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Pregnancy augments nitric oxide-dependent dilator response to acetylcholine in the human uterine artery

Sharon H.Nelson[1,3], Odd S.Steinsland[1], Maya S.Suresh[2] and Nicole M.Lee[1]

[1]Departments of Anesthesiology and Pharmacology, The University of Texas Medical Branch, Galveston, TX 77555 and [2]Department of Obstetric Anesthesia, Baylor College of Medicine, Houston, TX 77030, USA

[3]To whom correspondence should be addressed

The influence of pregnancy on the dilator effects of acetylcholine in the isolated human uterine artery was investigated. Acetylcholine (0.1 nM to 0.1 μM) produced concentration- and endothelium-dependent relaxation of norepinephrine (3 μM)-induced contraction. The relaxation was greater in arteries from pregnant patients (P arteries) than from non-pregnant patients (NP arteries). The maximal relaxation was 53.5 ± 3.4% (n = 21) in P arteries and 23.5 ± 2.5% (n = 35) in NP arteries. In both P and NP arteries the cholinergic relaxation was increased in the presence of superoxide dismutase and greatly reduced in the presence of the nitric oxide synthase inhibitors, N^G-mono-methyl L-arginine (L-NMMA) and L-nitro-arginine-methylester (L-NAME). The effect of these nitric oxide synthase inhibitors was reversed by L-arginine. We conclude that pregnancy enhances acetylcholine-induced nitric oxide synthesis and release in the human uterine artery.

Key words: human uterine artery/nitric oxide/NOS inhibitors/pregnancy/vascular reactivity

Introduction

Acetylcholine produces an endothelium-dependent relaxation of blood vessels (Furchgott and Zawadski, 1980; Furchgott, 1983). This relaxation is mediated primarily by nitric oxide (NO) which is synthesized from the terminal guanidine nitrogen of the amino acid L-arginine in the vascular endothelial cells (Palmer *et al.*, 1988). Besides acetylcholine, endothelium-derived NO can be generated and released by many endogenous substances and by physical stimuli such as increased blood flow and shear stress (see review by Rubanyi, 1993).

About 30 years ago Christopher Bell (1968) reported that the dilatory response to acetylcholine was minimal in the uterine vascular bed of non-pregnant guinea pigs but increased markedly (~10-fold) during pregnancy. The physiological significance of these findings was not understood for several years because there was no definitive evidence for a functional cholinergic innervation of the uterine vasculature. However, the knowledge that NO serves as a common mediator of the effect of acetylcholine and many other vasodilators kindled the hypothesis that the pregnancy-induced sensitization to acetylcholine could reflect a general NO-dependent mechanism involved in the increased utero–placental perfusion that is required for normal fetal development. The purpose of the present study, which was begun ~11 years ago, and prolonged because of the difficulty in obtaining uterine arteries from pregnant patients, was to determine the effect of pregnancy on acetylcholine-induced relaxation of the isolated human uterine artery. Some of the results have been reported (Nelson and Steinsland, 1987a,b; Johnson *et al.*, 1993).

Materials and methods

Preparation of arterial rings

Specimens of the ascending branch of the uterine artery were obtained from multiparous patients undergoing hysterectomy for various medical reasons. Use of uterine arteries obtained from patients after undergoing hysterectomy was approved by the Institutional Review Boards for the University of Texas Medical Branch, Galveston, Texas and Baylor College of Medicine, Houston, Texas. Uterine arteries were obtained from 21 pregnant patients and 35 non-pregnant patients. The mean age of the pregnant patients was 31.2 ± 1.6 years; the mean age of the non-pregnant patients was 35 ± 0.5 years. The mean arterial blood pressure of the pregnant patients was 81.4 ± 3.4 mmHg; the mean arterial blood pressure of the non-pregnant patients was 86.2 ± 2.5 mmHg. None of the pregnant patients were diagnosed with hypertension or pre-eclampsia. The pregnant patients were near-term gestation (36.4 ± 0.3 weeks). Reasons for the Caesarean/hysterectomy included fibroids, repeat Caesarean section, permanent sterilization, abnormal placentation (placenta accreta, marginal placenta previa), severe dysplasia and cervical carcinoma. The patients delivered healthy babies with no apparent growth retardation. The non-pregnant patients were in different phases of the menstrual cycle. Reasons for the hysterectomy included: fibroids, permanent sterilization, severe dysplasia, and enlarged uterus. According to the patients' charts, most of the pregnant and non-pregnant patients were not taking any medication. None of the patients included in the present study were on oestrogen and/or progesterone therapy. A few patients had taken ibuprofen or aspirin. The patients received diazepam or midazolam, morphine sulphate, and atropine, scopolamine, or glycopyrrolate for preanaesthetic medication. The pregnant patients also received an antacid and ranitidine for aspiration prophylaxis. Anaesthesia was induced with sodium thiopental and maintained with nitrous oxide–oxygen and either isofluorane or fentanyl. Some of these drugs have vascular actions, but the assumption was made that continuous suffusion of the tissue preparations removes most of the drugs. That the contractile responses to norepinephrine did not change during the experiments implies that either the drugs were washed out before commencement of the experiment or any residual effects of the drugs did not affect the contractile responses to norepinephrine.

Sections of the uterus containing the ascending branch of the uterine artery were placed in Krebs–bicarbonate solution equilibrated

with 95% O_2 and 5% CO_2 immediately after hysterectomy but before being taken to the laboratory. The preparation of isolated arterial rings and the suffusion techniques have been previously described in detail (Nelson and Suresh, 1988). Briefly, the ascending branches of the uterine artery on the lateral surfaces of the uterus were dissected. Only untraumatized uterine artery was used. The arteries were cut into rings of 4 mm length, as measured by a dissecting stereomicroscope with an ocular micrometer. Five or six rings were obtained from each uterine artery. The rings were carefully handled to prevent damage to the endothelium. The rings were then mounted between two tungsten wires (0.38 gauge) in four 5 ml volume suffusion chambers. Changes in isometric tension were measured by means of a force-displacement transducer (Statham; Astro-Med Inc, Glass Instruments, West Warwick, RI, USA) connected to the upper mounting wire and recorded (2400 Brush Model; Gould Inc, Valley View, OH, USA).

The mounted arteries were suffused continuously with gassed (95% O_2 and 5% CO_2) Krebs–bicarbonate solution at a flow rate of 4 ml/min delivered by a peristaltic pump (Gilson Medical Electronics Inc, Middleton, WI, USA). The Krebs–bicarbonate solution had the following composition (mM): NaCl, 119; $NaHCO_3$, 25; $MgSO_4$, 1.2; KCl, 3.6; KH_2PO_4, 1.2; $CaCl_2$, 2.5; glucose, 11; ascorbic acid, 0.005; and disodium ethylenediaminetetraacetate, 0.03. The bath solution was maintained at 37°C with a pH of 7.4. The drugs were administered in the inflowing suffusion fluid.

The preparation was allowed a stabilization period of 1–2 h before the experiment began. The optimal resting tension was determined from length–active tension relationships in initial experiments in uterine arteries from pregnant and non-pregnant (multiparous) patients (Nelson and Suresh, 1988). The rings were progressively stretched (i.e. exposed to increased tension) and norepinephrine (1 μM) was repeatedly administered for 5 min every 15 min until a maximal contraction to norepinephrine was elicited. The optimal resting tension of 1 g, determined in these initial experiments, was employed in all subsequent experiments and did not vary more than ±0.1 g in uterine arteries from pregnant and non-pregnant patients. As previously reported, we found that the diameters and wet weights of uterine arteries from multiparous patients with uncomplicated pregnancy were not significantly different from uterine arteries from multiparous non-pregnant patients (Nelson *et al.*, 1995). We did not obtain any uterine arteries from nulliparous patients.

Procedures

Except where indicated, the same experimental procedures were used in uterine arteries from pregnant (P arteries) and non-pregnant patients (NP arteries). After the equilibration and stabilization of the baseline at 1 g resting tension, all rings were exposed to potassium chloride (KCl, 75 mM) for 5 min to determine the viability of the tissue. A concentration of 75 mM KCl produced ~60–80% of the maximal contractile response to KCl which was obtained at 100 mM. Only rings that contracted in response to KCl were used.

In a few experiments, after washout of KCl, acetylcholine and other vasodilators were added. After washout of these drugs, contraction was induced by norepinephrine (3 μM). This concentration of norepinephrine was used for P and NP arteries, because we have previously shown that it produced 50–60% of the maximal contractile response to norepinephrine which was obtained at a concentration of 30 μM (Nelson and Suresh, 1988; Suresh *et al.*, 1985). Once the response to norepinephrine had stabilized, bradykinin (1 μM) was administered for 10 min to determine the presence of functional endothelium. In a few experiments, the endothelium was intentionally removed by gently rolling the arterial ring around the tip of a pair of no.1 forceps for 60 s. The presence of the endothelium in the arterial ring was confirmed at the end of the experiment by exposing the tissue to a silver stain (Furchgott and Zawadzki, 1980) and viewing the endothelium under a microscope.

After washout of bradykinin and with the return of the norepinephrine-induced precontraction to control levels, acetylcholine was administered at different concentrations (0.1 nM to 1 μM). Each concentration was administered until a steady-state level of relaxation was obtained or for 15 min. After maximum relaxation was produced, acetylcholine was washed out and the tissue was allowed to return to the original stable precontraction level. At this time the tissue was suffused with N^G-mono-methyl arginine (L-NMMA, 10 μM) or L-nitro-arginine-methylester (L-NAME, 100 μM) to inhibit the production of NO (Rees *et al.*, 1990). The blockers were administered for 30 min, until the precontraction became stable before starting the second concentration–response study. After washout of acetylcholine, L-arginine, the precursor of NO, was administered for 15 min. The acetylcholine concentration–response study was repeated in arterial rings from the same patient but not exposed to the nitric oxide synthase (NOS) inhibitors to determine time-dependent changes in sensitivity to acetylcholine. These studies were carried out in the absence of indomethacin, a cyclo-oxygenase inhibitor, because in preliminary experiments we determined that the concentration–response curves to acetylcholine were superimposable whether done in the absence or presence of indomethacin (10 μM). At the end of each experiment sodium nitroprusside (10 μM), a NO donor, was administered for 15 min to determine whether the precontracted artery relaxed in response to exogenous NO donors.

In another set of experiments, the arterial ring was precontracted by norepinephrine (3 μM) and after stabilization of the contraction, acetylcholine (10 nM) was administered for 15 min. After washout of acetylcholine and the norepinephrine-induced contraction had returned to control levels, superoxide dismutase (150 IU/ml), shown to decrease oxygen free radicals, was administered for 30 min. Administration of acetylcholine (10 nM) was then repeated.

Inhibition by acetylcholine of the norepinephrine-induced contraction was expressed as percentage relaxation of the stable contraction before acetylcholine was administered. Norepinephrine-induced tonic contractions were often associated with phasic contractions (drug-induced oscillatory activity). When this occurred, the lowest tension was used as the control. Thus, the magnitude of the rhythmic activity was not included in the measurements. The contractions induced by norepinephrine reflect active force development. All data are expressed as mean ± SEM for n separate experiments. Results were analysed using analysis of variance and non-parametric tests (Kruskal–Wallis and the Mann–Whitney U-test or the Wilcoxon signed ranks test). A two tailed P value of $\leqslant 0.05$ was considered to be statistically significant.

All dilutions of drugs were made in Krebs bicarbonate solution and prepared immediately before needed. L-Arterenol bitartrate, acetylcholine iodine, L-NAME, L-NMMA, superoxide dismutase (SOD), L-arginine, D-arginine, and sodium nitroprusside were all obtained from Sigma Chemical Company, St Louis, MO, USA.

Results

After the uterine arteries were mounted in the suffusion chamber and the resting tension was applied, tension gradually increased for 5–15 min, then decreased for periods of up to 30 min. The decrease in tension was often associated with some spontaneous phasic contractile activity. After the baseline stabilized, there was, as a general rule, little or no spontaneous contractile activity. KCl (75 mM)

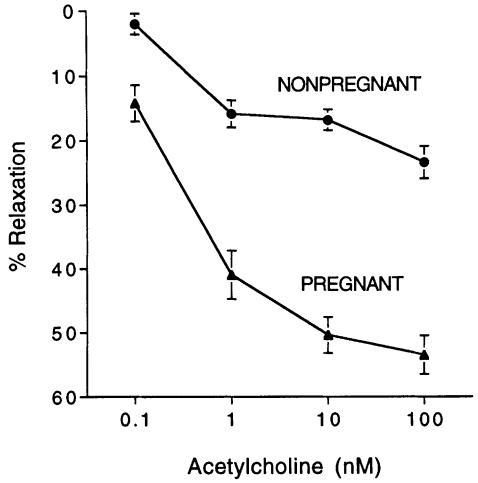

Figure 1. Concentration–response curves for acetylcholine (ACH)-induced relaxation of precontracted uterine arterial rings from pregnant (filled triangles, $n = 21$) and non-pregnant (filled circles, $n = 35$) patients. The arteries were precontracted by norepinephrine 3 μM, a concentration that produced 50–60% of the maximal contraction produced by norepinephrine 30 μM. Relaxation is expressed as percentage of initial active contraction.

induced contractions of 11.6 ± 1.3 g ($n = 21$) and 12.8 ± 2.1 g ($n = 35$) respectively in P and NP arteries.

Acetylcholine (0.1–30 μM), as well as other vasodilators such as sodium nitroprusside (10 μM), had no demonstrable effect on the resting tension in the uterine arteries from either pregnant patients (P arteries, $n = 4$) or non-pregnant patients (NP arteries, $n = 4$).

In order to study the relaxation induced by acetylcholine, contractile tone was first induced by norepinephrine (3 μM). The developed tension induced by norepinephrine in P arteries was 2.38 ± 0.21 g ($n = 21$) and in NP arteries was 1.98 ± 0.13 g ($n = 35$; not significant). Acetylcholine produced a concentration-dependent relaxation which was significantly greater in the P arteries compared to the NP arteries (Figure 1). The maximal relaxation was produced by 0.1 μM of acetylcholine in the P arteries (53.5 ± 3.4%, $n = 21$) whereas in the NP arteries the same concentration of acetylcholine produced only 23.5 ± 2.5% ($n = 35$) relaxation. All of the P arteries responded to acetylcholine. On the other hand, eight out of the 35 NP arteries did not relax in response to acetylcholine; these results were included in the data as plotted in Figure 1. Excluding the data from the non-responding arteries, the maximal relaxation produced by acetylcholine (0.1 μM) was 28.6 ± 1.4% and remained significantly less ($P < 0.001$) than that found in the P arteries. When the endothelium (endo) was removed, acetylcholine (10 nM) was almost completely abolished in both the P arteries (+endo = 54 ± 4%; –endo = 5 ± 3%; $n = 4$) and NP (+endo = 17 ± 3%; –endo = 8 ± 4%; $n = 4$) arteries. Superoxide dismutase (150 IU/ml) increased the acetylcholine (10 nM)-induced relaxation from 31.5 ± 2.3% to 46.8 ± 4.5% ($n = 4$, $P < 0.05$) in P arteries and from 17.3 ± 6.2% to 29 ± 9% ($n = 4$) in NP arteries. Sodium nitroprusside (10 μM) completely inhibited the norepinephrine-induced precontraction in the P arteries ($n = 4$) and NP arteries ($n = 4$; not significant). Histological examination

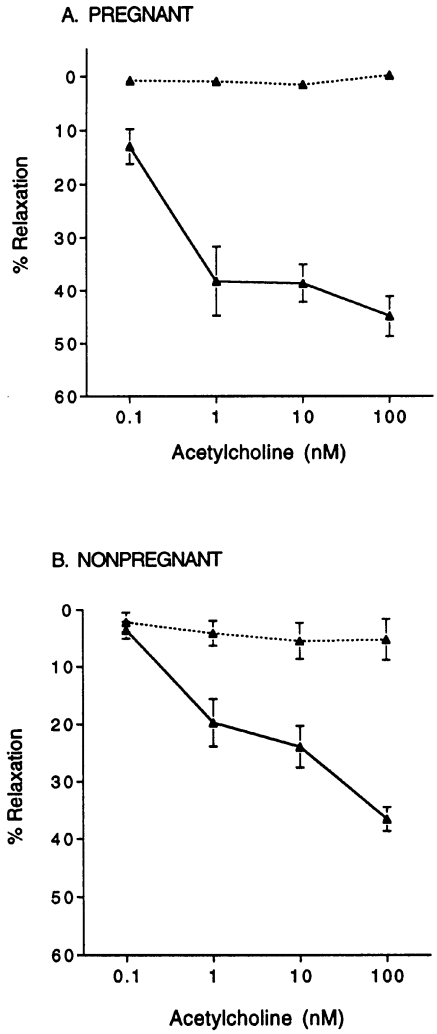

Figure 2. Reversal by the nitric oxide synthase (NOS) inhibitor N^G-mono-methyl L-arginine (L-NMMA) of acetylcholine-induced relaxation of norepinephrine (3 μM)-precontracted uterine arterial rings from (**A**) nine pregnant patients and (**B**) six non-pregnant patients. The relaxation is expressed as a percentage of initial active contraction. Acetylcholine was administered for 15 min or until the response stabilized at each concentration (solid line with filled triangles) and L-NMMA (10 μM) was administered for 30 min before repetition of the acetylcholine concentration–response determination (dotted line with filled triangles). Paired concentration–response curves in the absence and presence of L-NMMA are shown. Vertical bars show SEM.

at the end of the experiment demonstrated the presence of intact endothelium and did not reveal any obvious difference in cell size or shape in the endothelium in uterine arteries from pregnant and non-pregnant (multiparous) patients.

The NOS inhibitors L-NMMA (10 μM) (Figure 2) and L-NAME (100 μM) (Figure 3) reversed the relaxations induced by acetylcholine (0.1 nM to 0.1 μM) in both P arteries and NP arteries. In P arteries L-NMMA (10 μM) inhibited the acetylcholine (0.1 μM)-induced relaxation by 98 ± 3.3% ($n = 9$) (Figure 2A) and by 85.2 ± 0.5% ($n = 6$) in NP arteries (Figure 2B). L-NAME (100 μM) decreased the maximal acetylcholine (0.1 μM)-induced relaxation by 71 ± 1% ($n = 5$) in the P arteries (Figure 3A) and by 74 ± 2% ($n = 6$) in the NP arteries (Figure

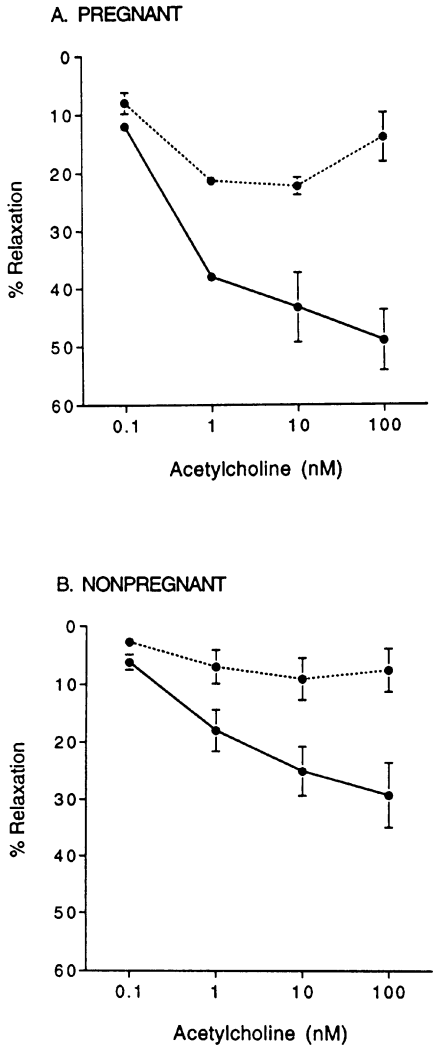

Figure 3. Reversal by the nitric oxide synthase inhibitor (NOS) L-nitro-arginine-methylester (L-NAME) of acetylcholine-induced relaxation of norepinephrine (3 μM)-precontracted uterine arterial rings from (**A**) five pregnant patients and (**B**) six non-pregnant patients. The relaxation is expressed as a percentage of initial active contraction. Acetylcholine was administered for 15 min or until the response stabilized at each concentration (solid line with filled circles) and L-NAME (100 μM) was administered for 30 min before repetition of the acetylcholine concentration–response determination (dotted line with filled circles). Paired concentration–response curves in the absence and presence of L-NAME are shown. Vertical bars show SEM.

3B). Higher concentrations of the NOS inhibitors did not produce greater inhibition of the relaxation in either P arteries or NP arteries. The substrate for NOS, L-arginine (0.1 mM), but not D-arginine, reversed the effect of the NOS inhibitors (data not shown). The NO-mediated relaxation induced by sodium nitroprusside (10 μM) was not inhibited by either of the NOS inhibitors.

Discussion

The results of this study show that acetylcholine produces relaxation in the human uterine artery and that this relaxation is much greater in arteries from pregnant than from non-pregnant patients (Figure 1). The acetylcholine-induced relaxation was markedly attenuated in the presence of NOS inhibitors (Figures 2 and 3), and was almost abolished after removal of the endothelium. The relaxation was enhanced by addition of superoxide dismutase which is known to indirectly interfere with NO inactivation. Previously we have reported (Nelson and Suresh, 1988) and we confirm in the present study that there is no difference between pregnant and non-pregnant uterine arteries in the sensitivity to the vasorelaxing effect of nitroprusside, a NO donor that acts directly on guanylate cyclase. Thus, it appears that the augmented dilatory response to acetylcholine in uterine arteries from pregnant patients is due to an increased synthesis and release of NO.

This conclusion is supported by considerable work showing that NO, synthesized by NOS, contributes to maternal systemic vasodilatation and regulates uterine blood flow during pregnancy (for review see Sladek *et al.*, 1997). For example, increases in endothelium-derived NOS (eNOS) activity and eNOS protein concentrations in uterine arteries in pregnant sheep have been demonstrated by Magness *et al.* (1997). Furthermore, the mRNA for NOS in the endothelium of uterine arteries is greater in pregnant than non-pregnant sheep (Bird and Magness, personal communication). These pregnancy-related responses appear to be limited to the uterine vasculature since the systemic (omental) artery endothelium showed only minimal or no significant changes in eNOS with pregnancy (Magness *et al.*, 1997; Bird and Magness, personal communication). An elevated eNOS activity in intact uterine arteries obtained from pregnant versus non-pregnant guinea pigs has also been reported by Weiner *et al.* (1994). In our own laboratory we have found that there is a pregnancy-related rise in eNOS activity and increased expression of eNOS in uterine arteries from pregnant patients compared with non-pregnant patients (Nelson *et al.*, 1997).

It should be noted that in other tissues, such as the uterus and the cervix, NOS expression and activity change during pregnancy. In the uterus NO synthesis, reflected in nitrite production, increases in gestation compared with the non-pregnant state and decreases during term labour (Buhimschi *et al.*, 1996). Conversely, cervical NO production is low in the non-labouring pregnant rats but markedly increases during term labour (Buhimschi *et al.*, 1996). In cultured myometrial cells isolated from rats on day 18 of gestation (22 days = full gestation) NO, as reflected in nitrite concentrations, was produced and there was abundant eNOS protein; but there was no detectable presence of other isoforms of NOS (inducible NOS or neuronal NOS) (Gangula *et al.*, 1997a).

Studies on human umbilical arteries which are fetal, not maternal, blood vessels show an increased responsiveness to NO at mid-gestation but a decrease at term (Izumi *et al.*, 1995). We have not been able to compare mid-gestation with term gestation sensitivity to acetylcholine and NO production in human uterine arteries. However, Magness *et al.* (1997) found no significant alteration in NOS protein expression in the endothelium of uterine arteries from preterm (110 days) and near-term (142 days; term = 145 ± 3 days) sheep.

Results of studies on the effect of pregnancy on the response to acetylcholine in the uterine vasculature from animals are

controversial. Bell (1968) found that pregnancy causes a 10-fold increase in the vasodilatory effect of acetycholine in the perfused, precontracted vascular bed of the guinea pig. In addition, Weiner *et al.* (1989, 1992) reported that in the isolated uterine artery of the guinea pig the vasodilatory effect of acetylcholine was greater in the arteries removed from pregnant than from non-pregnant animals. Similar results were obtained on the isolated uterine artery of the rat (Ni *et al.*, 1997). On the other hand, Jovanovic *et al.* (1994) and Matsumoto *et al.* (1992) found no pregnancy-induced changes in the potency or efficacy of acetylcholine in causing relaxation of the uterine arteries of guinea pigs and dogs respectively. The inconsistency of these results could be due to methodological differences (see review by Sladek *et al.*, 1997).

The uterine arterial preparation that we used for these studies exhibits a higher sensitivity to acetylcholine than that found in other uterine arterial preparations from either non-pregnant human patients (Jovanovic *et al.*, 1994; Azuma *et al.*, 1995) or experimental animals such as the guinea pig (Weiner *et al.*, 1989, 1992). Although we have no definitive explanation, we suggest that the unusually high sensitivity to acetylcholine in our preparation may be due to the fact that the arterial rings were superfused with acetylcholine continually flowing through the tissue chamber. This procedure allows less time for the metabolism of acetylcholine by tissue cholinesterases than in procedures using tissue baths without flow, exposing a given concentration of acetylcholine to longer periods of enzymatic metabolism.

The mechanism(s) controlling the specific increases in sensitivity to acetylcholine, in NOS protein expression and activity in the uterine artery, in other vasculatures, and in non-vascular tissues such as the uterus and cervix during pregnancy are unclear. In the uterine vasculature during normal pregnancy, shear stress and flow are known to be potent stimulators of NO production (review by Sladek *et al.*, 1997). In addition, the control of the NOS protein expression and activity and, hence, acetylcholine-induced relaxation may involve the sex steroid hormones. Circulating concentrations of oestrogen and progesterone are elevated in human pregnancy and may chronically influence production of NO (for review see Sladek *et al.*, 1997).

The available data regarding the effects of oestrogen and progesterone on the vasculature are confusing and difficult to interpret. These problems may be related to differences in blood vessels, species and methods used for the studies. Numerous studies have been done on the effects of oestrogen on vascular reactivity (for reviews see Chwalisz *et al.*, 1996; Farhat *et al.*, 1996; Sladek *et al.*, 1997). Results have been reported showing an increased responsiveness to acetylcholine in uterine arteries from non-pregnant patients at times in the menstrual cycle with elevated concentrations of oestrogen but not with low concentrations of oestrogen. However, in uterine arteries with intimal thickening from non-pregnant patients with high oestrogen concentrations, a decreased responsiveness to acetylcholine has also been demonstrated (Azuma *et al.*, 1995). Consistent with the notion that oestrogen plays a role in sensitizing the uterine arteries to acetylcholine, we have reported that human uterine arteries removed from post-menopausal patients (low oestrogen concentrations) exhibited a much lower response to acetylcholine than the arteries removed during the follicular and luteal phases (Johnson *et al.*, 1993). Data obtained on experimental animal preparations strongly indicate that oestrogen is involved in the up-modulation of the effect of acetylcholine on the uterine vasculature. For example, Bell (1968) observed that in the uterine vascular bed of the guinea pig the time course of development of responsiveness to acetylcholine is associated with the increase of plasma oestradiol concentrations during pregnancy. Oestrogen, administered *in vivo*, directly increases uterine blood flow in sheep (Naden and Rosenfeld, 1985; Van Buren *et al.*, 1992; Rosenfeld *et al.*, 1996) and also enhances NO-mediated relaxation in in-vitro sheep uterine arteries, but not in omental arteries (Rosenfeld *et al.*, 1996) or renal arteries (Veille *et al.*, 1996). On the other hand, there are fewer studies investigating the role of progesterone on vascular reactivity during pregnancy or in the non-pregnant condition. In these studies, progesterone has variable effects on the vasculature. For example, progesterone by itself has no effect on uterine blood flow in sheep but partially inhibits the vasodilatory actions of oestrogen (Resnick *et al.*, 1977). However, progesterone, at the plasma concentration found in pregnancy, has been shown to reduce arterial pressure in non-pregnant sheep (Roesch and Keller-Wood, 1997) and rat (Liao *et al.*, 1996). Progesterone increases the vasodilator effect of calcitonin gene-related peptide on blood pressure in the rat (Gangula *et al.*, 1997b) but decreases the sensitivity to acetylcholine in the in-vitro canine coronary artery (Miller and Vanhoutte, 1991) and rat aorta (Vedernikov *et al.*, 1997). Progesterone, in pharmacological concentrations, directly relaxes KCl-induced contractions in in-vitro omental arteries from pregnant and non-pregnant patients (Belfort *et al.*, 1996). In myometrial arteries from non-pregnant patients, pharmacological concentrations of progesterone (1–40 μM) and oestriol (10–40 μM), inhibit KCl-induced and vasopressin-induced contractions, whereas at physiological concentrations (0.02–0.2 μM) progesterone enhances vasopressin-induced contractions (Kogtrzewska *et al.*, 1993). To our knowledge, there are no data available on the effects of progesterone alone or on acetylcholine-induced relaxation in human uterine arteries during pregnancy. Hence, the influence of progesterone in the pregnancy-induced sensitization to acetylcholine in the human uterine artery cannot be ruled out.

It is well known that oestrogen and progesterone have non-vascular effects in the uterus and cervix. For example, progesterone inhibits spontaneous delivery and increases in NO production in the rat cervix at labour (Buhimschi *et al.*, 1996). Interestingly, however, in rat myometrial smooth muscle cells, both oestrogen and progesterone have no significant effect on nitrite production (used as an indicator of NO production by eNOS) (Gangula *et al.*, 1997b).

It is thought that genomic effects of oestrogen develop within hours whereas the non-genomic effects occur rapidly in minutes. In the present study the sensitivity to acetylcholine in the uterine arteries from pregnant and non-pregnant patients is long-lasting and persists for the duration of the experiments (10–12 h), hence indicating a genomic effect of oestrogen. Furthermore, a genomic effect of oestrogen is suggested in

the study of Bell and Coffey (1982) showing a block by cycloheximide, a protein inhibitor, of oestrogen-increased uterine blood flow.

We did not measure the oestrogen concentrations in the patients, nor did we always have available a clear indication of the phase of the menstrual cycle at the time of surgery. Also, we did not examine the uterine arteries for intimal thickening. As noted above, all of these factors could influence the reactivity of the uterine arteries to acetylcholine and thus account for the lack of response to acetylcholine that we observed in the arteries from eight of the 35 non-pregnant patients; however the data from these experiments which were included in the data plotted in Figure 1 cannot account for the significant difference in the responses to acetylcholine between pregnant and non-pregnant uterine arteries. Excluding the data obtained from the non-responding arteries, the relaxation of the uterine arteries from the pregnant patients remained significantly greater than that obtained from the non-pregnant patients.

It is unlikely that acetylcholine *per se* plays a functional role in the regulation of the tone of the human uterine artery (Toda *et al.*, 1994; Nelson *et al.*, 1995). Thus, the significance of the response to acetylcholine lies in the assumption that its effect reflects the responsiveness of the human uterine artery to other endogenous substances or stimuli. Accordingly, our finding that there is an increase in the acetylcholine-induced vasodilation of the human uterine artery during pregnancy provides strong support for a growing body of evidence (Chwalisz *et al.*, 1996; Magness *et al.*, 1996; Sladek *et al.*, 1997; Magness *et al.*, 1997) showing that endothelium-derived NO is involved in the pregnancy-associated increase in uterine blood flow.

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