Stimulated nitric oxide release and nitric oxide sensitivity in forearm arterial vasculature during normotensive and preeclamptic pregnancy

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OBJECTIVE: We sought to determine whether the enhanced forearm vascular activity of nitric oxide during pregnancy and preeclampsia is associated with altered smooth muscle sensitivity to nitric oxide or with stimulated nitric oxide release.

STUDY DESIGN: Forearm blood flow responses to brachial artery infusion of glyceryl trinitrate (a nitric oxide donor), serotonin (an endothelium-dependent nitric oxide–mediated agonist), and ritodrine (a β-adrenergic receptor agonist) were studied in 10 nonpregnant women, 12 pregnant women, and 7 women with preeclampsia by means of strain-gauge plethysmography. Responses to each drug (maximum dilator response and the sum of the percentage of dilator responses to each drug) were compared by analysis of variance.

RESULTS: Compared with nonpregnant women, pregnant subjects showed reduced responses to serotonin (summary response, 117 ± 19 vs 221 ± 30; $P < .05$). Responses to serotonin were reduced in the group with preeclampsia compared with those in the nonpregnant group (summary response, 71 ± 28; $P < .05$) but did not differ from the responses in pregnant women. There were no differences between responses to glyceryl trinitrate and responses to ritodrine in any of the groups.

CONCLUSION: Vascular smooth muscle sensitivity to nitric oxide is not altered in normal pregnancy or preeclampsia, but dilator responses to serotonin appear blunted. Alterations in serotonin receptor coupling to nitric oxide synthase, or a limitation of availability of the substrate for nitric oxide synthase (L-arginine) during pregnancy, could account for the reduction in stimulated nitric oxide release. (Am J Obstet Gynecol 1999;181:1479-85.)

Key words: Nitric oxide, pregnancy, preeclampsia, vascular resistance, serotonin

Peripheral vascular resistance falls dramatically during human pregnancy.[1] Nitric oxide, a potent endothelium-derived vasodilator, has been implicated in this adaptation.[2] We previously demonstrated increased constrictor responses to nitric oxide synthase inhibition in the dorsal hand vein of puerperal women.[3] More recently, we reported similar findings in the forearm arterial vasculature during late pregnancy,[4] confirming an earlier report of enhanced constrictor responses to nitric oxide synthase inhibition in the hand circulation during pregnancy.[5] Increased maternal serum and placental concentration of nitric oxide metabolites and second messengers, and the finding of increased endothelial nitric oxide synthase expression in various tissues in the guinea pig during pregnancy,[6] suggest that increased vascular nitric oxide synthesis is the underlying mechanism.[7] Alternatively, or in addition, pregnancy could be associated with enhanced vascular smooth muscle sensitivity to nitric oxide. Wire myographic studies of isolated vascular rings from pregnant women found no change in sensitivity to exogenous nitric oxide,[8] but these observations have not been confirmed in vivo under physiologic conditions of flow and autonomic activity.

Preeclampsia is associated with high peripheral vascular resistance. The elevation of serum levels of factor VIII–related antigen and fibronectin in preeclampsia suggests that endothelial dysfunction is important in the pathogenesis.[9] It has been suggested that endothelial dysfunction may then lead to alterations in nitric oxide activity. We recently reported no difference in forearm

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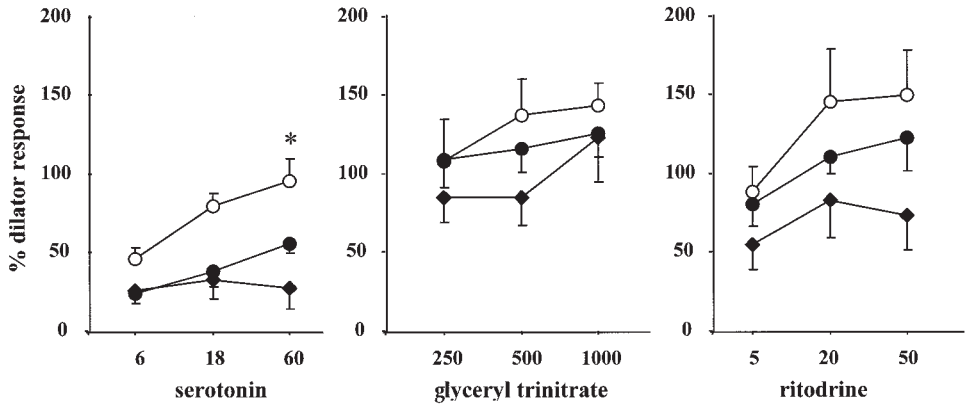

Fig 1. Percentage (mean and SE) of dilator forearm blood flow responses to glyceryl trinitrate (a nitric oxide donor), serotonin (an endothelium-dependent nitric oxide agonist), and ritodrine (a control vasodilator) in 10 nonpregnant women (*open circles*), 12 normotensive pregnant women *(filled circles)*, and 7 women with preeclampsia *(filled diamonds)*. Responses to serotonin were blunted in normotensive pregnant women and in women with preeclampsia, in comparison with responses in nonpregnant subjects. *Asterisk*, $P < .05$, by analysis of variance.

constrictor responses to nitric oxide synthase inhibition in untreated primigravid women with preeclampsia, in comparison with normotensive pregnant control subjects.[4] These results are consistent with in vitro observations that nitric oxide synthesis and nitric oxide metabolites are unchanged in preeclampsia.[7, 10] However, vascular smooth muscle sensitivity to nitric oxide has not been determined in vivo in women with preeclampsia. Furthermore, although no difference in basal vascular nitric oxide activity is evident, endothelial dysfunction could be manifested by more subtle impairments of the nitric oxide pathway, leading to reduced nitric oxide release in response to vasodilators that stimulate endothelial nitric oxide release.

The aims of the current study were to determine whether vascular smooth muscle sensitivity to nitric oxide and stimulated nitric oxide release are altered in normal pregnancy and untreated preeclampsia. Using the forearm technique of strain-gauge plethysmography, we examined responses to glyceryl trinitrate, an exogenous nitric oxide donor, and serotonin, an endothelium-dependent stimulator of nitric oxide release in the forearm in nonpregnant subjects.[11, 12] Responses were compared with responses to a control vasodilator, ritodrine, which acts predominantly through adenylate cyclase via β-adrenergic receptors.

Methods

Subjects. Twelve healthy primiparous women, 7 primiparous women with preeclampsia who had not received antihypertensive therapy, and 10 healthy nonpregnant women who were not receiving hormonal contraception were studied (Table I). Subjects were nonsmokers and refrained from alcohol and caffeine for a minimum of 4 hours before each study. Women with preeclampsia had persistently elevated blood pressure readings of at least 140 mm Hg systolic, and 90 mm Hg diastolic; proteinuria (≥300 mg protein in 24 hours or 2+ on dipstick) was also present. Written informed consent was obtained, and the University of Newcastle upon Tyne Joint Ethics Committee approved the studies.

Study protocol. All studies were carried out in a temperature-controlled quiet laboratory (25°C), with the subjects in a slight left lateral tilt to avoid caval occlusion. Forearm blood flow was measured simultaneously in both arms by mercury in silicone-rubber strain-gauge plethysmography. Drugs or physiologic sodium chloride solution was infused at 1 mL/min continuously into the brachial artery of the nondominant arm through a needle (27 standard wire gauge) introduced with the use of local anesthesia. The noninfused arm acted as a contemporaneous control, because small alterations in systemic blood pressure or sympathetic arousal affect blood flow in both arms uniformly. Furthermore, dominant forearm blood flow measurements were made to exclude a systemic effect from intra-arterial drug infusions. At the start of each study, after insertion of the needle, basal blood flow measurements were taken for 30 minutes (during infusion of sodium chloride solution) to establish resting control values. During the recording periods, circulation through the hands was excluded by inflating the wrist cuffs to a pressure of 200 mm Hg. The upper-arm congesting cuffs were inflated to 40 mm Hg for 10 seconds in five 15-second cycles. Blood pressure was measured in the control arm every 15 minutes with a semiautomated oscillometric device.

All drugs were dissolved in sterile physiologic sodium chloride solution immediately before infusion. After resting control forearm blood flow measurements had been obtained, each subject received 3 doses of serotonin (6,

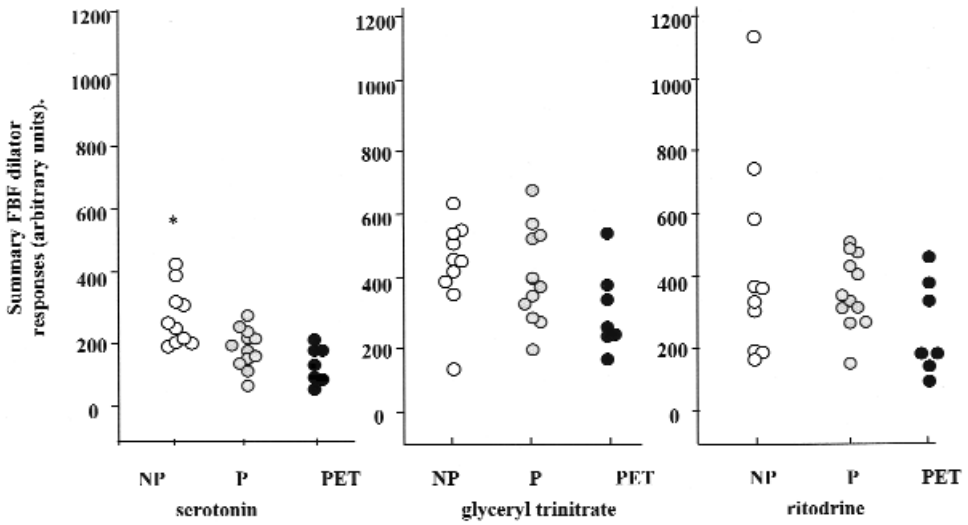

Fig 2. Summary forearm blood flow (*FBF*) responses to glyceryl trinitrate (a nitric oxide donor), serotonin (an endothelium-dependent nitric oxide agonist), and ritodrine (a control vasodilator) in 10 nonpregnant women *(NP)*, 12 normotensive pregnant women *(P)*, and 7 women with preeclampsia *(PET)*. Responses to serotonin were blunted in normotensive pregnant women and in women with preeclampsia, in comparison with nonpregnant subjects. *Asterisk*, $P < .05$, by analysis of variance.

18, and 60 ng min^{-1} each for 5 minutes). Sodium chloride solution was then infused until forearm blood flow returned to control values (median time, 15 minutes; range, 10-50 minutes). Subjects then received 3 doses of glyceryl trinitrate (250, 500, and 1000 ng min^{-1} each for 5 minutes). After return of forearm blood flow to resting values with sodium chloride solution, 3 doses of ritodrine (5, 20, and 50 μg min^{-1}) were administered, each dose being given for 5 minutes to produce a cumulative dose response. Blood flow was recorded during the last 3 minutes of each 5-minute drug infusion period. The order of administration of the 3 study drugs was not randomized because of the persisting effects of ritodrine observed in preliminary studies.

To overcome the potential confounding effects of elevated flow on nitric oxide release and of the differential dilutional effects of infused drugs between study groups, we reduced forearm blood flow in the pregnant subjects and in the subjects with preeclampsia to nonpregnant levels by initial infusion of norepinephrine (median dose, 10 ng min^{-1}; range, 5-30 ng min^{-1}). The dose of norepinephrine required to achieve this reduction was then coinfused with serotonin, glyceryl trinitrate, and ritodrine, as described above.

Data capture and statistical analysis. Data were recorded directly onto a computer with a MacLab (registered trademark of ADInstruments Pty Ltd, Castlehill, Australia) system with on-line slope analysis to determine forearm blood flow. The average of the 5 slopes for each measurement period was derived to determine forearm blood flow. Forearm blood flow was expressed as milliliters per 100 mL of forearm per minute according to the method of Whitney.[13] Forearm vascular resistance was derived from mean arterial pressure and baseline forearm blood flow. Differences in baseline heart rate, blood pressure, forearm blood flow, and forearm vascular resistance between groups were compared by analysis of variance. Where there was a difference between groups, the studentized range was used to determine which groups differed significantly. Within-subject differences in forearm blood flow in the control arm were assessed by repeated-measures analysis of variance. Within-subject differences in forearm blood flow in the infused arms were similarly assessed, and further analysis was undertaken if analysis of variance suggested a statistically significant change in forearm blood flow in time. For comparison of drug responses between groups, forearm blood flow responses were expressed as a percentage of forearm blood flow in the infused arm during baseline infusion of sodium chloride solution or norepinephrine. The overall response to each drug in each subject was assessed by 2 summary measures (according to the principles described by Mathews et al[14]), the maximum response and an overall response, calculated as the summation of the percentage of dilator responses for the 3 doses of the infused drug (in arbitrary units). The dilator responses were normalized to the baseline during infusion of sodium chloride solution in the experimental nondominant arm. Values are expressed as mean and SEM and were compared by analysis of variance. $P < .05$ was considered statistically significant.

Instruments and drugs. Mercury strain gauges and plethysmographs were supplied by D.E. Hokansen Inc (Bellevue, Wash), and the twin cuff inflators were ob-

Table I. Subject details

Characteristics	Nonpregnant (n = 10)	Pregnant	
		Normotensive (n = 12)	Preeclamptic (n = 7)
Age (y, median and range)	24 (20-34)	24 (20-34)	25 (18-36)
Weight (kg, median and range)	60 (42-83)	72 (58-99)*	87 (75-93)*†
Systolic blood pressure (mm Hg, mean ± SE)	125 ± 4	129 ± 4	164 ± 6‡§
Diastolic blood pressure (mm Hg, mean ± SE)	67 ± 2	68 ± 2	95 ± 4‡§
Forearm vascular resistance (mm Hg · mL^{-1} · min^{-1} · 100 mL^{-1}, mean ± SE)	37 ± 5	19 ± 1‡	37 ± 6*
Forearm volume (mL, mean ± SE)	760 ± 51	803 ± 46	806 ± 63
Forearm length (cm, mean ± SE)	25 ± 0.4	25 ± 0.4	25 ± 0.5
Gestational age (wk, median and range)	—	32 (29-38)	35 (29-38)
Forearm blood flow (mL · 100 mL forearm · min, mean ± SE)			
Basal	3.3 ± 0.3	4.9 ± 0.5*	4.1 ± 0.7*†
With norepinephrine	—	3.8 ± 0.4	2.8 ± 0.4

Statistical significance was determined by 1-way analysis of variance.
*$P < .05$, nonpregnant versus pregnant subjects; pregnant subjects versus subjects with preeclampsia.
†$P < .05$, subjects with preeclampsia versus nonpregnant subjects.
‡$P < .01$, nonpregnant versus pregnant subjects; pregnant subjects versus subjects with preeclampsia.
§$P < .01$, subjects with preeclampsia versus nonpregnant subjects.

tained from Techno-Medical Services Ltd (Surrey, United Kingdom). The continuous syringe infusion pumps were obtained from Welmed Limited (Bramley, United Kingdom). The needles (27 standard wire gauge) were obtained from Coopers Needleworks Ltd (Birmingham, United Kingdom), and 5-hydroxytryptamine was obtained from Clinalfa AG (Laufelefingen, Switzerland). Glyceryl trinitrate was obtained from David Bull Laboratories (Warwick, England). Norepinephrine (Levophed) was obtained from Sanofi Winthrop Laboratories (Surrey, United Kingdom).

Results

Compared with nonpregnant subjects, pregnant subjects had reduced forearm vascular resistance but similar systemic blood pressure. Systemic blood pressure and forearm vascular resistance were higher in subjects with preeclampsia than in pregnant subjects. Forearm volume and length did not differ between the study groups. Forearm blood flow did not vary significantly in the noninfused arm in any of the groups during the study. Blood pressure did not vary significantly within the time course of each experiment. Basal forearm blood flow did not differ between pregnant subjects and the subjects with preeclampsia but was lower in nonpregnant women. Norepinephrine lowered forearm blood flow in pregnant and preeclamptic subjects to levels comparable to those in nonpregnant subjects (Table I).

All 3 drugs produced a dose-dependent increase in forearm blood flow (Fig 1, repeated-measures analysis of variance). Summary and maximum dilator responses are shown in Table II and Fig 2. Compared with nonpregnant subjects, the pregnant subjects showed reduced dilator responses to serotonin. Dilator responses to serotonin were also reduced in the group with preeclampsia in comparison with nonpregnant subjects but did not differ from the pregnant group. There were no differences between dilator responses to glyceryl trinitrate and responses to ritodrine in any of the groups.

Comment

This is the first study to determine vasodilator responses in the human forearm during pregnancy. The results demonstrate that vascular smooth muscle sensitivity to nitric oxide is not altered in normal pregnancy or preeclampsia. Thus gestation-related increases in vascular nitric oxide activity are most likely mediated by increased endothelial nitric oxide production. An unexpected finding of our study was that dilator responses to serotonin appear blunted during both normotensive and preeclamptic pregnancy.

Our observations that forearm vascular responsiveness to glyceryl trinitrate is unchanged during the third trimester of normal pregnancy and during preeclampsia are consistent with previous in vitro studies in which maximum dilator responses to sodium nitroprusside were similar in subcutaneous arteries from nonpregnant, pregnant, and preeclamptic subjects.[8, 9, 15] Both glyceryl trinitrate and sodium nitroprusside are transformed to nitric oxide, which binds to guanylate cyclase in vascular smooth muscle, leading to vasorelaxation.[16] Thus increased nitric oxide synthase activity, rather than enhanced responsiveness to nitric oxide as a result of upregulation of guanylate cyclase, is likely to account for the increased vascular nitric oxide activity that we and others have previously observed during normal pregnancy.[4, 5] Mechanisms possibly responsible for increased nitric oxide synthase activity include estrogen,[6] blood flow,[16] and fluid shear stress.[17] The normal dilator response to glyceryl trinitrate in preeclampsia suggests that

Table II. Summary and maximum dilator responses

	Nonpregnant subjects	*Pregnant subjects*	*Subjects with preeclampsia*
Maximum dilation with serotonin (%, mean ± SE)	95 ± 17	56 ± 6*	33 ± 12*†
Summary response to serotonin (arbitrary units, mean ± SE)	221 ± 30	117 ± 19*	71 ± 28*†
Maximum dilation with glyceryl trinitrate (%, mean ± SE)	143 ± 15	125 ± 15	122 ± 28
Summary response to glyceryl trinitrate (arbitrary units, mean ± SE)	388 ± 46	349 ± 43	292 ± 60
Maximum dilation with ritodrine (%, mean ± SE)	163 ± 43	123 ± 21	83 ± 23
Summary response to ritodrine (arbitrary units, mean ± SE)	409 ± 103	313 ± 33	211 ± 60

Statistical significance was determined by analysis of variance.
*$P < .05$, pregnant versus nonpregnant subjects.
†$P < .05$, subjects with preeclampsia versus nonpregnant subjects.

end-organ sensitivity to nitric oxide[16] is also not altered in this condition and provides further evidence that alterations in the L-arginine–nitric oxide pathway are not responsible for the increased peripheral vascular resistance seen in this condition.

We studied stimulated nitric oxide activity with serotonin to determine the ability of the endothelium to increase nitric oxide production above basal levels. The early sustained forearm dilator responses to serotonin are dependent on endothelium and completely inhibited by the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine,[11, 12] which suggests that they are mediated entirely by nitric oxide.[11, 12] Our choice of serotonin for the present studies was based on the assumption that its vasodilator response in pregnant subjects is also entirely mediated by nitric oxide. However, this has not been proved, and it is plausible that the coupling of agonists to the different signaling pathways to produce vasodilation may be altered in pregnancy. Responses to serotonin vary between species and vascular beds.[11] Molecular studies have identified 5 subtypes of the serotonin receptor, but the receptor mediating nitric oxide–dependent vasodilation in the human forearm is unknown.[12] In vivo responses to serotonin were reduced in both groups of pregnant subjects. In contrast, responses to ritodrine, a selective β_2-adrenergic receptor agonist that acts predominantly via cyclic adenosine monophosphate,[18] were unchanged, suggesting that pregnancy is not associated with a nonspecific attenuation of vasodilator responsiveness. A number of mechanisms could account for the reduced dilator response to serotonin during pregnancy. Reduced endothelial serotonin receptor numbers or altered serotonin receptor coupling to nitric oxide synthase may be important. Acetylcholine and bradykinin, other endothelium-dependent nitric oxide agonists, are coupled to nitric oxide synthase by specific G proteins.[19] It is possible that pregnancy-induced modification of G protein activity may account for our observations. Alternatively, if nitric oxide synthase in the pregnant woman's endothelial cells is nearly maximal, the dilator response to administration of serotonin may be less than in nonpregnant subjects. Panza et al[20] found that at basal levels of blood flow the infusion of L-arginine (the substrate for nitric oxide production) had no effect on forearm blood flow in healthy subjects. In contrast, L-arginine significantly enhanced vasodilator responses to the agonist acetylcholine. These findings suggest that substrate availability could be a rate-limiting step for endothelium-dependent vascular relaxation, particularly in states of high basal nitric oxide activity and protein anabolism such as pregnancy. Whatever the mechanism of the reduced response to serotonin in normal pregnancy, we have no evidence to suggest that it is altered in preeclampsia, which suggests that endothelial dysfunction in this disease does not dramatically affect the serotonin signal transduction pathway.

Previous human studies of stimulated nitric oxide release have all been performed on isolated small vessels. McCarthy et al[8] reported that dilation to acetylcholine was unaltered in subcutaneous arteries from pregnant women. The same group subsequently found enhanced dilation to bradykinin in vessels of pregnant women compared with those of nonpregnant women.[15] Responses to both acetylcholine and bradykinin were impaired in arteries from women with preeclampsia.[9, 15] However, Pascoal et al[21] found no alteration in endothelium-dependent vasodilation to bradykinin in omental arteries from healthy pregnant women or in those from women with preeclampsia. The authors found, however, complete absence of relaxation to acetylcholine. Direct comparison of these results with those reported here is difficult. Vasodilation in response to acetylcholine and bradykinin is only partly mediated by nitric oxide,[22, 23] and changes in the non–nitric oxide component of vasodilation could be modified in these vessels during pregnancy. Indeed, Pascoal and Umans[24] reported that relaxation in the omental arteries of pregnant women in response to acetylcholine and bradykinin was completely insensitive to nitric oxide synthase inhibition. Furthermore, responses of vessels studied in vivo may differ from those of explanted vessels, which are not exposed to physiologic levels of shear stress, the principal stimulus to endothelial nitric oxide synthesis.

We reduced baseline forearm blood flow to nonpreg-

nant levels in the pregnant subjects using norepinephrine. This technique minimizes both the potential confounding effect of increased flow on the response to nitric oxide synthase inhibition and the differential dilutional effects of the infused drugs. Norepinephrine induces endothelium-independent vasoconstriction predominantly through α_1-adrenergic receptor stimulation and therefore has been extensively used as a nitric oxide–independent constrictor in the forearm vascular bed. However, in some species norepinephrine has been reported to induce nitric oxide–mediated vasorelaxation through endothelial α_2-adrenergic receptors. Any such effect in the forearm vasculature is likely to be small, as we have found that forearm blood flow responses to nitric oxide synthase inhibition during normal pregnancy is unaffected by concomitant infusion of norepinephrine.[25] Serotonin is known to enhance the contractile response to norepinephrine and other vasoconstrictor agents in isolated vessels from several vascular beds.[26] However, such potentiation does not appear to occur in vivo in the forearm vasculature[27] and is therefore unlikely to account for the differences in response between our pregnant and nonpregnant subjects. Norepinephrine has no apparent effect on endothelium-dependent vasodilation in response to methacholine in the human forearm.

In conclusion, smooth muscle sensitivity to nitric oxide is not altered during normotensive and preeclamptic pregnancy. Thus an increase in production of nitric oxide by nitric oxide synthase is the most likely explanation for our previous finding of increased vascular nitric oxide activity during pregnancy. Forearm vasodilator responses to serotonin are blunted during normotensive and preeclamptic pregnancy. This may result from pregnancy-related alterations in the signal transduction pathway of serotonin-induced forearm vasodilation. Alternatively, if endothelial cells are maximally producing nitric oxide during pregnancy, there may be a reduced response to agonist-stimulated nitric oxide production. The signaling pathways for agonist-induced vascular effects during pregnancy and preeclampsia remain to be fully determined. However, our findings do not suggest any major abnormality in resting or stimulated nitric oxide activity and nitric oxide sensitivity in preeclampsia.

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