Nitric oxide in the endometrium

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Nitric oxide (NO) is an important mediator of paracrine interactions, especially within the vascular system. It is a powerful inhibitor of platelet aggregation and a potent vasodilator. NO is also a neurotransmitter and it plays a role in cell-mediated cytotoxicity. NO-generating enzymes (nitric oxide synthases, NOS) have been described in the endometrium of a number of species, suggesting that NO might be involved in endometrial function. In human endometrium, endothelial NOS and inducible NOS have been localized to glandular epithelium in the non-pregnant uterus. Weak inducible NOS immunoreactivity has been observed in decidualized stromal cells. NO might participate in the initiation and control of menstrual bleeding. Furthermore, it may play a part in the inhibition of platelet aggregation within the endometrium, where menstrual haemostasis is thought to occur primarily by vasoconstriction rather than clot organization. Endometrially derived NO could also suppress myometrial contractility. Recent attention has focused on the part that NO might play in maintaining myometrial quiescence during pregnancy. NO also appears to relax the non-pregnant myometrium, an action which could be exploited for the medical treatment of primary dysmenorrhoea.

Key words: endometrium/human/nitric oxide

Introduction

The endometrium plays a pivotal role in female reproductive physiology. Clinical disorders of endometrial function comprise a range of gynaecological problems, from menstrual dysfunction to infertility and recurrent miscarriage. Furthermore, abnormalities of implantation and placentation may be implicated in the pathogenesis of complications of pregnancy, including placental abruption and intrauterine growth restriction.

Nitric oxide (NO) is an important mediator of paracrine interactions, especially within the vascular system. Endothelial-derived NO is a powerful inhibitor of platelet aggregation and a potent vasodilator (Palmer et al., 1987). NO also functions as a neurotransmitter in the central and peripheral nervous systems and plays a role in cell-mediated cytotoxicity (for reviews, see Lowenstein and Snyder, 1992; Knowles and Moncada, 1994; Green, 1995). The actions of NO are effected by stimulating soluble guanylate cyclase to increase cyclic GMP concentrations. NO-generating enzymes have recently been described in the endometrium of a number of species, suggesting that NO might be involved in the local control of endometrial function.

Nitric oxide

The diverse functions of NO can be attributed to its physical and chemical properties, and to the range of cells in which it is synthesized. As a free radical with an unpaired electron, NO reacts with a variety of targets, including proteins such as guanylate cyclase and cytochrome P-450 enzymes. NO has a short half-life in vivo. Oxidation leads to the formation of nitrate (via nitrite) excreted in urine. Oxidized NO may also form nitrosylated molecules with sulphhydryl-containing compounds, resulting in biologically active derivatives more stable than NO itself (Stamler et al., 1992).

NO reacts with superoxide anions (\(\cdot\)O\(^2\)-) to form peroxynitrite (ONOO\(^-\)) and nitrate. Thus, the effects of NO are reduced in the presence of compounds that generate
superoxide anions (such as xanthine and xanthine oxidase), and the action of NO is potentiated by superoxide dismutase (SOD), a widely distributed enzyme system that inactivates superoxide.

**Nitric oxide synthase**

NO synthase catalyses the oxidation of L-arginine to nitric oxide (NO) and citrulline. The enzyme exists in at least three forms: neuronal NOS [nNOS, b (brain) NOS or type I NOS], endothelial NOS (eNOS or type III NOS) and inducible NOS (iNOS or type II NOS). Neuronal NOS and eNOS are dependent on calcium and calmodulin for their activity; iNOS is calcium-independent. Influx of calcium in the neurone at the site of NO generation or into the endothelial cell cytoplasm activates the production of NO (Knowles and Moncada, 1994). Other cells within the endometrium that express nNOS and eNOS would therefore be expected to respond in a similar way. Neuronal NOS and eNOS were originally described as constitutive enzymes, generating small amounts of NO for neurotransmission and to maintain vascular tone. By contrast, cytokine induction of iNOS results in the release of large quantities of NO, often associated with disease. Whether NO plays a regulatory or cytotoxic role appears to be determined by the magnitude and duration of NO synthesis (Green, 1995). For example, induced NO has been implicated in the hypotension, vasodilatation and vascular hyporeactivity seen in patients with septic shock (Kilbourn et al., 1990).

Most studies that have attempted to localize NOS protein have used immunocytochemistry with antibodies raised against one of the isoforms of NOS. An alternative approach has been to assess the distribution of NADPH diaphorase activity (NADPH-d). This is based on the knowledge that NOS catalyses the conversion of nitroblue tetrazolium (NBT) to NBT formazan, detected by the development of a blue dye. However, care is needed in the interpretation of these data, as diaphorase activity is a property of many other enzymes besides NOS.

**Nitric oxide synthase in the endometrium**

**Rat**

Recent studies have examined the endometrium to address whether the potent vasodilatory and anti-platelet actions of NO may play a role in implantation, or whether endometrially derived NO might act as a relaxant for vascular and myometrial smooth muscle. Initial work focused on the localization of NOS in the rat uterus. Schmidt et al. (1992) studied the distribution of nNOS, soluble guanylate cyclase and NADPH-d in a range of organs from adult rats. Immunocytochemistry was carried out with a polyclonal antibody raised in rabbits against rat cerebellar NOS. As expected, nNOS and NADPH-d were localized to tissues in the central and peripheral nervous systems, but in addition, localization was also seen to epithelial cells in the lung, kidney, stomach and endometrium. Schmidt et al. suggested that uterine NO might be involved in the process of implantation. An increased concentration of cyclic AMP and cyclic GMP had been demonstrated in implantation sites in the rat some years earlier (Vilar-Rojas et al., 1982).

Shew et al. (1993) also used NADPH-d to assess NOS in the rat uterus. In contrast to the results obtained by Schmidt et al. (1992), endometrial NADPH-d was demonstrated in nerve fibres near small blood vessels, but not in epithelial cells. Contractility studies suggested that NO was involved in the myometrial-relaxant effect of calcitonin gene related peptide (CGRP). Another group investigated NOS in the rat uterus under varying hormonal conditions, using NADPH-d, and rabbit antisera against nNOS and iNOS (Suburo et al., 1995). Diaphorase activity was found in nerve fibres, endothelium and rounded cells (identified as oestrogen-dependent eosinophils) in all uterine layers. Neuronal NOS-like immunoreactivity, using two different antibodies, was strictly confined to nerve fibres. Inducible NOS-like immunoreactivity was seen in isolated cells near the uterine lumen. These cells did not stain with NADPH-d, and were morphologically similar to some uterine cells that possessed the macrophage–monocyte marker ED-1 (Suburo et al., 1995). The neural distribution of nNOS in the rat uterus was studied using retrograde axonal tracing with fluorogold, NADPH-d and immunocytochemistry with an antibody raised in rabbits against rat cerebellar nNOS (Papka et al., 1995). This work demonstrated that autonomic and sensory NO-synthesizing nerves in the rat uterus may play a role in the control of the myometrium and uterine vasculature.

The NO system has been demonstrated in the rat Fallopian tube (Bryant et al., 1995). Calcium-dependent and calcium-independent NOS activity was observed by measuring the conversion of $[^3]H$arginine to $[^3]H$citrulline. Immunocytochemistry was performed using antibodies against nNOS, iNOS and eNOS. The endothelial isoform was localized to epithelium and endothelium, nNOS was seen in epithelium and parts of the connective tissue, and iNOS was confined to the epithelial cell lining. Western blotting of whole tissue revealed a protein band of 125 kDa that was recognized by all three antibodies, but bands were not seen at the expected molecular weights for nNOS, iNOS or eNOS.
In summary, there is functional and immunological evidence for the presence of NOS in the rat uterus. There is, however, some inconsistency about the precise localization of the enzymes. One major cause of this discrepancy is likely to be the specificity of different antibodies that have been used to identify particular isoforms of NOS. In addition, NADPH-d cannot be assumed to be NOS in every case.

**Mouse**

The cellular localization and hormonal regulation of iNOS were investigated in the mouse uterus using immunocytochemistry and NADPH-d (Huang et al., 1995). Inducible NOS immunoreactivity was present in uterine epithelial cells, macrophage-like endometrial stromal cells, mast cells and myometrial smooth muscle. These studies suggested differential hormonal regulation of iNOS in mast cells and epithelial cells. In ovariectomized animals treated with oestradiol, iNOS was seen in mast cells, but it was undetectable in epithelial cells. After treatment with progesterone, there was no staining in mast cells, but weak staining in uterine epithelial cells.

**Human**

NO synthase isoforms have also been found in human endometrium (Tseng et al., 1996; Telfer et al., 1997; Ota et al., 1998). Tseng et al. collected endometrium from 20 premenopausal women who underwent hysterectomy for medical reasons. Localization of NOS activity was investigated using NADPH-d. Messenger RNA for eNOS and iNOS was quantified by Northern analysis on intact tissue and separated epithelial glands and stromal cells. Diaphorase activity was confined to epithelial cells and blood vessels, with increased activity in the late secretory phase of the cycle. Northern analysis revealed that glandular epithelial cells expressed eNOS mRNA. Expression of message was greatest in the late secretory phase of the cycle. Messenger RNA for iNOS was only seen in isolated epithelial glands at the time of menstruation (Tseng et al., 1996).

Telfer et al. (1997) used immunocytochemistry on wax and cryostat sections, and reverse transcription–polymerase chain reaction (RT-PCR) on isolated gland fragments to examine the distribution of eNOS (human monoclonal) and iNOS (murine polyclonal) in the endometrium. In agreement with Tseng et al. (1996), both eNOS and iNOS were localized to glandular epithelium (Figure 1). Qualitatively observed variations in the intensity of immunostaining between sections was not related to the stage of the menstrual cycle. Nor was it related to the degree of menstrual blood loss in nine women for whom this had been quantified. Expression of these NOS isoforms in endometrial glandular epithelium was supported by the demonstration of mRNA for eNOS and iNOS in isolated glands. The difference between the observations of Telfer et al. for iNOS mRNA and those of Tseng and colleagues might be due to the different sensitivities of Northern analysis and RT-PCR. Immunoreactivity for eNOS and iNOS was not present in endometrial stroma in tissue obtained from non-pregnant women. Weak iNOS immunoreactivity was seen in decidualized stromal cells both following treatment with synthetic progestagens in vivo and in tissues obtained from women in the first trimester of pregnancy (Telfer et al., 1997). These data suggest either up-regulation of iNOS by progesterone, or an association between iNOS expression and the process of decidualization itself. Thus, in human endometrium, both eNOS and iNOS appear to be localized...
to glandular epithelium in the non-pregnant uterus. Direct comparison with the rodent is difficult in the absence of studies localizing nNOS in the human.

Further support for the localization of eNOS to endometrial surface and glandular epithelium has been provided by Ota et al. (1998). Immunostaining for eNOS was most intense in endometrium collected from normal fertile women in the mid-secretory phase of the cycle. Furthermore, expression of eNOS was persistently greater in endometrium from women with endometriosis and adenomyosis, when compared with the control group.

In pregnancy, iNOS immunoreactivity is present in decidualized stromal cells. A similar distribution has recently been reported for SOD (Sugino et al., 1996). Endometrial glandular epithelium showed positive immunoreactivity for cytoplasmic Cu/Zn and mitochondrial Mn SOD throughout the menstrual cycle. Weak to moderate staining was observed in decidualized stromal cells in the late secretory phase of the cycle. Intense immunostaining for both Cu/Zn and Mn SOD was demonstrated in decidual cells in early pregnancy. SOD might therefore provide a local mechanism to enhance the actions of NO in the endometrium, by reducing superoxide-mediated inactivation of NO.

A NO-dependent relaxation system has been shown in the human Fallopian tube (Ekerhovd et al., 1997). Immunocytochemistry revealed iNOS-like immunoreactivity (using a polyclonal antibody raised in rabbits against iNOS) in tubal epithelium and smooth muscle cells, but also vascular endothelium.

**NO in autocrine and paracrine signalling**

NO can be released from vascular endothelium by a range of stimuli, including endothelin (ET) binding to the ETő receptor on the endothelial cell itself (Takayanagi et al., 1991). Endothelin immunoreactivity has been demonstrated on vascular endothelium and glandular epithelium in human endometrium (Cameron et al., 1992; Salamonsen et al., 1992). Furthermore, ETő receptors have been localized to the same cell type as NOS immunoreactivity in human endometrium, namely glandular epithelium (Collett et al., 1996). Therefore, as is the case in vascular endothelium, one of the effects of ET binding to ETő receptors on endometrial glandular epithelium might be to release NO.

Another potentially important interaction is the relationship between NO and prostaglandins. NO has been shown to enhance cyclo-oxygenase (COX) enzyme activity (Salvemini et al., 1993). In tissues such as human endometrium, in which both NOS and COX are present predominantly in glandular epithelium, there may be NO-mediated prostaglandin production. In turn, this could modify the response to NO itself. For example, although NO was shown to relax the spontaneously contracting rat uterus, the action of NO on the quiescent uterus was to stimulate myometrial contractions, and this effect was attenuated by the COX inhibitor indomethacin (Franchi et al., 1994).

**Role of NO in the endometrium**

The presence of NO isoforms within the endometrium suggests that NO might participate in the initiation and control of menstrual bleeding. The onset of menstruation is thought to be preceded by endometrial regression, vasoconstriction and then vasodilatation (Markee, 1940). NO could contribute to these events as the most potent known vasodilator. Next, along with the vasodilatory prostaglandins E2 and I2, NO might play a role in determining the degree of menstrual bleeding (Smith et al., 1981), though a relationship between the intensity of NOS immunostaining and objectively measured menstrual blood loss has not been demonstrated (Telfer et al., 1997).

In addition, NO may play a part in the inhibition of platelet aggregation within the endometrium. During the first few days of bleeding, haemostasis is achieved mainly by vasoconstriction and not by the deposition of platelet–fibrin plugs (Markee, 1940; Christiaens et al., 1980).

Endometrially derived NO could also contribute to the local control of myometrial contractility. Attention has focused on the role of NO in the maintenance of myometrial quiescence during pregnancy, suggesting the administration of NO donors as a therapeutic approach for the treatment of pre-term labour (see Norman and Cameron, 1996, for review; Thomson et al., 1997a). Recent work has looked at NO in the process of cervical ripening (Ali et al., 1997; Thomson et al., 1997b). NO also appears to relax the non-pregnant myometrium, an action which has been exploited in a preliminary study for the medical treatment of primary dysmenorrhoea (Pittrof et al., 1996).

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**References**


