Nitric oxide synthesis is increased after dehydroepiandrosterone sulphate administration in term human pregnancy

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The purpose of this study was to evaluate the role of nitric oxide in the vasodilative effect of dehydroepiandrosterone sulphate (DHEA-S) in term pregnant women. Circulating nitrite, nitrate and oestriadiol concentrations were measured on 10 normal full-term pregnant women before (-30 min) and after (10, 30, 60, 90 and 120 min) administration of a 200 mg i.v. dose of DHEA-S dissolved in 20 ml of 5% dextrose (DHEA-S group). Ten normal full-term pregnant women received 20 ml of 5% dextrose as controls (control group). Maternal blood pressure and heart rate were also recorded. The median oestriadiol concentration increased significantly after the infusion in DHEA-S group (P < 0.001), whereas there was no significant change in plasma oestriadiol in the control group. In the DHEA-S group, plasma circulating nitrite and nitrite increased significantly at 10 and 30 min after DHEA-S administration respectively (P < 0.05). In the control group, there was no change in plasma nitric oxide (NO) metabolites. No change was found in heart rate or mean arterial blood pressure in the control or DHEA-S groups. These results suggest there may be a link between increased NO and increased oestriadiol after DHEA-S injection but their peak values did not coincide. Both may be associated with vasodilation in term pregnant women.

Key words: dehydroepiandrosterone sulphate/nitric oxide/oestriadiol/term pregnancy/vasodilative effect

Introduction

Nitric oxide (NO) is a potent vasodilator produced coincident with the metabolism of L-arginine to L-citrulline by nitric oxide synthase (NOS) in endothelial cells (Palmer et al., 1988; Nathan, 1992). NOS activity (enzyme activity) in maternal tissues rises early in pregnancy and an increased NOS plays a role in adaptations of vascular and gastrointestinal muscle to pregnancy in guinea pig (Weiner et al., 1994). Messenger RNA for NOS is expressed in a variety of cell types in the non-pregnant human uterus and different forms of constitutive NOS are present in human endometrium and myometrium (Cameron and Campbell, 1998). Further locally synthesized NO may play a role in the control of both the uterine vascular bed and myometrial contractility (Buhimschi et al., 1995; Telfer et al., 1995; Garfield et al., 1998). Ovarian steroid hormones regulate inducible NOS expression in both leukocytes and several types of uterine cells in mice and NO plays a role in uterine cyclicity and preparation for pregnancy (Huang et al., 1995; Dong et al., 1996, 1998). An earlier study showed that deprivation of NO by use of competitive inhibitor led to fewer ovulations, reduced accumulation of nitrate, a decreased neutrophil count in the theca of pre-ovulatory follicles, and reduced oestradiol secretion, while progesterone release remained unaffected in rats (Bonello et al., 1996). NO synthesis increases during pregnancy (Yallampalli et al., 1993, 1998), and inhibition of NO synthesis has been shown to affect fetal growth in rats (Yallampalli and Garfield, 1993; Diket et al., 1994). Recent studies show that NO synthesis is an important regulator of uteroplacental blood flow (Rosenfeld et al., 1996) and that maternal and fetal NO synthesis are decreased in pregnancies with small for gestational age infants compared with appropriate for gestational age infants (Hata et al., 1998a).

Dehydroepiandrosterone sulphate (DHEA-S) of both maternal and fetal origin is converted to oestriadiol in the placenta. The metabolic clearance rate of DHEA-S in normal pregnant women is markedly elevated compared with that of non-pregnant subjects (Gant et al., 1971). The blood oestriadiol concentration rapidly increases after an i.v. injection of DHEA-S to women in late pregnancy (Tulchinsky et al., 1976). DHEA-S induces a significant decrease in the uterine artery pulsatility index (PI), which suggests a possible decrease in uterine vascular impedance in term pregnancy (Hata et al., 1995). Oestriadiol induces notable uterine vasodilation in non-pregnant sheep (Killam et al., 1973; Resnik et al., 1974; Van Buren et al., 1992; Rosenfeld et al., 1996). Van Buren et al. (1992) showed that oestriadiol-induced increases in uterine blood flow in non-pregnant sheep are mediated mainly by NO. A more recent study also found that placental blood flow increased markedly after DHEA-S injection using power Doppler imaging in term human pregnancy (Hata et al., 1998b).

In this study it is postulated that a rapid decrease in uterine arteryvascular impedance after bolus injection of DHEA-S may be mediated by a rapid increase of NO after an increase of oestriadiol.

Materials and methods

Twenty normal pregnant women were recruited (10 normal controls and 10 treated with DHEA-S) ranging from 37 to 42 weeks gestation.
Table I. Clinical characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>DHEA-S group</th>
<th>Control group</th>
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<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
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<tr>
<td>Maternal age (years)</td>
<td>27.2 ± 2.8</td>
<td>27 ± 3.3</td>
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<tr>
<td>Gastational age at examination (weeks)</td>
<td>38.7 ± 1.5</td>
<td>38.6 ± 0.9</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>77.2 ± 9.1</td>
<td>77.5 ± 5.0</td>
</tr>
<tr>
<td>Birth age (weeks)</td>
<td>39.8 ± 1.4</td>
<td>39.7 ± 0.8</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3152 ± 346</td>
<td>3052 ± 219</td>
</tr>
<tr>
<td>Apgar score at 1 min</td>
<td>8.8 ± 0.4</td>
<td>8.7 ± 0.5</td>
</tr>
<tr>
<td>Blood pH of umbilical artery</td>
<td>7.28 ± 0.05</td>
<td>7.27 ± 0.04</td>
</tr>
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DHEA-S = dehydroepiandrosterone sulphate.
Data are presented as mean ± SD.

The clinical characteristics of subjects in both groups are shown in Table I. These women were non-smokers with no evidence of maternal complications of pregnancy or substance abuse. Gestational age was estimated from the first day of the last menstrual period and confirmed by first-trimester and early second-trimester ultrasound examinations (crown–rump length, biparietal diameter and femur length measurements). Birth weights in all babies were within normal ranges (between the 10th and 90th percentile) of the standard growth curve for the Japanese population (Sato et al., 1982). All babies were delivered vaginally. No neonates had congenital malformations or genetic disorders. The study was approved by the local ethical committee of Shimane Medical University and standardized informed consent was obtained from each patient.

At 10.00 h after overnight fasting for 15 h (Zaidi et al., 1995), the subjects were examined in the semi-Fowler’s position (the position in which the head of the patient’s bed is raised about 10 inches). Maternal blood sampling to measure plasma oestradiol concentrations, nitrite and nitrate concentrations and recordings of blood pressure and heart rate were performed using impedance cardiology (NCCOM3-R7; Biomed Medical Instruments, Irvine, CA, USA) before (–30 min) and after administration (10, 30, 60, 90, 120 min) of a 200 mg i.v. dose of DHEA-S dissolved in 20 ml of 5% dextrose for the experimental group (DHEA-S group) or a 20 ml dose of 5% dextrose for the control group (control group). A 200 mg i.v. dose of DHEA-S is usual for cervical ripening (Sasaki et al., 1982; Chwalisz and Garfield, 1998). After centrifugation of these blood specimens [3000 revolutions (1000 g)/min for 10 min], plasma samples were stored at –80°C until analysis of circulating nitric oxide metabolite. Plasma oestradiol concentrations were measured immediately after centrifugation by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (IMMULYZE Estradiol®; Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma nitrite and nitrate concentrations were measured using methods adapted from those published (Misko et al., 1993). Briefly, plasma was filtered through a Centrifon 10 (Amicon, Beverly, MA, USA) for 1 h at 4°C at 3000 g to remove contaminating haemoglobin. A 50 µl sample of plasma was incubated with 40 µmol/l NADPH and 14 µlU of nitrate reductase (from Aspergillus niger; Sigma, St Louis, MO, USA) in a final volume of 50 µl of 20 mmol/l Tris, pH 7.6. The reaction was terminated after 5 min at 20°C by addition of 10 µl of 2.3-diaminonaphthalene (0.05 mg/ml in 0.62 mol/l HCl). After a 10 min incubation at 20°C, 5 µl of 2.8 N NaOH was added and the intensity of the fluorescence was measured using a Hitachi 850® Fluorescence spectrophotometer (Hitachi Co Ltd, Tokyo, Japan). Nitrite standards (>98% pure; Sigma) were routinely made fresh, dissolved in double-deionized water, and kept on ice prior to use. Nitrite was detectable at a concentration of 10 pmol/ml.

Statistical analysis for comparison of maternal age, gestational age at examination, mean arterial pressure, birth age, birth weight, Apgar score and blood pH of umbilical artery between both groups were done using an unpaired t-test. Data for experimental values for oestradiol and nitrite and nitrate were analysed by Kruskal–Wallis one-way analysis of variance by ranks, and multiple comparisons. P < 0.05 was considered significant.

Results

There were no significant differences in maternal age, gestational age at examination, mean arterial pressure, birth age, birth weight, Apgar score at 1 min and blood pH of umbilical artery in both groups (Table I). There was no significant change in maternal blood pressure or heart rate nor did the women have side-effects (e.g. discomfort, shock) in either group. The median oestradiol increased significantly after the administration of DHEA-S (DHEA-S group, Control group, P < 0.001) (Table II). In the DHEA-S group, plasma circulatory nitrate and nitrite increased significantly at 10 and 30 min after DHEA-S administration respectively (P < 0.05) (Table III). In the control group, there was no change in plasma nitric oxide metabolites.

Discussion

DHEA-S is converted to oestrogen in the placenta and the maternal blood oestradiol concentration rapidly increases after administration.
i.v. injection of DHEA-S in term pregnant women (Tulchinsky et al., 1976). In pregnant ewes the maximum concentration of oestrogens occurred ~30 min after the administrations of DHEA-S, and uterine blood flow increased ~90 min after the maximum concentration of oestrogens (Pupkin et al., 1975). In our previous investigation (Hata et al., 1995), bolus injection of DHEA-S in full-term pregnant women reduced the PI of the uterine artery by ~36% after 10 min, and a lowering of the resistance in the vascular bed in uterine circulation after DHEA-S administration was suggested (Hata et al., 1995). Moreover, increased power Doppler enhancement of the placenta after DHEA-S injection was evident in term pregnancy, and returned to the baseline imaging within 60 min (Hata et al., 1998b). Oestrogen production by the fetoplacental unit and uteroplacental blood flow play important roles in the preservation of pregnancy and in the outcome of the fetus. However, very little is known about the mechanism of the vasodilative effect of DHEA-S during pregnancy. Therefore, this study was designed to investigate the interrelation between oestrogen synthesis by the fetoplacental unit and the mechanism of the vasodilative effect of DHEA-S in term pregnant women.

In this investigation, there were no significant changes in maternal blood pressure and heart rate after DHEA-S administration, regardless of the vasodilative effect of DHEA-S. A previous report showed that maternal cardiac output increased by 20% and mean increase in stroke volume was 25% after DHEA-S administration (Hata et al., 1996). One possible explanation is that DHEA-S might increase cardiac output and stroke volume, without altering blood pressure and heart rate, by a change in the systemic vascular resistance.

The effect of oestrogens on NO metabolism has already been investigated (Rosenfeld et al., 1996; Cinicelli et al., 1998). In post-menopausal women, plasma concentrations of NO metabolites 24 h after transdermal oestradiol administration were significantly higher than baseline concentrations (Cinicelli et al., 1998). In non-pregnant ewes, acute oestrogen-induced increases in the uterine blood flow were associated with NO-dependent increases in cyclic GMP synthesis (Rosenfeld et al., 1996). In this investigation, bolus injection of DHEA-S in full-term pregnant women increased significantly the NO metabolites at 10 and 30 min respectively. However, nitrate and nitrite concentrations returned to baseline (i.e. no statistical difference over pre-injection) at 60 min after injection, while the peak oestradiol concentration occurred at 60 min. If it is suggested that the sequence of events is DHEA-S converted to oestradiol which stimulates production of NO, then one would expect the peak of NO metabolites (nitrate and nitrite) to occur after the oestradiol peak. The reason for this discrepancy between oestradiol peak and the peak of NO metabolites is currently unknown. One possibility is that DHEA-S may alter peak nitrate and nitrite concentrations by a mechanism other than via oestradiol. In our previous investigation (Hata et al., 1995), uterine artery pulsatility index decreased from baseline by 26% after 5 min, and the mean reduction was 36% after 10 min and 15% after 30 min. The pulsatility index returned to the baseline value 60 min later. Moreover, increased power Doppler enhancements of the placenta after DHEA-S injection were evident in each case studied; however, these power Doppler enhancements returned to the baseline imaging within 60 min (Hata et al., 1998b).

These results suggest that a rapid decrease in uterine artery vascular impedance (Hata et al., 1995) after bolus injection of DHEA-S should be mediated by a rapid increase of NO following an increase of oestradiol. This improved uterine perfusion after DHEA-S administration might play an important role for the preservation of the pregnancy and in the outcome of the fetus. It seems that this vasodilative effect of DHEA-S is expected to be a new possible therapeutic agent in high-risk fetuses with decreased uteroplacental blood flows. However, the direct effect of DHEA-S on uterine vascular tone is still unknown, and further study is needed to clarify the direct effect of DHEA-S on uterine circulation during pregnancy. Caution should be exercised since DHEA-S (Sasaki et al., 1982), oestradiol (Gordon and Calder, 1977; Allen et al., 1989; Magann et al., 1995), and NO (Chwalisz et al., 1997; Thomson et al., 1997; Thomson et al., 1998), can each produce effective ripening of the pregnant human cervix. Recently, NO donors have been shown to improve uteroplacental blood flow in severe fetal growth restriction and pre-eclampsia ( Ramsay et al., 1994; Cacciato et al., 1998).

Further study is needed to clarify the relationship between oestradiol production and NO synthesis after DHEA-S injection in human pregnancy.

References
Dehydroepiandrosterone sulphate and nitric oxide


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