Nitric oxide in the human uterus

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Nitric oxide (NO) plays a crucial role in many biological systems. Recent evidence indicates that NO is found in the human uterus. During pregnancy, it is produced in the placenta, the decidua and the myometrium. In the nonpregnant state, nitric oxide synthase, the enzyme that catalyses the production of NO, has been identified in both the myometrium and the endometrium. Potential roles for NO in the human uterus are speculative, but include vaso-dilatation (both before implantation, and in the uteroplacental and systemic circulation during pregnancy), inhibition of platelet activation during menstruation, and suppression of myometrial contractility during pregnancy. Nitric oxide may also be involved in uterine pathology. Excessive NO production by the uterus during menstruation could lead to menorrhagia. During pregnancy, a change in NO production may be implicated in pre-eclampsia, and animal studies have shown that inhibition of NO production leads to intrauterine growth retardation. If a role for NO is confirmed in these various uterine conditions, pharmacological modification of NO activity may lead to novel therapeutic applications. However, these notions are still conjectural, and extensive work is required before such treatments can be introduced into clinical practice.

In 1987, the gas nitric oxide (NO) was shown to possess the physical and pharmacological properties of Furchgott’s endothelium-derived relaxing factor (EDRF). It is now recognized that this ubiquitous molecule plays a crucial role in a host of biological systems (Furchgott and Zawadzki, 1980; Palmer et al., 1987; Moncada and Higgs, 1993; Anggard, 1994). Nitric oxide is a smooth muscle relaxant in the vascular system, lungs and the gastrointestinal tract. It is a neurotransmitter in the brain and peripheral nervous system, and is involved in activities as diverse as memory and insulin release from islet cells. Furthermore, NO is produced by macrophages and other cells of the immune system in large quantities as part of the defence mechanism against foreign organisms and malignant cells.

Given the pervasive nature of NO, it is not surprising that it is produced by and intimately involved in the functions of the female reproductive tract. For example, it may play a role in regulating blood loss during, and preventing the development of intrauterine adhesions following, menstruation. In pregnancy, NO may be involved in the regulation of blood flow through the fetoplacental circulation and it may be pivotal for the maintenance of myometrial quiescence.

This review aims to examine the sites of production and the function of NO in the human uterus. In each situation in which a role for NO has been proposed in women, results will be supported by studies using animal models.

Synthesis of nitric oxide

Nitric oxide is derived from L-arginine by the action of nitric oxide synthase (NOS; Fig. 1). This enzyme exists in three main forms (Table 1). A constitutive, calcium-dependent form is found in endothelial cells (eNOS) and neurones (bNOS), while a calcium-independent, inducible form (iNOS) is present in macrophages, neutrophils and other cell types, including vascular smooth muscle. These three forms of NOS are encoded by separate genes, located on chromosomes 7 (enOS), 12 (bnOS) and 17 (iNOS) (see Knowles and Moncada, 1994 for review). The deduced amino acid sequences of the different NOS forms share 50–57% similarity both between constitutive and inducible NOS and between species (mouse, rat and human).

Whereas the action of constitutive NOS releases small quantities of NO in response to a range of stimuli, such as acetylcholine, bradykinin, endothelins and shear stress, activation of iNOS by factors including lipopolysaccharide and gamma interferon results in a marked amplification of NO activity.

The production of NO by the action of NOS can be inhibited competitively by analogues of L-arginine, including Nω-monomethyl-L-arginine (L-NMMA) and Nω-nitro-L-arginine methyl ester (L-NAME) (Fig. 1). These agents have provided powerful and specific tools to determine the role of NOS in physiology and pathology, and their use has been exploited therapeutically for the management of septic shock. The effects of L-NMMA and L-NAME on the synthesis of NO can be attenuated by large doses of L-arginine when endogenous supplies of this amino acid are limited.

Nitric oxide has a short half life. It is rapidly oxidized to nitrite (NO2−) both in vivo and in vitro. Measurement of this oxidation product has offered a practical and reliable assay for NO, and can be performed using the Griess reaction (Green et al., 1982). Briefly, nitrite reacts with the Griess reagent (one part 0.1% naphthylethylenediamine hydrochloride and one part 1% sulfanilamide in 5% concentrated H3PO4) to form a purple azo dye, the absorbance of which at 546 nm can be detected by UV spectrophotometry and compared with a known standard. Nitrate can be converted to nitrite before analysis by reduction in a cadmium column. Nitric oxide also reacts with superoxide...
anions ($O_2^-$) to form peroxynitrite (ONOO$^-$), which in turn is oxidized to nitrate ($NO_3^-$). Thus the effects of NO are reduced in the presence of compounds that generate superoxide anions (such as xanthine and xanthine oxidase), and they are potentiated in the presence of superoxide dismutase (SOD), a widely located enzyme that inactivates superoxide anions.

The cellular actions of NO are mediated by causing guanylate cyclase to increase cyclic GMP concentrations. In smooth muscle cells, cGMP activates cGMP-dependent protein kinase, leading to a reduction in intracellular Ca$^{2+}$ concentrations and relaxation. Quantitation of cGMP concentrations therefore provides another, albeit nonspecific, measure of NO activity.

Measurement of nitric oxide activity

Direct measurement of NO is difficult. Some studies have examined nitrite and nitrate concentrations in urine or serum. However, results have to be interpreted with caution unless specimens are collected after fasting.

Nitric oxide synthase activity can be assessed directly by measuring the conversion of L-arginine to L-citrulline (Fig. 1). In addition, the distribution of protein and mRNA for the different NOS forms can be determined in vitro by immunocytochemistry and in situ hybridization.

The knowledge that NOS exhibits diaphorase activity suggests that the distribution of NOS might be determined by localizing NADPH diaphorase (which produces a blue precipitate from nitroblue tetrazolium in the presence of NADPH). However, although NOS co-localizes with NADPH diaphorase activity, NADPH diaphorase activity is not all NOS.

**Nitric oxide in the nonpregnant uterus**

**Localization**

Nerve fibres synthesizing nitric oxide have been demonstrated in the uteri of nonpregnant rats and mice by both co-localization with NADPH diaphorase, and by immunoreactivity using antibodies raised against pig and rat bNOS, and iNOS (Papka and McNeill, 1992; Schmidt et al., 1992; Shew et al., 1993; Grozdanovic et al., 1994; Huang et al., 1995; Papka et al., 1995; Suburo et al., 1995). The density of nerve fibres was greatest in the uterine cervix, although some nerve fibres were also present in the body of the uterus. Detailed studies to identify the source of

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**Table 1. The location and properties of the different forms of nitric oxide synthase (NOS)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Name</th>
<th>Ca$^{2+}$ dependency</th>
<th>Location</th>
<th>Gene location</th>
</tr>
</thead>
<tbody>
<tr>
<td>bNOS</td>
<td>NOS-I</td>
<td>Ca$^{2+}$ dependent</td>
<td>Brain</td>
<td>Chromosome 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NANC neurones</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>NOS-II</td>
<td>Ca$^{2+}$ independent</td>
<td>Macrophages</td>
<td>Chromosome 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neutrophils</td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td>NOS-III</td>
<td>Ca$^{2+}$ dependent</td>
<td>Vascular smooth muscle</td>
<td>Chromosome 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vascular endothelium</td>
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NANC: non-adrenergic, non-cholinergic
uterine-projecting neurones suggested that the nerves were autonomic (probably mainly parasympathetic) and sensory (Papka et al., 1995). Some evidence also indicates the presence of NOS in rat and mouse uteri in non-neuronal cells; bNOS-like immunoreactivity was demonstrated in glandular epithelium of rat endometrium, while iNOS was seen in adluminal epithelium (Schmidt et al., 1992; Suburo et al., 1995).

NADPH diaphorase activity was demonstrated in the uterus of nonpregnant humans (Telfer et al., 1995; Yoshida et al., 1995) where sections of endometrium and myometrium were taken from women undergoing hysterectomy. NADPH diaphorase activity was identified in all sections, and the greatest intensity of staining was in endometrial glandular epithelium and myometrial blood vessels throughout the menstrual cycle. Nitric oxide synthase-like immunoreactivity was also demonstrated in human uterine sections (Telfer et al., 1995). A polyclonal antibody raised against bovine eNOS was used and NOS-like immunoreactivity was detected in blood vessels and in some endometrial glandular epithelia in the secretory phase of the cycle (Fig. 2). Nitric oxide synthase-like immunoreactivity was also seen in endometrial stroma. Endothelial NOS-like immunoreactivity was not found in myometrial smooth muscle cells. Messenger RNA was detected in endometrial glandular epithelium, stroma, myometrium and myometrial blood vessels by in situ hybridization using riboprobes made from NOS endothelial cDNA (Telfer et al., 1995).

**Fig. 2.** Nonpregnant human endometrium stained with an antibody to nitric oxide synthase found in endothelial cells (eNOS). Staining is seen predominantly in glandular epithelium and blood vessels. Scale bar represents 10 µm.

The mechanism of menstruation: a role for nitric oxide?

The demonstration of NOS in the human uterus suggests that it may have a role in uterine physiology. Vasodilatation and the inhibition of platelet aggregation are fundamental processes for normal endometrial function. Local vasodilatation within the uterus is essential for the initiation of successful implantation and placentation, and the production of a powerful anti-aggregatory agent in basal endometrium is of crucial evolutionary advantage in menstruating species for preventing the formation of intrauterine adhesions.

Nitric oxide may also play a part in the pathogenesis of excessive menstrual bleeding (menorrhagia), a condition that presents a considerable burden to health services in developed countries, and which, in the United Kingdom, is one of the most common reasons for patient referral from the community to hospital specialists (Coulter et al., 1989). The precise cause of menorrhagia is unclear, but the excessive local production of vasodilator agents such as prostacyclin and prostaglandin E₂ is thought to be important (Smith et al., 1981; Cameron et al., 1987).

**Nitric oxide in the pregnant uterus**

**Release of nitric oxide and localization of nitric oxide synthase**

In pregnancy, most animal models have focused on the possible role of NO in the control of myometrial contractility. For example, a variety of studies have shown that uterine tissues from pregnant rats can generate large amounts of nitrite and nitrate, and that the uterus can convert l-arginine to l-citrulline (Yallampalli et al., 1993a, b).

Studies in humans have concentrated on the demonstration of NO-generating systems in the placenta. In the normal placenta
at term, eNOS-like immunoreactivity is localized to vascular endothelium and syncytiotrophoblast (Myatt et al., 1993a; Buttery et al., 1994). This distribution was also seen for mRNA encoding eNOS using in situ hybridization (Conrad et al., 1993). Similarly, in tissue collected in the first trimester of pregnancy after spontaneous miscarriage, NOS was indicated in syncytiotrophoblast, but not cytotrophoblast, using NADPH diaphorase activity and immunohistochemistry with an antibody raised against eNOS (Eis et al., 1995). These studies suggest that the expression of NOS may not occur until after the differentiation of cytotrophoblast to syncytiotrophoblast.

In placental blood vessels, eNOS-like immunoreactivity has been demonstrated in umbilical cord, chorionic plate and stem villous vessels (Myatt et al., 1993a). Endothelial cells from umbilical cord and stem villous vessels have also been shown to release NO in culture (Myatt et al., 1993b).

Unlike the placenta, maternal decidua do not appear to be a major site of NO production at term. When the conversion of L-[^14]C]arginine to L-[^14]C]citrulline was used as a measure of NO production at term, eNOS-like immunoreactivity is localized to vascular endothelium and syncytiotrophoblast (Myatt et al., 1993a; Buttery et al., 1994). This distribution was also seen for mRNA encoding eNOS using in situ hybridization (Conrad et al., 1993). Similarly, in tissue collected in the first trimester of pregnancy after spontaneous miscarriage, NOS was indicated in syncytiotrophoblast, but not cytotrophoblast, using NADPH diaphorase activity and immunohistochemistry with an antibody raised against eNOS (Eis et al., 1995). These studies suggest that the expression of NOS may not occur until after the differentiation of cytotrophoblast to syncytiotrophoblast.

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Unlike the placenta, maternal decidua do not appear to be a major site of NO production at term. When the conversion of L-[^14]C]arginine to L-[^14]C]citrulline was used as a measure of NOS, low activity was found in the placental bed compared with the placenta itself (Morris et al., 1993).

Summary

In the third trimester of pregnancy, the main source of NO in the human uterus is probably:

- the vascular endothelium of large placental vessels
- the syncytiotrophoblastic shell

The potential functions of NO during pregnancy are to:

- limit platelet aggregation at the interface between maternal and fetal circulations
- regulate placental blood flow
- suppress contractions of the underlying myometrium

The role of nitric oxide in the physiology of normal pregnancy

Effect of nitric oxide on systemic vascular resistance.

Endogenous production of NO is thought to play an important role in nonpregnant women in controlling systemic blood pressure by maintaining resistance vessels in a constant state of relaxation. Since systemic vasodilatation is known to occur in pregnancy, it has been suggested that, in concert with other vasodilator agents such as prostacyclin, NO may be implicated in the mechanism of pregnancy-induced vasodilatation. In guinea-pigs, eNOS is upregulated in a variety of tissues during pregnancy (Weiner et al., 1989, 1991). Recent studies have indicated that this upregulation may occur after oestradiol-mediated induction of constitutive eNOS (see below). However, limited information in human pregnancy does not support a similar increase in NO activity. In a recent study in vitro, small arteries isolated from the subcutaneous fat of women undergoing either Caesarean section or gynaecological surgery were mounted on a myograph (McCarthy et al., 1994). While L-NNAME attenuated acetylcholine-induced relaxation in arteries from both pregnant and nonpregnant women, no differences were observed between pregnant and nonpregnant vessels. Furthermore, the response to sodium nitroprusside (SNP, a donor of NO)-induced vasodilatation did not differ in vessels from either group of women.

However, further studies are required on larger resistance and capacitance vessels from different vascular beds before a role for NO can be excluded in the circulatory adaptation to normal pregnancy.

Nitric oxide and the uteroplacental circulation.

Endogenous NO production appears to contribute to the maintenance of vasodilatation in the human fetoplacental circulation. The NO donors glycyltrinitrate (GTN) and S-nitroso-N-acetylpenicillamine (SNAP) reduced the pressure required to perfuse preconstricted term placental cotyledons in vitro (Myatt et al., 1991). The observed reduction in perfusion pressure was diminished by both methylene blue (an inhibitor of guanylate cyclase) and pyrogallol (which generates superoxide anions to inactivate NO). In addition, when NO production was inhibited or its effects reduced by methylene blue, L-NMMA or L-NAME, the resting pressure required to perfuse the cotyledons was increased, indicating that endogenous NO production may contribute to the maintenance of placental vasodilator tone (Myatt et al., 1992). Recent studies demonstrated that SNAP is more potent than prostacyclin in the relaxation of human umbilical and chorionic plate arteries (Chaudhuri et al., 1993).

It is likely that release of NO from umbilical vessels in vivo is stimulated by blood flow itself, to provide a mechanism to maintain the fetoplacental circulation in a state of relative vasodilatation (Rubayani et al., 1986; Van De Voorde et al., 1987; Chaudhuri et al., 1991, 1993). Furthermore, the administration of exogenous NO has been shown to increase placental blood flow. Flow through uterine and umbilical arteries can be measured semiquantitatively using Doppler ultrasound to describe the ‘resistance index’, a ratio of flow velocity during systole to flow velocity during diastole. Infusion of 20 µg GTN min–1 reduced the resistance index (hence improved blood flow) both in patients undergoing termination of pregnancy at 8–10 weeks, and in women with reduced placental blood flow at 24–26 weeks gestation (Ramsay et al., 1994). In contrast, infusion of 150 ng prostacyclin min–1 to women with reduced placental blood flow at 24–26 weeks of gestation had no effect on blood flow.

Thus, NO is a powerful dilator of the fetoplacental circulation, where it is produced locally to maintain vessel relaxation. When blood flow is reduced, exogenous NO appears to increase blood flow. However, in the absence of any evidence to suggest that the uterine or fetoplacental circulation is more sensitive than the systemic circulation to the vasodilatory effects of NO, administration of exogenous NO might be expected to result in a change in maternal blood pressure, unless other homeostatic mechanisms come into play. The concept that fetoplacental blood flow is reduced as a result of a reduction in endogenous NO production is probably oversimplified, for it may be that in some pathological states that are known to be associated with decreased placental perfusion (such as pre-eclampsia and intrauterine growth retardation), the generation of endogenous NO is upregulated as a compensatory mechanism to maintain homeostasis (Cameron et al., 1993).

Nitric oxide inhibits myometrial contractions.

Nitric oxide is a powerful myometrial relaxant. During pregnancy, the placenta may provide an abundant source of NO to relax the underlying uterine smooth muscle. In addition, a reduction in endogenous
NO production at term could be implicated in the mechanism of parturition.

Several studies have examined the effects of NO, L-arginine and cyclic GMP on spontaneous or induced contractions in isolated strips of myometrium from pregnant rats. Both NO gas and SNP-derived NO inhibit spontaneous myometrial contractions at term (Yallampalli et al., 1993a). Contractions are also inhibited by 8-bromo cyclic GMP, a cell-permeable analogue of cGMP (Yallampalli et al., 1993b). Spontaneous myometrial activity is inhibited by L-arginine, as are contractions stimulated by the muscarinic agonist carbachol, but not those caused by potassium chloride, which induces voltage-dependent contractions after membrane depolarization (Izumi et al., 1993). The inhibitory effects of L-arginine on myometrial relaxation were abolished by 1-NAME and methylene blue, confirming that the observed effects were mediated specifically by NO (Yallampalli et al., 1993a). In other studies, the spontaneous decline in myometrial contractions seen when myometrium from oestrogen-treated rats was examined in vitro was inhibited by 1-NAME, suggesting that this reduction in uterine contractility is caused by NO (Franchi et al., 1994). Taken together, these data show that NO is a powerful inhibitor of myometrial contractility and that this inhibition is effected predominantly by the production of cyclic GMP.

The role of L-arginine in myometrial contractions was also studied in women using tissue obtained at Caesarean section. In keeping with the animal experiments, spontaneous myometrial contractions were inhibited by L-arginine (0.3 mmol l\(^{-1}\)), and predictably, this inhibitory effect was prevented by the arginine analogue L-\(N^\text{\textsuperscript{o}}\)-nitro-arginine (Izumi et al., 1993). Inhibition of spontaneous myometrial contractions was also achieved with each of GTN (10\(^{-4}\) mol l\(^{-1}\)), SNP (10\(^{-6}\) mol l\(^{-1}\)), diethylenetriamine/NO (10\(^{-7}\) mol l\(^{-1}\)) and streptozotocin (10\(^{-5}\) mol l\(^{-1}\)) under UV radiation (Buhimschi et al., 1995; Lee and Chang, 1995; Norman et al., 1995).

Preliminary studies have started to address whether exogenous NO can be administered to suppress myometrial contractions in vivo with the intention of exploiting this action therapeutically for the treatment of preterm labour. A study investigated the effect of GTN patches (Deponit 10\(^{3}\)) in 20 episodes of preterm labour in 13 women (Lees et al., 1994). Only one woman delivered during the treatment period, and in this case delivery was attributed to cervical incompetence. Although the authors suggested that NO donors might prevent preterm delivery, the results must be interpreted with caution as the study was uncontrolled.

The mechanism whereby NO suppresses myometrial contractility in vivo was evaluated by administering an intravenous infusion of GTN (20 \(\mu\)g min\(^{-1}\) for 15 min) or placebo (normal saline) to 12 women undergoing therapeutic termination of pregnancy at between 12 and 16 weeks of gestation (Norman et al., 1995). Uterine contractions were induced using the progesterone receptor antagonist mifepristone (200 mg) administered 48 h previously and quantitated using an intrauterine pressure transducer. In this study, there was no significant difference between the groups in terms of uterine activity expressed as a percentage of pretreatment activity, either during or after the infusion of GTN or saline. Parallel studies using myometrium collected from women undergoing elective Caesarean section showed that, although GTN was effective at suppressing spontaneous myometrial activity in vivo at high concentrations, the dose required to achieve this effect in vivo would be inappropriate because of systemic side effects (Fig. 3; Norman et al., 1995).

Is nitric oxide involved in the initiation of parturition? Results from animal studies suggest that the myometrial relaxant effects of NO may play an important role in parturition. In rat uteri that were homogenized after removal of the fetus, membranes and placenta, NOS activity was greater during pregnancy than in labour (Natuzzi et al., 1993). In rabbit decidua, NOS activity decreased progressively during the last four days of gestation (Slaad et al., 1993), and in rat uteri, concentrations of nitrate and nitrite were lower at the time of delivery compared with at day 18 of gestation. Furthermore, the concentration of cyclic GMP was high during pregnancy, but attenuated at the time of delivery (Yallampalli et al., 1993a, 1994). Corresponding experiments in women have not been published.

Nitric oxide synthase activity and sex steroids

An accumulating body of evidence suggests that NO production may be augmented by oestrogens, and that induction by oestrogen could account for some of the increase in NO production observed during pregnancy. Administration of oestradiol in vivo increased calcium-dependent, but not calcium-independent, NOS activity in guinea-pig heart, kidney, skeletal muscle and cerebellum, with a maximum response after treatment for 10 days (Weiner et al., 1994a). An increase in skeletal muscle mRNA encoding eNOS and bNOS was also observed after treatment with oestradiol. The pregnancy-induced
increase in NOS activity in guinea-pig heart was prevented by the oestrogen antagonist tamoxifen (Weiner et al., 1994b). Administration of oestradiol to ovariectomized mice results in increased iNOS immunoreactivity in mast cells but not in epithelial cells; the reverse is observed after treatment with progesterone alone (Huang et al., 1995). Further direct evidence for a role of oestrogen in the regulation of NOS is provided by an ovine model which demonstrates that oestradiol-induced uterine vasodilatation is attenuated by the inhibition of NOS in vivo (Van Buren et al., 1992).

Ramsay et al., (1995) investigated the effects of oestrogen status on NO production in women: 30 women of reproductive age were given monthly depot injections of the GnRH analogue decaptyl. After one month, oestradiol (2 mg daily) or placebo was added to the treatment regimen, and the treatment arms were reversed in the third month. Fasting concentrations of nitrate in plasma were significantly higher during the oestradiol treatment phase, suggesting that oestradiol stimulates NO production. Nitrite concentrations did not differ between the two groups.

Nitric oxide and pregnancy-associated pathology

Inhibition of nitric oxide synthesis during pregnancy

The effects of inhibition of NOS during human pregnancy have not been described, but animal experiments suggest that such intervention would have major consequences. Pregnant rats were infused with the arginine analogue L-nitro-arginine from day 18 of gestation to 24 h after birth (Molnar et al., 1994). A 50% increase in mean arterial pressure was observed by the second day of infusion. Urinary albumin excretion increased by two- to sevenfold and a 40% decrease in mean birth mass of the pups was observed. These effects are similar to those seen in pre-eclampsia. A reduction in pup size was also observed in a study in which L-NAME was fed to pregnant rats from day 13 to day 19 or 20 of pregnancy (Diket et al., 1994). There were no significant effects on maternal blood pressure or urine biochemistry, but the pups exhibited haemorrhagic necrosis of the hind limbs, the incidence of which was proportional to the dose and duration of L-NAME treatment, rising to a maximum of

Fig. 4. Nitric oxide (NO) in the human uterus. Nitric oxide generating systems have been localized to blood vessels, glandular epithelium and endometrial stroma in the nonpregnant human uterus, where the molecule may play a role in the control of menstrual bleeding by limiting platelet aggregation and causing vasodilatation of the spiral arterioles. In pregnancy, NO appears to be produced by blood vessels and syncytiotrophoblast. It may be a crucial factor determining blood flow through the placenta and may play a pivotal role in suppressing myometrial activity. S: endometrial stroma; G: endometrial gland; M: myometrium; D: decidua; syn.: syncytiotrophoblast; c: cytotrophoblast bv: blood vessel.
53%. Co-administration of sodium nitroprusside reduced the incidence of these limb abnormalities (Diket et al., 1994). Whether similar problems would result from NO deficiency in human pregnancy is a matter of conjecture.

Pre-eclampsia

A variety of studies have examined the potential role of NO in pregnancy-induced hypertension. The theory that reduced NO synthesis may be responsible for the increase in systemic blood pressure observed in pre-eclampsia is attractive. This appeared to be confirmed in a study showing lower concentrations of nitrite in serum in a group of 26 women with pregnancy-induced hypertension in the third trimester, compared with matched normotensive controls (Seligman et al., 1994). However, this study did not control for the influence of maternal diet. Greater concentrations of an endogenous inhibitor of NO synthesis were also reported in the peripheral circulation of women with pre-eclampsia compared with healthy pregnant women (Fickling et al., 1993). More convincing evidence for a reduction in NO synthesis during pre-eclampsia was obtained by determining the ability of endothelial cells from human umbilical vessels to generate EDRF in response to bradykinin, which revealed that cells from women whose pregnancies were complicated by pre-eclampsia generated lower concentrations of EDRF than did cells taken from normotensive controls (Pinto et al., 1991). This difference was maintained despite infusion of L-arginine, suggesting that pre-eclampsia may be associated with either reduced NOS activity or altered numbers of endothelial cells. Whether this reduced NO production merely reflects endothelial cell damage, or whether it contributes to the pathogenesis of pre-eclampsia is not known. However, recent work assessing nitrite and nitrate concentrations in umbilical cord blood samples from babies of women with and without pre-eclampsia suggested increased NO activity in the samples from the babies of the hypertensive women (Lyall et al., 1995). Again, the data should be interpreted with caution owing to the confounding effects of maternal diet. However, the results may imply that in pre-eclampsia a compensatory mechanism is activated in the fetoplacental circulation to limit the effects of circulating vasoconstrictors.

Therapeutic applications

To date, clinical interest has focused on the use of NO as an agent to inhibit uterine contractions, and randomized trials assessing the efficacy of NO donors for the suppression of preterm labour are ongoing. Another exciting possibility is that administration of NO could be exploited to improve feto-placental blood flow, either acutely to relieve fetal distress or chronically to improve fetal growth. Reduced placental blood flow has been implicated in the mechanism of intrauterine growth retardation, a condition that leads to both increased perinatal loss and increased mortality from cardiovascular and other degenerative disease in adult life (Barker, 1992). The development of a safe therapy to improve fetomaternal blood flow would have far-reaching implications. Similarly, the administration of NO might alleviate the maternal hypertension and reduced placental blood flow seen in pre-eclampsia. However, before the initiation of clinical trials, further animal studies are essential to ensure that maternal NO administration has no long term adverse effects on the fetus.

Conclusion

Although increasing evidence suggests that NO may play an important part in uterine physiology and pathology, our knowledge remains patchy (Fig. 4). Nevertheless, the foreseeable future is likely to herald major advances in the understanding of the role of this ubiquitous molecule, which could suggest novel therapies for common conditions that are resistant to currently available therapies. However, clinical studies should be accompanied by basic investigation of the mechanism of action of NO in health and disease, to target pharmacological modification of endogenous NO more effectively.

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