Nitric oxide metabolite concentrations in maternal plasma decrease during parturition: possible transient down-regulation of nitric oxide synthesis

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To elucidate the possible involvement of nitric oxide (NO) in parturition, we measured the maternal plasma concentrations of the NO metabolites, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and guanosine 3′,5′-cyclic phosphate (cGMP) in pregnant women at various gestational ages including those at vaginal and elective Caesarean deliveries. The plasma cGMP and NO metabolite concentrations at vaginal delivery were significantly lower than those of the pregnant women in the third trimester of pregnancy. These concentrations remained low until 4 h after delivery but returned 24 h after delivery to values similar to those of the non-pregnant women. Such suppressions of plasma cGMP and NO metabolite concentrations were not observed in the women who underwent elective Caesarean section before the onset of labour. Moreover, no significant changes were observed in the plasma ANP and BNP concentrations at the time of vaginal and Caesarean deliveries, except that a slight but significant elevation of the plasma ANP concentration was observed 1 h after Caesarean delivery. In conclusion, the plasma concentrations of cGMP and NO metabolites significantly decreased at vaginal delivery but not at Caesarean delivery. These changes were independent of the plasma ANP and BNP concentrations, suggesting the possible down-regulation of maternal NO synthesis during parturition.

Key words: atrial natriuretic peptide/brain natriuretic peptide/cGMP/nitric oxide/parturition

Introduction

Guanosine 3′,5′-cyclic phosphate (cGMP) is the intracellular second messenger that decreases the intracellular calcium ion concentration and causes a relaxation of smooth muscle cells, including vascular smooth muscle cells and uterine myometrium (Nakao et al., 1992a; Garbers et al., 1994; Yallampalli et al., 1994a; Wedel et al., 1995). cGMP is produced from guanosine triphosphate (GTP) by two distinct intracellular pathways, i.e. the soluble guanylate cyclase (sGC) pathway and the particulate guanylate cyclase (pGC) pathway (Nakao et al., 1992a; Garbers et al., 1994; Wedel et al., 1995). The ligand of sGC is nitric oxide (NO). NO is synthesized by NO synthases (NOS), a family of isoenzymes (eNOS, iNOS and nNOS) that catalyse the oxidation of L-arginine to L-citrulline and NO. eNOS was first identified as a constitutive NO-producing enzyme in vascular endothelial cells and is calcium dependent like nNOS, which was originally identified in neuronal tissues. iNOS, first identified in macrophage, is inducible by cytokines or lipopolysaccharide (LPS) and is not dependent on calcium ion concentration. These three isoforms have been reported to be present in non-pregnant and/or pregnant uteri (Dong et al., 1996b; Rosselli et al., 1996; Rosselli et al., 1997; Telfer et al., 1997). NO is synthesized by endothelial NO synthase (eNOS) in the vascular wall and secreted as an endothelium-derived relaxing factor (EDRF). NO activates sGC in the cytosol of adjacent vascular smooth muscle cells in a paracrine manner and the activated sGC produces cGMP which finally results in vasodilatation (Mageness et al., 1996a). Conrad et al. (1993) first reported that the urinary secretion of NO metabolites (nitrate and nitrite) and cGMP was increased in pregnant rats, suggesting the possible involvement of NO in the modulation of blood flow during pregnancy (Conrad et al., 1993; McLaughlin et al., 1995). Begum et al. (1996) reported a similar increase in urinary NO metabolites in pregnant women. Magness et al. (1996a) recently reported that eNOS expression in the ovine uterine artery was 2–4-fold up-regulated during pregnancy and that the local cGMP production in the uterine vascular bed increased 38-fold during pregnancy, strongly suggesting the possible involvement of eNOS in the marked increase in the local blood flow of uterine vessels during pregnancy (Magness et al., 1996b; Rosenfeld et al., 1996).

The uterine myometrium relaxes markedly during pregnancy. Recent studies showed that sGC as well as SOS were present in the pregnant human uterus and that the cGMP content in the myometrium increased during pregnancy (Izumi et al., 1993; Yallampalli et al., 1994a; Buhimschi et al., 1995). Thus, the NO–sGC system has been postulated to be involved in various marked changes in the pregnant uterus, such as the increase in the uterine blood flow, and myometrial relaxation, among others. To our knowledge, however, only a few in-vivo studies have examined the possibility of a direct involvement of NO in the initiation of parturition (Yallampalli et al., 1994a;
Buhimschi et al., 1995). In rats, the production of NO is increased during mid-gestation and is markedly decreased during spontaneous delivery and postpartum (Conrad et al., 1993; Izumi et al., 1993; Yallampalli et al., 1994a). In rabbit, similar changes in the NO–cGMP system were reported (Sladek et al., 1993). The NO–cGMP system is also present in the human uterus, is up-regulated during pregnancy and is down-regulated at term (Buhimschi et al., 1995). All these results suggest that the NO–cGMP system may contribute to the maintenance of uterine quiescence during pregnancy and that the down-regulation at term may initiate labour (Sladek et al., 1997).

Particulate guanylate cyclase (pGC) is activated by the natriuretic peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). The biologically active natriuretic peptide receptors, ANP-A receptor and ANP-B receptor, are present in the cell membrane and constitute a major component of the pGC pathway, which is another distinct guanylate cyclase pathway causing cGMP-mediated vasodilatation (Naka[o et al., 1992a,b]. Thus, natriuretic peptides exert natriuretic and vasodilative activities via cGMP (Naka[o et al., 1992b). Both ANP and BNP are secreted from the heart into the circulation as cardiac hormones, and act on the ANP-A receptor, the predominant pGC, in the vascular smooth muscle cells. The activated pGC produces cGMP from GTP, which finally results in vasodilatation (Nakao et al., 1992a,b). We previously reported that the maternal plasma concentrations of BNP and ANP were increased in pregnant women complicated with pregnancy-induced hypertension (Itoh et al., 1993, 1997). We subsequently have demonstrated that both the ANP-A and ANP-B receptor genes are expressed in human pregnant myometrium and that ANP-A receptor is the dominant type in this tissue (Itoh et al., 1994). 8-Bromo-cGMP, a non-hydrolysable cGMP analogue that permeates the cell membrane, produced relaxation of both non-pregnant and pregnant rat myometrium in vitro (Yallampalli et al., 1994a). These results lead us to speculate that ANP and/or BNP in the maternal blood may reach myometrium, produce cGMP and finally results in relaxation of myometrium. To our knowledge, there has been no report of an investigation of both the plasma concentrations of NO metabolites, the specific ligand of sGC, and those of ANP and BNP, the specific ligands of pGC-A, in the same study population during pregnancy and parturition in any species. This is in spite of the fact that these two distinct guanylate cyclase pathways are present in both pregnant myometrium and vascular wall.

In the present study, to clarify the possible involvement of the NO–sGC system in parturition, we measured the plasma concentrations of cGMP, NO metabolites, ANP and BNP of pregnant women at various gestational ages and of women at vaginal deliveries and postpartum. In addition, the plasma concentrations of these substances at vaginal deliveries were compared with those at elective Caesarean deliveries.

Materials and methods

The subjects of the present study were: 11 normal non-pregnant women [28.4 ± 7.9 years (mean ± SD)]; 20 pregnant women in the first trimester (29.2 ± 4.6 years); 28 pregnant women in the second trimester (30.7 ± 3.4 years); 56 pregnant women in the third trimester (30.2 ± 4.3 years); 14 pregnant women at spontaneous vaginal delivery (27.0 ± 3.7 years, 39.6 ± 1.4 weeks of gestation at delivery); and 11 pregnant women who underwent elective Caesarean section before the onset of labour (29.4 ± 3.7 years, 38.1 ± 1.3 weeks of gestation at operation). In the latter two groups of women blood specimens were obtained at 1–2 h before the delivery or Caesarean section, and at 1, 4, 24 and 48 h after the delivery or Caesarean section. Indications for elective Caesarean section were breech presentation or previous Caesarean section. Table I summarizes the ages, gestational ages and mean blood pressure of each group. The blood pressure was measured at the time of blood collection. In cases of vaginal delivery, blood pressure was measured between the active labour pains. All of the non-pregnant and pregnant women studied were Japanese, and informed consent was obtained from all of the subjects after a full explanation of the purpose and nature of the study. None of the subjects received any kind of medications other than iron and vitamin B₁₂ until the time of the collection of blood specimens. There are several reports that the plasma NO metabolite concentrations are influenced by diet (Jungersten et al., 1996), pain, vitamins, anaesthesia, fetal status and placenta size (Neri et al., 1995). The nutritional status, fetal status and placental sizes were not different between the two groups. All the women who underwent Caesarean section received epidural anaesthesia. The major difference between the two groups was that labour was occurring in the vaginal delivery group but not in the Caesarean section group, all of which were carried out before the onset of labour. Peripheral venous blood was taken from the antecubital vein after 15 min of bed rest. In addition, the retroplacental pooled blood was collected at the time of recoveries of the placenta from 11 other pregnant women at the spontaneous vaginal deliveries (26.9 ± 3.5 years, 39.6 ± 1.4 weeks of gestation at delivery) and from 13 other pregnant women at elective Caesarean section before the onset of labour (28.5 ± 3.2 years, 38.0 ± 1.2 weeks of gestation at Caesarean section). To minimize the contamination of other fluid from the vaginal tract, retroplacental blood was collected from the haematoma capsuled with placenta and amniochorionic membrane, which was formed by controlled cord traction. In cases of Caesarean section, the placenta was also removed by controlled cord traction after uterine contraction was induced by massage on the uterine fundus, and the blood specimen was collected from capsuled retroplacental blood. The peripheral blood samples were immediately transferred to chilled siliconized glass tubes containing aprotinin (1000 units/ml) (Ohkura Pharmaceutical Co., Kyoto, Japan) and EDTA (1 mg/ml), and centrifuged at 1200 g for 20 min at 4°C. The plasma thus obtained was aliquoted and stored at –20°C until assayed. The retroplacental blood obtained at the vaginal and Caesarean deliveries was prepared in the same manner as the peripheral blood. The plasma cGMP concentrations were determined by a radioimmunoassay as described previously (Steiner et al., 1972) using a commercially available kit (YAMASA cyclic GMP assay kit; Yamasa-Shoyu Co., Chiba, Japan). The inter- and intra-assay variations of this system were <8%. The plasma NO metabolites nitrate and nitrite were measured using Griess Reagent (Conrad et al., 1993) in a commercially available kit (Cayman’s Nitrate/Nitrite Assay Kit; Cayman Chemical Co., Ann Arbor, MI, USA). The inter- and intra-assay variations of this system were <10%. Plasma ANP and BNP concentrations were determined by specific immunoradiometric assays as described previously using commercially available kits (Shionoria ANP and Shionoria BNP; Shionogi Pharmaceutical Co., Osaka, Japan) (Tsui et al., 1994a,b). The inter- and intra-assay variations of these systems were both <7%.

To examine the effects of steroid hormones on the changes in
Plasma NO metabolite concentrations during parturition

Table I. Ages, gestational ages, and mean blood pressure (MBP) data for the subject groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>Gestational age (weeks)</th>
<th>MBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>11</td>
<td>28.4 (7.9)</td>
<td>–</td>
<td>79.1 (7.8)</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st trimester</td>
<td>20</td>
<td>29.2 (4.6)</td>
<td>9.4 (2.4)</td>
<td>81.2 (9.3)</td>
</tr>
<tr>
<td>2nd trimester</td>
<td>28</td>
<td>30.7 (3.4)</td>
<td>23.5 (4.2)</td>
<td>78.7 (8.2)</td>
</tr>
<tr>
<td>3rd trimester</td>
<td>56</td>
<td>30.2 (4.3)</td>
<td>34.3 (4.0)</td>
<td>81.2 (8.1)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h before VD</td>
<td>14</td>
<td>27.0 (3.7)</td>
<td>39.6 (1.4)</td>
<td>88.1 (7.8)*</td>
</tr>
<tr>
<td>1 h after VD</td>
<td>14</td>
<td>27.0 (3.7)</td>
<td>39.6 (1.4)</td>
<td>80.7 (8.9)</td>
</tr>
<tr>
<td>24 h after VD</td>
<td>14</td>
<td>27.0 (3.7)</td>
<td>39.6 (1.4)</td>
<td>76.6 (9.0)</td>
</tr>
<tr>
<td>48 h after VD</td>
<td>14</td>
<td>27.0 (3.7)</td>
<td>39.6 (1.4)</td>
<td>79.4 (15.6)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h before CS</td>
<td>11</td>
<td>29.4 (3.7)</td>
<td>38.1 (1.3)</td>
<td>83.6 (10.7)</td>
</tr>
<tr>
<td>1 h after CS</td>
<td>11</td>
<td>29.4 (3.7)</td>
<td>38.1 (1.3)</td>
<td>82.5 (8.4)</td>
</tr>
<tr>
<td>4 h after CS</td>
<td>11</td>
<td>29.4 (3.7)</td>
<td>38.1 (1.3)</td>
<td>86.5 (5.6)</td>
</tr>
<tr>
<td>24 h after CS</td>
<td>11</td>
<td>29.4 (3.7)</td>
<td>38.1 (1.3)</td>
<td>83.8 (2.9)</td>
</tr>
<tr>
<td>48 h after CS</td>
<td>11</td>
<td>29.4 (3.7)</td>
<td>38.1 (1.3)</td>
<td>80.2 (3.3)</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD).

*Significantly (P < 0.05) different from the value of pregnant women in the third trimester.

VD = vaginal delivery; CS = Caesarean section.

plasma NO metabolite concentrations, we measured oestradiol and progesterone concentrations in the plasma of pregnant women at term 1 h before, and at 1 and 4 h after vaginal deliveries, then compared these with the results obtained from women who underwent Caesarean section before labour onset. Plasma concentrations of oestradiol and progesterone were measured by specific radioimmunoassay.

The data are expressed as means ± SD. Statistical analyses were performed by Mann–Whitney U-test for comparisons of the means of two groups, and by analysis of variance (ANOVA) followed by Fisher’s protected least significant difference test for comparisons of the means of more than two groups. Linear regression analysis was performed with a least squares method. Probability values less than 0.05 were accepted as significant.

Results

Figure 1 summarizes the changes in the plasma concentrations of cGMP, NO metabolites, ANP and BNP during the pregnancies, and vaginal and Caesarean deliveries. The plasma cGMP concentrations in the second and third trimesters were significantly higher than that of the non-pregnant women (P = 0.036, 0.047, respectively). The plasma NO metabolite concentration in the second trimester (46.0 ± 28.8 µM) was significantly higher than that of the nonpregnant women (P = 0.045), although the plasma NO metabolite concentration was slightly decreased to 39.6 ± 24.5 µM in the third trimester. The plasma concentrations of ANP and BNP, the ligands of pGC, increased as gestation advanced.

The plasma concentrations of cGMP, NO metabolites, ANP and BNP at various stages of spontaneous vaginal deliveries were compared with those of pregnant women in the third trimester. At 1–2 h before the vaginal deliveries, and at 1 and 4 h after the vaginal deliveries, the plasma cGMP and NO metabolite concentrations were significantly lower than those of the pregnant women in the third trimester (cGMP: P = 0.0001, P = 0.0001, P = 0.0001 respectively; NO metabolites: P = 0.0086, P = 0.005, P = 0.0076 respectively). Both the plasma cGMP and NO metabolite concentrations remained

Figure 1. Changes in the concentrations of guanosine 3’5’-cyclic phosphate (cGMP), nitric oxide metabolites (NOx), atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in the maternal peripheral plasma during pregnancy and vaginal and Caesarean deliveries, shown in A, B, C and D respectively. The hatched columns represent the values of non-pregnant women. The closed columns represent the values of pregnant women at various stages of pregnancy and at vaginal deliveries. The open columns represent the values of pregnant women at elective Caesarean sections. The vertical bars represent the SD. *Significantly (P < 0.05) different from the value of non-pregnant women. +Significantly (P < 0.01) different from the value in the third trimester. Statistically significant differences between the values of vaginal delivery group and those of Caesarean section group are shown in the figure.
low until 4 h after the deliveries, but they returned to the concentrations of non-pregnant women by 24 h after delivery (Figure 1). The plasma ANP and BNP concentrations generally did not show any significant changes, except that the plasma ANP concentration was significantly decreased at 24 h after delivery \( (P = 0.0024) \). In contrast to that observed with vaginal deliveries, the plasma cGMP and NO metabolite concentrations were not suppressed in the patients who underwent elective Caesarean section before the onset of labour (Figure 1). The plasma cGMP and NO metabolite concentrations around the time of the Caesarean sections were similar to those of pregnant women in the third trimester. The plasma ANP and BNP concentrations did not generally show any significant changes around the time of Caesarean section, except that the plasma ANP concentration increased to 73.2 ± 18.1 pg/ml 1 h afterwards, a value significantly \( (P = 0.0046) \) higher than the third trimester concentration (Figure 1). We also compared the plasma concentrations of cGMP, NO metabolites, ANP and BNP in the vaginal delivery group with those in Caesarean section group. Plasma cGMP and NO metabolite concentrations at 1 h before and 1 and 4 h after vaginal delivery were significantly lower than those in Caesarean section group. On the other hand, plasma ANP concentrations in the Caesarean section group at 1–24 h after Caesarean section were significantly higher than those in vaginal delivery group.

To elucidate the role of NO, ANP and BNP in the marked decrease in cGMP concentrations during vaginal delivery, the plasma cGMP concentrations of the pregnant women in the third trimester and of the women during vaginal deliveries were compared with those of NO metabolite, ANP and BNP (Figure 2A,B). A significant positive correlation was observed between the plasma cGMP concentrations and those of NO metabolites \( (r = 0.37, \ P < 0.01) \) (Figure 2A). No significant correlations were observed between the plasma cGMP concentrations and those of ANP or BNP (Figure 2B). No significant correlations were observed between the mean blood pressure and the plasma concentrations of cGMP, NO metabolites, ANP or BNP (Figure 3A,B).

Oestradiol and progesterone concentrations in the plasma of pregnant women at 1 h before vaginal delivery at term \( (n = 14) \) were 18.7 ± 15.2 and 44.2 ± 29.0 ng/ml respectively. Both oestradiol and progesterone concentrations decreased markedly at 1 h after vaginal delivery to 5.6 ± 4.1 and 15.0 ± 4.9 ng/ml, at 4 h after vaginal delivery to 2.0 ± 1.9 and 8.2 ± 3.0 ng/ml, and at 24 h after delivery to 0.53 ± 0.46 and 2.7 ± 1.0 ng/ml respectively. In women who underwent Caesarean section before labour onset \( (n = 11) \), plasma oestradiol and progesterone concentrations were 14.6 ± 6.2 and 44.5 ± 19.5 ng/ml respectively, and were not significantly different from those in the women at 1 h before vaginal delivery. Plasma oestradiol and progesterone concentrations at 1 and 4 h after Caesarean delivery decreased in the same manner as that observed in vaginal delivery: 6.8 ± 1.6 and 24.0 ± 17.2 ng/ml at 1 h after Caesarean section, 1.4 ± 0.8 and 11.2 ± 7.1 ng/ml at 4 h after Caesarean section respectively. All these values were not significantly different from those at the same time course of vaginal delivery.

To investigate the involvement of intrauterine tissues in the changes in the cGMP and NO metabolite concentrations during vaginal deliveries, the concentrations of cGMP and NO metabolites in the retroplacental blood obtained at the time of vaginal and Caesarean deliveries were compared. The NO metabolite concentration in the retroplacental blood obtained at the time of the vaginal deliveries \( (25.8 ± 16.3 \text{ } \mu M, \ n = 11) \) was significantly \( (P = 0.0345) \) lower than that obtained at the time of the Caesarean sections \( (55.5 ± 38.9 \text{ } \mu M, \ n = 13) \) (Figure 4). These values were 1.5-fold higher than those in the peripheral plasma obtained at delivery. The cGMP concentrations in the retroplacental blood obtained at the time of the vaginal deliveries and Caesarean sections were 1.7 ± 1.0 and 2.4 ± 1.1 nM, respectively. These values were slightly lower than those in the peripheral plasma. The ANP and BNP concentrations in the retroplacental blood obtained at the vaginal deliveries and Caesarean sections were both slightly lower than those in the peripheral blood (data not shown).

**Figure 2.** Relationship between concentrations of plasma guanosine 3',5'-cyclic phosphate (cGMP) and nitric oxide metabolites (NOx), atrial natriuretic peptide (ANP) or brain natriuretic peptide (BNP) concentrations in pregnant women in the third trimester and in women 1 h before and 1 h after vaginal deliveries. (A) Open triangles represent the plasma NO metabolite concentrations. A positive correlation was observed between plasma cGMP concentrations and NO metabolite concentrations \( (y = 20.917 + 3.694x, \ r = 0.37, \ P < 0.01, \ n = 84) \). (B) Open circles and closed triangles represent the plasma ANP and BNP concentrations respectively. No significant correlations were observed between plasma cGMP concentrations and either ANP or BNP concentrations.
Plasma NO metabolite concentrations during parturition

Discussion

The present study is the first to demonstrate that the plasma NO metabolite concentrations of women at vaginal deliveries were significantly lower than those of pregnant women in the third trimester and that this low NO metabolite concentration returned to the concentration in non-pregnant women by 24 h after delivery (Figure 1B). The plasma concentrations of cGMP, the second messenger of NO, at the vaginal deliveries were also significantly lower than those of the pregnant women in the third trimester (Figure 1A). A significant correlation was observed between the maternal plasma concentrations of NO metabolites and those of cGMP (Figure 2A). In contrast, no significant decreases in the plasma concentrations of NO metabolites and cGMP were observed in the pregnant women who underwent elective Caesarean section before the onset of labour (Figure 1A,B). Moreover, plasma NO metabolite and cGMP concentrations at 1–2 h before and 1 h after vaginal delivery were significantly lower than those of Caesarean delivery. These results indicate that the decreases in the plasma NO metabolite and cGMP concentrations were phenomena specific to vaginal delivery.

Some of the results of the present study on the changes in NO metabolites during Caesarean and vaginal deliveries conflict with the recent report by Okutomi et al. (1997) in which maternal nitrite concentration decreased after both Caesarean section and vaginal delivery. They measured only plasma nitrite concentrations. In contrast, we measured the summation of plasma nitrate and nitrite concentrations. Because NO is rapidly metabolized to both nitrate and nitrite in vivo, plasma concentrations of both nitrate and nitrite may represent more accurately the production of nitric oxide. On the other hand, in their report, the plasma nitrite concentration before vaginal delivery (160 pmol/mg protein, mean of 17 patients) is lower than that of Caesarean delivery (216 pmol/mg protein, mean of 13 patients). This result is consistent with the present finding that in the vaginal delivery group, plasma NO metabolite concentration at 1 h before delivery was lower than that of the Caesarean section group.

At present, it is difficult to distinguish the exact origin of the plasma cGMP during pregnancy. The systemic vascular wall is one of the most plausible candidates of the major source of plasma cGMP, since maternal circulating blood is surrounded by vascular wall, and both sGC and pGC are present in vascular wall (Nakao et al., 1992a; Magness and Zheng, 1996b). Indeed, when ANP or BNP was administered to patients with cardiovascular disorders, an increase in the plasma cGMP concentration was noted together with a reduction in blood pressure (Yoshimura et al., 1991). When hypertension was induced in pregnant ewes by norepinephrine infusion, the administration of sodium nitroprusside, an NO donor, partially offset its hypertensive effect (Wheeler et al., 1980). These findings suggest that the vascular tone is actually regulated by both sGC and pGC pathways. In the present study, however, the plasma concentrations of cGMP, NO metabolite, ANP and BNP showed no significant correlation with mean blood pressure, although mean blood pressure in pregnant women at 1 h before vaginal delivery was significantly

Figure 3. Relationship between the mean blood pressure (MBP) and plasma guanosine 3',5'-cyclic phosphate (cGMP), nitric oxide metabolites (NOx), atrial natriuretic peptide (ANP) or brain natriuretic peptide (BNP) concentrations in pregnant women in the third trimester and in women 1 h before and 1 h after vaginal delivery. (A) Closed circles and open triangles represent the plasma cGMP and NO metabolite concentrations, respectively. No significant correlation was observed between MBP and either plasma cGMP or NO metabolite concentrations. (B) Open circles and closed triangles represent the plasma ANP and BNP concentrations, respectively. No significant correlation was observed between MBP and either plasma ANP or plasma BNP concentrations.

Figure 4. Comparison between the nitric oxide metabolite (NOx) concentrations in the retroplacental blood obtained at vaginal delivery and those obtained at Caesarean section before the onset of labour. The NO metabolite concentrations in the retroplacental blood obtained at the vaginal delivery were significantly ($P = 0.0345$) lower than those obtained at Caesarean section.
pregnancy (Buhimschi et al., 1993; Weiner et al., 1994b; Yallampalli et al., 1994a; Buhimschi et al., 1995). The contractility of myometrial strip was reported to be attenuated by treatment with sodium nitroprusside, an NO donor (Yallampalli et al., 1994), although the myometrial NO activity in pregnant women in labour was higher than those not in labour (Ramsey et al., 1996). Ramsey et al. (1996) also reported that the myometrial endogenous NO activity was less than one-fifth of that of placental trophoblast, suggesting that contribution of myometrial NOS to total NO production in the uterus is small. On the other hand, Bansal et al. (1997) reported that iNOS expression in the human myometrium is increased during pregnancy, and declines towards term or with labour. Francoual et al. (1995) reported that the urinary cGMP concentrations decreased during uterine contraction. Thus, the uterine myometrium is another possible source of maternal plasma cGMP. However, it is very difficult to estimate the degree of the contribution of pregnant myometrium to the concentrations of NO metabolites and cGMP in peripheral plasma. To examine the contribution of myometrium, we collected the retroplacental blood at the time of both vaginal and Caesarean deliveries. Retroplacental blood may include substances secreted from placenta, decidua and myometrium, which have been reported to express NOS (Buhimschi et al., 1996; Dong et al., 1996b; Purcell et al., 1997). The cGMP concentration in the retroplacental blood obtained at the vaginal deliveries was 77.3% of that in the peripheral plasma, while the NO metabolite concentration in the retroplacental blood at the vaginal deliveries was 146% of that of the peripheral plasma obtained at the same time. The NO metabolite concentration in the retroplacental blood obtained at the time of the Caesarean sections before the onset of labour was 161% of that in the peripheral plasma, and was significantly ($P < 0.05$) higher than that obtained at the vaginal deliveries. These findings suggest that a substantial amount of NO metabolites is released from intrauterine tissues including placenta, decidua and myometrium to the systemic circulation and that the change in the NO activity in these tissues might at least partly contribute to the changes in the concentration of the peripheral plasma NO metabolites during delivery. The time between fetal delivery and placental delivery in Caesarean section is less than that in vaginal delivery. In the present study, we did not compare the oxygen tension in retroplacental blood. Thus, the possibility cannot be excluded that the oxygen tension of retroplacental blood collected from vaginal delivery may be lower than that collected from Caesarean section, and that this might affect the NO/cGMP concentrations in the samples. This point must be elucidated by future study.

It is reported that oestrogen (Weiner et al., 1994) and progesterone (Yallampalli et al., 1994b; Liao et al., 1996) have great impact on the plasma NO metabolite concentration. To examine the effects of steroid hormones on the changes in plasma NO metabolite concentrations before and after delivery, we measured oestradiol and progesterone concentrations in the plasma of pregnant women 1 h before vaginal delivery at term and at 1 and 4 h after vaginal deliveries. In the present study, plasma concentrations of oestradiol and progesterone at 1 h before Caesarean section and at 1 and 4 h after Caesarean section were not different from those at the same time course of vaginal delivery, although the plasma NO metabolite concentrations at 1 h before and 1 h after vaginal delivery were significantly lower than those in Caesarean section. Moreover, the plasma NO metabolite concentration in the vaginal delivery group was significantly lower than that in Caesarean section group at 1 h before vaginal delivery when the plasma concentrations of oestradiol and progesterone were still elevated in both groups. These facts suggest that the decrease in plasma NO metabolite concentration observed in vaginal delivery is not related with changes in steroid hormone concentrations. The difference between the two groups was the presence of labour, which is associated with an increase in plasma prostaglandin E2 and F2α concentrations. Prostaglandin E2 is reported to inhibit interleukin-1β-induced NO production by rat uterine tissue (Dong et al., 1996a). Thus, it is interesting to speculate that the increased prostaglandin E2 production during labour suppresses NO synthesis in the
uterus, offsets the uterine relaxation and finally results in the acceleration of uterine contraction. However, further investigation of the direct effect of prostaglandin E2 on the NO synthesis in intrauterine tissues is required to confirm this hypothesis.

In the present study, the plasma cGMP concentrations paralleled the NO metabolite concentrations during the course of pregnancy; both were increased in the second and third trimester of pregnancy, decreased at vaginal delivery and remained low until 4 h after delivery. Subsequently, the plasma cGMP concentrations remained low until 48 h after delivery, although the plasma NO metabolite concentrations returned to concentrations similar to those of pregnant women in the third trimester by 24 h after delivery. In addition, the cGMP concentration in the retroploental blood was lower than that in the peripheral blood, although the retroploental NO metabolite concentration was higher than that in the peripheral blood. These results suggest that the local sGC activity in the uterus is temporarily suppressed during parturition. However, further investigations are required to confirm this possibility.

In summary, we have demonstrated that the maternal plasma concentrations of cGMP and NO metabolites decreased during spontaneous vaginal deliveries but not during Cesarean deliveries. These changes were independent of the ANP and BNP concentrations. The concentrations of NO metabolites in the retroploental blood obtained at the Cesarean sections were higher than those in the retroploental blood at the vaginal deliveries. These results suggest that the maternal NOS activity in the intrauterine tissues including the local uterine vessels, myometrium, decidua and placenta are down-regulated during parturition. Further investigation of the mechanism of the down-regulation of NOS in these tissues may provide information clarifying the exact roles of the NO–sGC–cGMP pathway in the initiation of parturition.

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Plasma NO metabolite concentrations during parturition
H. Nanno et al.


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