely distributed in the cardiovascular tissues (Wimalawansa and MacIntyre, 1988b). It has also been reported that elevated blood pressure during pregnancy can be lowered with calcium supplementation (Knight and Keith, 1992). Recently it was reported that these blood pressure-lowering effects of oral calcium supplementation may be mediated through the synthesis and release of CGRP (Wimalawansa, 1993b). CGRP also seems to have a growth factor-like effect on the human umbilical vein endothelial cells (Datta et al., 1990) and possibly acts as an angiogenic factor in ischaemic conditions (Carter et al., 1993).

During pregnancy, the circulating immunoreactive CGRP (i-CGRP) concentration increases up to the time of delivery, with a sharp reduction in the post-partum period (Stevenson et al., 1986). NO synthase inhibitors have been shown to inhibit the relaxation of uterine arteries in pregnant rat, in comparison with non-pregnant rats (Nelson et al., 1993). In addition, during pregnancy the sensitivity of the uterine arteries to circulating endogenous CGRP seems to be higher, in comparison with non-pregnant uterine arteries (Nelson et al., 1993). Taken together, it is likely that CGRP is involved in regulating the utero-placental blood supply (and hence fetal survival and growth) during pregnancy (Wimalawansa, 1996). Here, we examine the effects of the administration of synthetic CGRP in reversing the pre-eclampsia-like signs induced by L-NAME.

Materials and methods

Animals

Adult nulliparous pregnant rats (300–325 g body weight) were purchased from Harlan Sprague-Dawley (Houston, TX, USA) and were received in the animal care facilities on day 16 of pregnancy (day 1 = day of positive sperm smear). All animals were given free access to food and water. All procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch, Galveston, TX, USA.

Induction of pre-eclampsia symptoms

Starting on day 17 of pregnancy, rats received osmotic minipumps which delivered specific NO synthase inhibitor L-NAME (Sigma, St Louis, MO, USA), dissolved in a sterile saline solution, at a rate of 50 mg/day/rat (Yallampalli and Garfield, 1993). Osmotic minipumps (Alza Corporation, Palo Alto, CA, USA; model 2ML1 with a pumping rate of 10 µl/h) were filled with vehicle with or without L-NAME and placed s.c. during halothane (Halocarbon Laboratories, North Augusta, SC, USA) anaesthesia. Human CGRP was synthesized by solid-phase chemistry by one of the authors (S.J.W.), purified, cyclized and fully characterized by amino acid analysis, sequencing and fast-atom bombardment mass spectrophotometry. Synthetic CGRP was administered in additional osmotic pumps inserted on day 17 of pregnancy (pump rate 10 µl/h, delivering 1 µg of CGRP/h) along with the pump with L-NAME or vehicle.

Blood pressure measurement

On day 17 of pregnancy, the systemic blood pressure was measured with a pneumatic tail-cuff device (Narco-BioSystems, Houston, TX, USA) in animals that were prewarmed in a metal chamber maintained at ~30°C. Blood pressure values obtained from three consecutive measurements were averaged and recorded as the mean systolic blood pressure of a given rat.

The measurement of the systolic blood pressure of all the animals was carried out until delivery and for 2 days post-partum by one investigator. All animals delivered on day 22 of gestation, the expected day of delivery. After delivery of the pups (within 1 h), the number, weight and condition of the pups were recorded.

Statistical analysis

The mean (±SEM) values were calculated for blood pressure on a specified day of pregnancy and during the post-partum period, and pup weights and mortality rates were averaged. Differences in the blood pressure values were analysed using an analysis of variance (ANOVA) followed by Dunnett's test comparing all experimental groups against the control group. Pup weights and mortality rates were analysed using ANOVA followed by an unpaired Student's t-test. A P value <0.05 was considered significant.

Results

Effects of CGRP on the L-NAME-induced hypertension in pregnant rats

Figure 1 depicts the systolic blood pressure measured in rats beginning on day 17 of gestation and up to post-partum day 2. The systolic blood pressure in the L-NAME-treated group was significantly elevated during pregnancy and the post-partum period. Blood pressures were measured on days 17, 18, 19 and 22 of gestation and on days 1 and 2 post-partum. CGRP alone significantly reduced the systolic blood pressure measured on days 18, 19 and 22 of gestation and on day 1 post-partum when compared with the control animals, but no differences in the blood pressure values were seen on post-partum day 2. The co-infusion of CGRP reversed the L-NAME-induced increase in blood pressure throughout the pregnancy. However, the L-NAME-induced increase in blood pressure during the post-partum period was not reversed by CGRP. These results indicate that CGRP reverses the L-NAME-induced elevation in blood pressure during pregnancy.

Effects of CGRP on the fetal weight and perinatal mortality in L-NAME-treated animals

All animals delivered pups spontaneously at term on the afternoon of day 22 of gestation. L-NAME infusion in pregnant rats had a significant fetal mortality (18.4 ± 3.5%) compared with the controls (0.0 ± 0.0%). When CGRP was co-administered with L-NAME, a substantial decrease in mortality was observed (6.4 ± 1.8%; P < 0.05). This suggested that CGRP may have reversed the high fetal mortality associated with hypertensive disorders during pregnancy. No change in mortality was seen with CGRP alone (0.0 ± 0.0%). The
average birth weight of pups in L-NAME-treated animals was substantially lower (5.14 ± 0.07 g; P < 0.01) compared with controls (6.2 ± 0.06 g) or the CGRP-treated (6.35 ± 0.06 g) groups. The weight of the pups in the CGRP + L-NAME group was 5.31 ± 0.06 g, which was not statistically different from the l-NAME-treated animals, indicating that CGRP by itself may not reverse the fetal weight decrease caused by L-NAME.

Discussion

Recently we have demonstrated that inhibition of the NO pathway during pregnancy in the rat produces signs similar to those of pre-eclampsia in humans (Yallampalli and Garfield, 1993). These animals exhibit hypertension, fetal growth retardation, increased fetal mortality and proteinuria. In this animal model of human pre-eclampsia, the infusion of l-arginine can prevent the onset of this condition (Buhimschi et al., 1995b). Using this animal model, we further demonstrated that progesterone can also partially counteract the occurrence of hypertension and fetal growth retardation produced by l-NAME (Buhimschi et al., 1995b). In our study we demonstrated that CGRP can reverse the hypertension induced by l-NAME during pregnancy but not during the post-partum period. In addition, the l-NAME-induced increases in fetal mortality rate were also significantly attenuated by the administration of CGRP. For the first time these studies provide evidence that the potent vasodilator CGRP cannot only reverse the hypertension but can also decrease the fetal mortality associated with the pre-eclampsia-like condition in pregnant rats.

Several aetiological factors have been proposed in the pathogenesis of pre-eclampsia and there has been no consensus in this respect. There is now overwhelming evidence of the impaired function of the NO–cGMP-generating pathway in women with pre-eclampsia. NO synthase activity under basal conditions has been demonstrated in uterine arterial smooth muscle (Jovanovic et al., 1994a). The acetylcholine-induced relaxation, which is mediated by NO (Jovanovic et al., 1994b), is attenuated in arteries from pre-eclamptic patients (McCarthy et al., 1993). The decreased NO production in women with pre-eclampsia is demonstrated further by lower circulating nitrite concentrations (Seligman et al., 1994), and concentrations of an endogenous inhibitor of NO synthesis (asymmetric dimethylarginine), which was found to be lower in normal pregnancies compared with the non-pregnant state, is increased in women with pre-eclampsia (Fickling et al., 1993). Furthermore, the inhibition of NO synthesis by l-NAME in pregnant rats induces pre-eclampsia-like symptoms, indicating the involvement of an impaired NO system in this condition (Yallampalli and Garfield, 1993; present study). In addition to NO, changes in PGI2 and thromboxane (TxA2) concentrations have also been shown to be involved in the pathophysiology of pre-eclampsia.

It has been reported that CGRP is involved in regulation of the blood supply to critical organs (Wimalawansa, 1995). This also seems to be true in the case of pregnant uterus, where not only the l-CGRP concentrations are raised but also the sensitivity of the vascular smooth muscle of uterine arteries to CGRP (Nelson et al., 1993). In some arterial beds, researchers have shown that endothelium is essential for the vasodilatory actions of CGRP (Grace et al., 1987). This may also be the case in the uterine artery where CGRP may...
achieve its vasodilatory potentials through the local release of NO. However, recent studies have suggested that the vasodilatory effects of CGRP in the human uterine artery (i) are mediated by specific CGRP receptors, (ii) do not involve β-adrenoceptors, vasodilator prostanooids, EDRF or increased concentrations of cGMP (Nelson et al., 1993) and (iii) are independent of endothelium (Edvinsson et al., 1985; Bodelsson and Sternquist, 1992). Regardless of the mechanisms, our findings (that the blood pressure-lowering effects of CGRP were substantial during pregnancy when compared with that during labour and the post-partum period) support the hypothesis that the sensitivity to CGRP is elevated during pregnancy, thus enabling uterine vascular relaxation and increased blood flow to the uterus; this is lost during the parturition and postpartum periods. Present in-vivo studies in the rat and the gradual increase of i-CGRP concentrations demonstrated in the circulation during pregnancy, together with earlier in-vitro (Bodelsson and Sternquist, 1992; Nelson et al., 1993) studies in the human, imply not only a significant role for CGRP in the uterus but also a marked increase in the sensitivity of the uterine blood vessels and muscles to CGRP during pregnancy.

In this study we have demonstrated that the acute hypertension induced by l-NAME can be reversed by the concomitant administration of synthetic CGRP (1-37) in pregnant rats. The mechanism(s) involved in the CGRP-induced reversal of blood pressure increase caused by l-NAME is unclear at present. Although studies of the uterine explants indicated that the relaxant effects of CGRP were dependent upon NO formation, the vasorelaxing effects of CGRP on uterine vascular tissues appeared to be independent of NO formation (Nelson et al., 1993). Our studies demonstrated that in the rat, CGRP can effectively reverse the l-NAME-induced hypertension during pregnancy, indicating that the effects of CGRP are independent of NO. In addition, CGRP also decreases the high mortality rate associated with the inhibition of NO during pregnancy. Thus, CGRP may play an important role in the regulation of blood pressure, the blood supply to the utero-placental unit and fetal development, perhaps by inducing compensatory mechanisms. Whether these compensatory effects of CGRP could involve PG12 is not examined in this study, and therefore it is difficult to assess. Taken together, the fact that the sensitivity of uterine vascular tissue to CGRP is increased during pregnancy, the observed reversal of l-NAME-induced hypertension and the endothelium-independent action of CGRP on uterine vasculature suggest that the main beneficial effects seen in this study may be mediated by the relaxation of vascular smooth muscle and increased blood supply to the uterus.

Treatment with l-NAME resulted in an ~20% reduction in fetal weight compared with the controls. Although CGRP infusion reversed the l-NAME-induced hypertension and fetal mortality, it failed to correct fetal weight reduction. During pregnancy, both the systemic and utero-placental vascular compartments contribute significantly to the vascular resistance. Measuring fetal weights only addresses the resistance contributed by the utero-placental compartment (in particular, the fetal side). Therefore, we suggest that in this model, and by extrapolation also in human pre-eclampsia, these two compartments are differentially regulated by various agents and may show different sensitivities to CGRP regulating the blood pressure.

It is also possible that CGRP decreased the peripheral vascular resistance in the systemic compartment and is followed by a secondary reduction in the placental perfusion through a 'steal phenomena' (Ishc and Shumacker, 1975). However, it is more likely that the beneficial effects of CGRP are mainly or only on the maternal side because this peptide is unlikely to pass through the placental barrier into the fetus. Therefore, CGRP infusion appears to correct the defect on the maternal side of the placenta, but the adverse effects of l-NAME (i.e. the suppression of NO synthesis) persist on the fetal side. This may explain why the fetal weight was not improved, although hypertension and fetal mortality were corrected following the addition of CGRP.

In summary, these studies suggest that l-NAME-induced elevated blood pressure and increased fetal mortality can be reversed by CGRP. Thus CGRP may play an important role in the regulation of blood pressure and placental perfusion. Further studies are required to clarify the mechanisms involved in the correction of hypertension and improvement in the fetal mortality.

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