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In vivo regulation of vasomotricity by nitric oxide and prostanoids during gestation

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Abstract

Pharmacological studies using the Doppler technique revealed that pregnancy decreases the systemic blood pressure and enhances uterine blood velocity in rats. The reactivity of the uterine artery to α-adrenoceptor and muscarinic receptor agonists was higher than that of systemic arteries. Sodium nitroprusside increased uterine arterial blood velocity slightly during gestation and markedly in non-pregnant rats. N^G-L-Arginine methyl ester (L-NAME) decreased the uterine blood velocity mainly in gravid animals. The effect of diclofenac on uterine blood velocity was also more pronounced during pregnancy. The actions of sodium nitroprusside, L-NAME and diclofenac on systemic blood pressure were similar in pregnant and virgin rats. Altogether, these results indicate that pregnancy enhances nitric oxide (NO) and vasodilatory prostanoid production in the uterine vascular muscle which becomes less sensitive to exogenous NO. The uterine vasodilated status appears to be determined by conjugated actions of endothelial NO and vasodilator prostanoids of which the synthesis and the effects are weakly modified in systemic arteries during gestation.

Keywords: Pregnancy; Vasomotricity; Nitric oxide (NO); Prostanoid; Uterine artery; Arterial blood pressure

1. Introduction

In human and many animal species, pregnancy is characterized by significant changes in blood flow at the level of several organs, particularly in uterus of which the activity is crucial for fetal development (Rosenfeld, 1977; Johnson et al., 1985; Thaler et al., 1990). The uterine vasculature acts as a low-resistance shunt to facilitate a large increase in the uteroplacental blood flow which optimizes delivery of oxygen and substrates to the developing fetus (Magness and Zheng, 1996). The increase of uterine blood flow during gestation can partially be attributed to a growth of new vessels and/or remodeling of existing vessels by a loss of various smooth muscular structures (De Wolf et al., 1973; Pijnenborg et al., 1981). It is well documented that the elevated uterine blood flow is due to dilatation of the uterine artery provoked by reduction of vascular reactivity to endogenous vasoconstrictor agents such as catecholamines and angiotensin II (Magness and Rosenfeld, 1986; McLaughlin et al., 1989; Weiner et al., 1991, 1992). Therefore, many attempts have been made in order to characterize endothelial factors which may control vascular reactivity in pregnant animals. Various studies have demonstrated that endothelial nitric oxide (NO) production markedly increased in gravid animals, indicating that NO may play a key role in the attenuation of uterine artery reactivity to vasoconstrictors (Ahokas et al., 1991; Magness et al., 1997a,b; Xiao et al., 1999). It has also been shown that high amounts of prostacyclin, a potent vasodilator compound (Moncada and Vane, 1978), are synthesized in the vascular endothelium of gravid animals, suggesting that vasodilator prostaglandins participate in the regulation of vascular reactivity (Magness et al., 1992; Magness and Rosenfeld, 1993). However, the mechanisms responsible for the vasodilated status of the uterine artery during pregnancy are poorly understood. In particular, an important question which remains to be answered concerns the identification, under in vivo conditions, of the real contribution of NO and/or

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prostanoids in the increase of uterine blood flow during gestation.

In this study, we have used pharmacological tools and the Doppler technique to determine, in vivo, the effects of two endothelial factors, NO and prostaglandin, on the uterine arterial blood velocity and systemic blood pressure in pregnant (day 16) and virgin rats.

2. Materials and methods

2.1. Animal preparation

Sexually mature (3 months old) Wistar rats were placed overnight with a male breeder. The detection of sperm by microscopic examination of a vaginal smear the following morning confirmed day 1 of pregnancy (full term is 21–22 days). Female rats weighing between 200 and 300 g were divided into two groups: non-pregnant and pregnant in mid-gestation (day 16). The animals were anesthetized with Nesdonal (50–100 mg kg^{-1}, i.p.). A tracheotomy was performed after midline neck incision and the rats were mechanically ventilated with room air supplemented with low-flow oxygen using small-rodent ventilator. The rate of ventilation was fixed at 60 cycles/min and tidal volume was 10 ml kg^{-1}. Body temperature was maintained at 37 °C with a thermostatted heating blanket connected to a rectal thermometer. The polyethelen catheters (PE 50) were used for cannulations of the right jugular vein (injection of drugs) and the left carotid artery (recording of systemic blood pressure). Blood pressure was recorded on a Gould Windowgraph recorder.

Under strict aseptic condition, the uterus and associated vasculature were exposed through a midline incision in the abdomen. The uterus was kept moist during surgery by dripping warmed physiologic solution on the tissue. Care was taken to avoid stretching the uterus during surgery. The main uterine arterial blood velocity, proportional to the blood flow (Weiner et al., 1986), was measured by a Doppler. In all animals, a transit-time ultrasonographic flow probe (diameter, 7 mm) was placed gently around the main artery at the base of the left uterine horn in an oblique projection, proximal to the first bifurcation. The pulsed Doppler mode was used to obtain quantitative measurements of velocity. The peripheric resistance index was calculated with the systemic blood pressure and uterine arterial blood velocity values, i.e. peripheric resistance index = systemic blood pressure/uterine arterial blood velocity.

2.2. Drugs

Acetylcholine hydrochloride, sodium nitroprusside, N^G-L-arginine methyl ester (L-NAME), Diclofenac, Phenylephrine and Nesdonal were purchased from Sigma. Before use, all drugs were dissolved in 0.9% saline.

2.3. Protocols

The animals were monitored for 30 min to ensure stable physiological parameters. A complete stabilization of these parameters determined the beginning of baseline period recording in pregnant and virgin rats.

2.3.1. Effect of pregnancy on the vascular reactivity and integrity of the endothelium

The reactivity and integrity of the vascular endothelium were assessed by two classical tests using phenylephrine (an α-adrenoceptor agonist which is a potent vasoconstrictor) and acetylcholine (which is well known for its vasodilative endothelium-dependent effect). In a first step, the reactivity of the vascular muscle to α-adrenoceptor agonist was measured in pregnant and virgin rats after injection of a single dose of phenylephrine (10 μg kg^{-1}) in the jugular vein. The systemic blood pressure and uterine arterial blood velocity values were measured when the arterial blood pressure reached the peak of the hypertensive action provoked by the injected dose of phenylephrine.

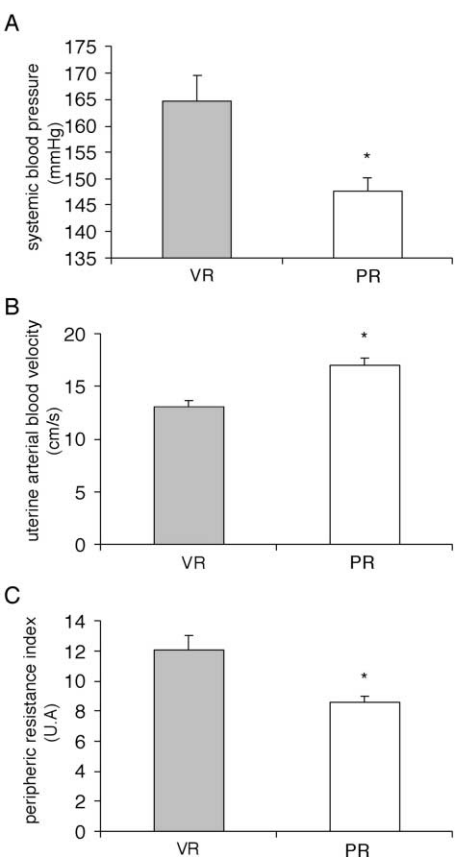

Fig. 1. Absolute values of (A) the systolic arterial pressure (mm Hg), (B) the uterine arterial blood velocity (cm s^{-1}) and (C) the peripheric resistance index measured in virgin (VR) and pregnant rats (PR). Values are mean ± S.E.M. of 12 animals in each group. * $P < 0.05$ in pregnant vs. virgin rats.

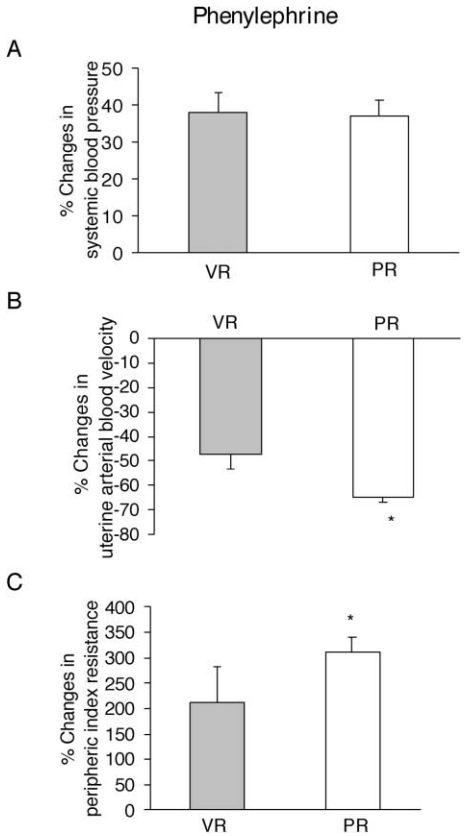

Fig. 2. Percent changes in (A) the systolic arterial pressure, (B) the uterine arterial velocity and (C) the peripheric resistance index in virgin (VR) and pregnant rats (PR), after administration of phenylephrine at the dose of 10 μg kg^{-1}. Values are mean ± S.E.M. of 12 animals in each group. $^{*}P < 0.05$ in pregnant vs. virgin rats.

Before measuring the effect induced by acetylcholine, a maximal vasoconstriction was prior provoked in pregnant and virgin rats by the administration of phenylephrine (10 μg kg^{-1}). Therefore, the injection of 1, 10 or 100 μg kg^{-1} acetylcholine, followed by systemic arterial blood pressure and uterine arterial blood velocity recording as well as the calculation of peripheric resistance index, made it possible to assess the vasodilator action of acetylcholine.

2.3.2. Effect of pregnancy on vascular smooth muscle sensitivity to nitrite compounds

To determine whether pregnancy status altered or not endothelium-independent relaxation, sodium nitroprusside (50 μg kg^{-1}) was administered as a single bolus. Since sodium nitroprusside is a vasodilator which acts instantaneously on the vascular smooth muscle, systemic blood pressure and uterine arterial blood velocity were rapidly recorded from the onset of sodium nitroprusside administration.

2.3.3. Action of NO on the vascular tone during pregnancy

The contribution of endogenous NO in the control of vascular tone was assessed in pregnant and virgin rats by administrating a single bolus of L-NAME (5 mg kg^{-1}), a specific inhibitor of NO synthases. The changes in systemic blood pressure and uterine arterial blood velocity values from baseline were generally measured 5 min after the administration of L-NAME.

2.3.4. Action of prostanoids on the vascular tone

The role of prostanoids in the induction of the vasodilatation effect during pregnancy was studied with diclofenac (5 mg kg^{-1}), a specific inhibitor of prostaglandin production. The changes in systemic blood pressure and uterine arterial blood velocity from baseline were measured 5 min after diclofenac administration.

2.4. Statistical analysis

The data are presented as percentage changes from the baseline values. The results are expressed as mean ± S.E.M. and were compared by unpaired *t*-tests or by repeated-measures analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant. The pregnant and virgin rats were randomized before the study.

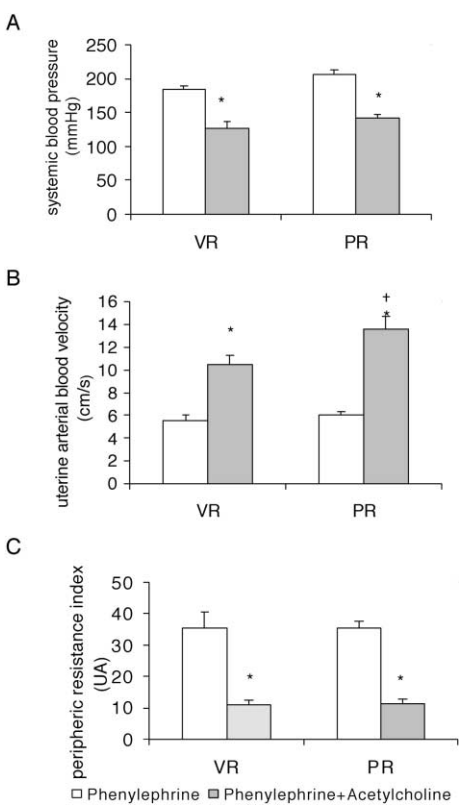

Fig. 3. Effect of acetylcholine administration (10 μg kg^{-1}) on (A) the systolic arterial pressure (mm Hg), (B) the uterine arterial blood velocity (Vs, cm s^{-1}) and (C) the peripheric resistance index measured in virgin (VR) and pregnant rats (PR) pretreated with phenylephrine. Values are mean ± S.E.M. of 12 animals in each group. $^{*}P < 0.05$ in acetylcholine vs. phenylephrine. †$P < 0.05$ in pregnant vs. virgin rats.

3. Results

3.1. Effect of pregnancy on the basal vascular tone

The baseline haemodynamic parameters recorded in virgin and pregnant rats during saline infusion are presented in Fig. 1. The systemic blood pressure values measured in virgin animals (165 ± 7 mm Hg) were significantly higher than in mid-pregnant rats (148 ± 3 mm Hg) (Fig. 1A). In contrast, uterine arterial blood velocity detected in non-pregnant rats (13.1 ± 0.6 cm s^{-1}) was lower than in gravid animals (17 ± 0.6 cm s^{-1}) (Fig. 1B). Therefore, it appeared that pregnancy decreased around 33% the basal peripheric resistance index value (Fig. 1C).

3.2. Effect of pregnancy on the vascular reactivity to α-adrenoceptor agonist

Injection of phenylephrine (10 μg kg^{-1}) increased by 40% the basal level of systemic blood pressure in virgin as well in pregnant rats (Fig. 2A). The uterine arterial blood velocity markedly decreased in both groups but the drop was much higher in gravid ($-64.7 \pm 2.3\%$) than in virgin ($-47 \pm 6\%$) rats (Fig. 2B). Consequently, the augmentation of the peripheric resistance index values provoked by phenylephrine was 300% during gestation vs. 200% in non-pregnant rats (Fig. 2C).

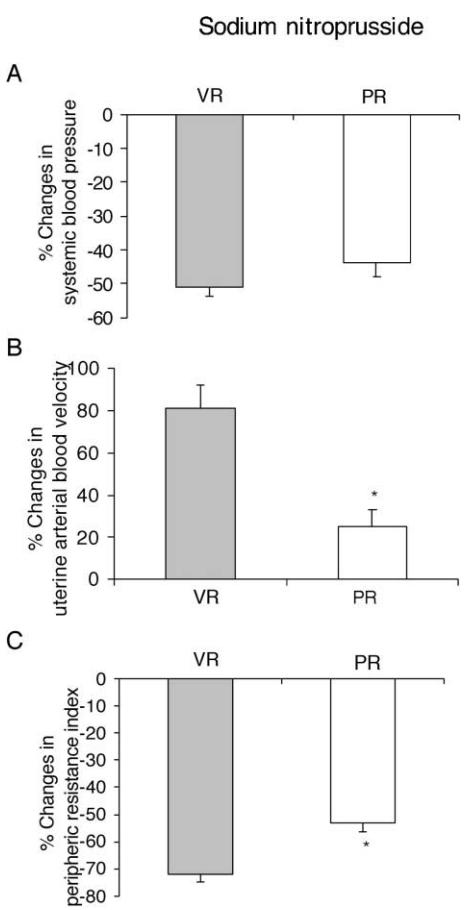

Fig. 4. Percent changes in (A) the systolic arterial pressure, (B) the uterine arterial blood velocity and (C) the peripheric resistance index after administration of sodium nitroprusside at the dose of 50 μg kg^{-1}, in virgin (VR) and pregnant rats (PR). Values are mean ± S.E.M. of 12 animals in each group. $^{*}P < 0.05$ in pregnant vs. virgin rats.

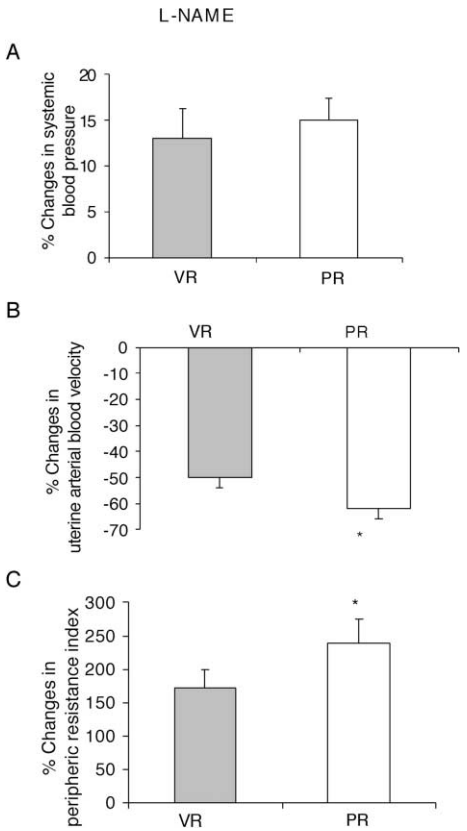

Fig. 5. Percent changes in (A) the systolic arterial pressure, (B) the uterine arterial blood velocity and (C) the peripheric resistance index after administration of N^{G}-L-arginine methyl ester (L-NAME) at the dose of 5 mg kg^{-1} in virgin (VR) and pregnant rats (PR). Values are mean ± S.E.M. of 12 animals in each group. $^{*}P < 0.05$ in pregnant vs. virgin rats.

3.3. Effects of pregnancy on the integrity of the endothelium

A vasoconstrictive status was prior induced by phenylephrine (10 μg kg^{-1}), which raised the basal level of systemic blood pressure and decreased uterine arterial blood velocity as described beyond (Fig. 2A, B, C). Following this status, the administration of acetylcholine (10 μg kg^{-1}) significantly reduced the basal level of systemic blood pressure in virgin and pregnant rats. The percentage of decrease (30%) was similar in both groups (Fig. 3A). In contrast, acetylcholine enhanced uterine arterial blood velocity with a more pronounced stimulatory effect in gravid (130%) than in non-pregnant (100%) rats (Fig. 3B). The peripheric resistance index values were similar in virgin ($-65.6 \pm 11\%$) and pregnant ($-67.3 \pm 3\%$) rats (Fig. 3C).

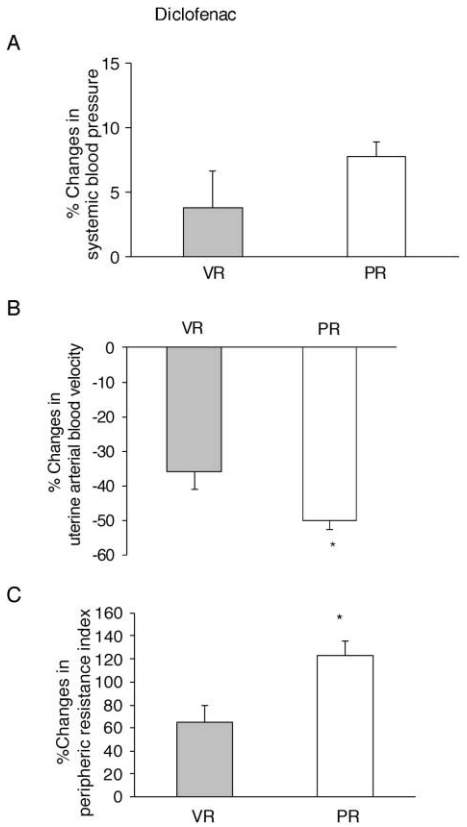

Fig. 6. Percent changes in (A) the systolic arterial pressure, (B) the uterine arterial blood velocity and (C) the peripheric resistance index after administration of diclofenac, at the dose of 5 mg kg^{-1}, in virgin (VR) and pregnant rats (PR). Values are mean ± S.E.M. of 12 animals in each group. * $P < 0.05$ in pregnant vs. virgin rats.

3.4. Effect of pregnancy on vascular smooth muscle sensitivity to nitrite compounds

Administration of sodium nitroprusside (50 μg kg^{-1}) reduced around 45% the basal systemic blood pressure in both virgin and pregnant rats (Fig. 4A). Sodium nitroprusside enhanced uterine arterial blood velocity slightly during gestation (25%) and markedly in non-pregnant animals (80%) (Fig. 4B). Calculation of peripheric resistance index values revealed a 75% and 50% decrease of basal peripheric resistance index values in virgin and gravid rats, respectively (Fig. 4C).

3.5. Effect of endogenous NO in maintaining basal vascular tone during pregnancy

Inhibition of endogenous NO formation by L-NAME (5 mg kg^{-1}) induced an increase of systemic blood pressure. The percentage of increase was similar (15%) in both groups (Fig. 5A). L-NAME also provoked a decrease of uterine arterial blood velocity, which was much higher in pregnant (−62%) than in (−50%) non-pregnant rats (Fig. 5B). Therefore, the increase of peripheric resistance index observed in non-pregnant and pregnant rats was 150% and 250%, respectively (Fig. 5C).

3.6. Effect of endogenous prostanoids on vascular tone

Inhibition of endogenous prostaglandin formation by diclofenac (5 mg kg^{-1}) provoked a modest increase of systemic blood pressure in virgin (4% of control) and pregnant (8%) rats (Fig. 6A). In contrast, the effect of diclofenac on uterine arterial blood velocity was markedly pronounced. Diclofenac reduced by 35% and 50% the basal level of utrine arterial blood velocity in non-pregnant and pregnant rats, respectively (Fig. 6B). The related increase of peripheric resistance index was 55% in virgin and 125% in pregnant rats (Fig. 6C). When the animals were prior treated with phenylephrine (10 μg kg^{-1}), the administration of diclofenac (5 mg kg^{-1}) attenuated the pressor effect of the α-adrenoceptor agonist in the virgin (−18 ± 3%) and pregnant (−9 ± 3%) rats (Fig. 7A). The changes provoked by phenylephrine on uterine arterial blood velocity and peripheric resistance index were not

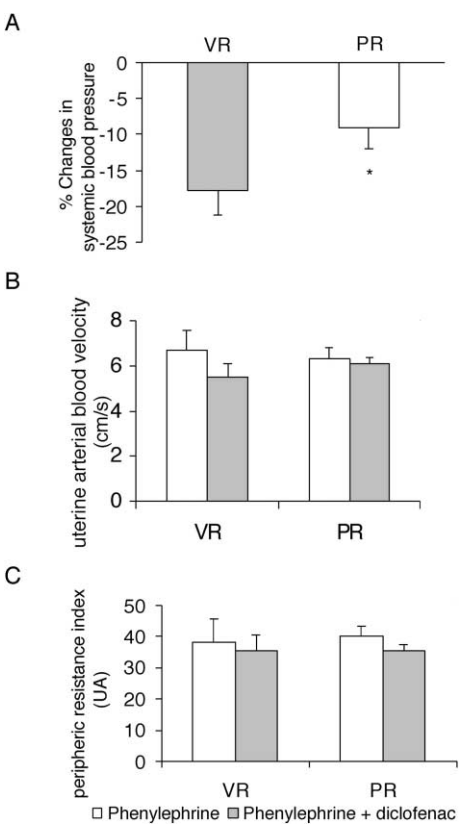

Fig. 7. Effect of diclofenac on the systolic arterial pressure (mm Hg), the uterine arterial blood velocity (cm s^{-1}) and the peripheric resistance index measured in virgin (VR) and pregnant rats (PR) pretreated with phenylephrine. Values are mean ± S.E.M. of 12 animals in each group. * $P < 0.05$ in pregnant vs. virgin rats.

modified by diclofenac in both gravid and non-pregnant animals (Fig. 7B, C).

4. Discussion

It is well established that the integrity of uterine circulation is crucial for fetal life and that a maternal vascular insufficiency is responsible for intrauterine growth retardation. However, the mechanisms involved in maintenance of vascular tone during pregnancy remain poorly studied.

This report provides in vivo evidence for key actions of NO and vasodilator prostaglandins in the control of systemic vascular tone and uteroplacental blood flow in gravid animals.

We have observed that, in rats, pregnancy induces a significant decrease of systemic blood pressure, which is associated with an increase of uterine arterial blood velocity. The peripheric resistance index value was therefore significantly reduced in pregnant animals. The drop of systemic blood pressure provoked by pregnancy may reflect physiological changes in the activity of kidney, in basal metabolism and/or in vascular reactivity. By using phenylephrine and acetylcholine, we have assessed the effect of gestation on the vascular reactivity and integrity of the endothelium. The stimulation of systemic blood pressure induced by phenylephrine was similar in both virgin and gravid animals. In contrast, phenylephrine provoked a marked decrease of uterine arterial blood velocity in pregnant rats, suggesting a modification of uterine vascular reactivity to α-adrenoceptor agonists during gestation. In support of this hypothesis, an increase of the sensitivity of isolated ovine uterine artery to α-adrenoceptor agonists was also observed during pregnancy in guinea-pig (Annibale et al., 1989). However, it should be noted that the human uterine artery seems to not react differently to adrenergic substances in non-pregnant and gravid women (Steele et al., 1993). These data may reflect the existence of specific influences of pregnancy on the vascular reactivity to adrenergic agonists according to animal species.

Following a contraction provoked by phenylephrine, the endothelial relaxation was assessed by the injection of acetylcholine. Pregnancy increased the sensitivity of uterine artery to acetylcholine as the vasorelaxation observed in gravid animals was much higher than in non-pregnant rats. The enhancement of uterine sensitivity to acetylcholine may be related to an increase of the affinity of muscarinic receptors to acetylcholine during pregnancy (Weiner et al., 1989, 1991) or to a stimulation of NO production by endothelial tissue (Ni et al., 1997). Therefore, we have studied the effect of pregnancy on the vascular smooth muscle sensitivity to sodium nitroprusside, a nitrite compound. This substance decreased systemic blood pressure in both virgin and pregnant animals but enhanced slightly or markedly the uterine blood velocity in gravid and non-pregnant rats, respectively. This result suggests an attenuation of the uterine artery vascular smooth muscle sensitivity to exogenous NO during pregnancy in rats. Sodium nitroprusside has been shown to relax the vascular smooth muscle via a release of NO which activates the guanylyl cyclase pathways (Kowulak et al., 1992). The administration of L-NAME (a specific inhibitor of NO synthase) produced a similar increase of systemic blood pressure in both pregnant and virgin rats. This effect is generally considered as a reflection of removal of the tonic vasodilator influence of NO. The hypertensive effect of L-NAME is associated with an important decrease of uterine arterial blood velocity and a stimulation of the peripheric resistance index which is more pronounced during gestation. In agreement with this observation, it has been shown that the expression and bioactivity of NO synthase increase in the endothelial tissue of uterine artery during pregnancy but not in the systemic endothelium (Magness et al., 1997b; Xiao et al., 1999). These data indicate that endogenous production of NO is stimulated in the uterine artery by pregnancy and could therefore be responsible, at least in part, for the vasodilated status observed in pregnant rats.

We have investigated the role of prostaglandins in the maintenance of vascular tone during pregnancy by using diclofenac, an inhibitor of prostanoid synthesis. The systemic blood pressure value was not significantly affected by diclofenac in both groups, suggesting that vasodilator prostanoids may not be involved in the maintenance of the systemic vascular tone in mid-pregnant rats as previously described in guinea-pig (Harrison and Moore, 1989). In contrast, diclofenac provoked a substantial decrease of uterine arterial blood velocity which was higher in pregnant than in virgin rats. These results suggest that, during pregnancy, the production of vasodilator prostaglandins may only increase in the uterine artery but not in systemic arteries. In support of this finding, recent studies have shown a significant increase of cytosolic phospholipase A_2 (a key enzyme for prostaglandin synthesis) and the expression of prostacyclin synthase in uterine artery (but not systemic) during gestation (Di et al., 1999; Magness et al., 2000). Moreover, we have observed that the pressor effect of phenylephrine was reduced by diclofenac slightly in pregnant animals and significantly in virgin rats.

Collectively, these results suggest that pregnancy may affect the biosynthetic pathways of prostanoids by increasing the production of vasodilator prostaglandins and reducing simultaneously the formation of vasoconstrictor prostanoids. Inhibition by diclofenac of vasopressive prostaglandins, of which the level was much higher in virgin rats, may be responsible for the high attenuation of the pressor effect of phenylephrine observed in these animals compared to pregnant rats.

Finally, our results demonstrate that uterine vascular reactivity is considerably modified during gestation in rats. This modification is associated with a decrease of systemic blood pressure. The gravid status increases both endoge-

nous NO synthesis and the sensitivity of the receptors which mediate NO production by endothelial cells, particularly in the uterine artery. In addition, our data indicate an involvement of the synthesis and/or release of vasodilator prostaglandins in the control of uterine vascular tone during pregnancy. Gestation affects mainly vasomotricity of the uterine artery while the systemic vascular reactivity is just weakly modified at mid-pregnancy. Therefore, endothelial NO and vasodilator prostanoids appear to be the two main or key factors which strongly determine the dilated status of the uterine artery during pregnancy.

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