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MINI-REVIEW

Nitric oxide and reproduction

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In the last decade, nitric oxide (NO) has assumed an important functional role in a variety of physiological systems and different pathways, therefore it is indisputable that such a polyvalent molecule should also play a decisive role in the reproductive system. NO is derived from L-arginine by the action of NO synthase (NOS), an enzyme existing in three isoforms. One of constitutive (cNOS) type [brain NOS (bNOS) or neuronal NOS (nNOS)] and one of endothelial NOS (eNOS) are together responsible for the continuous basal release of NO requiring Ca^{2+} /calmodulin. A third isoform is an inducible form (iNOS) which is expressed in response to cytokines and lipopolysaccharides but which is calcium independent. Basal generation of NO by cNOS plays an important role in the physiology of several organs. In the vascular system, NO induces vasodilation, inhibits platelet aggregation, prevents neutrophil/platelet adhesion to endothelial cells, inhibits smooth muscle cells proliferation and migration, and maintains endothelial cell barrier function. In the neural system, NO acts as a neurotransmitter, whereas increased expression of iNOS plays a key role under several pathological conditions (Moncada *et al.*, 1991). NO generated by macrophages in response to an immune reaction acts as a cytotoxic agent for the invading microbes. Since neurones, vasculature and cells of the immune system are an integral part of the reproductive organs, it is obvious that NO is an important regulator of the biology and physiology of the reproductive system.

NO was first recognized in the reproductive system by Ignarro *et al.* (1990), who demonstrated that NO was generated in response to non-adrenergic/non-cholinergic neurotransmission-mediated penile erection. Following this finding, the presence of cNOS and iNOS has been demonstrated in both male and female reproductive organs suggesting that apart from the immune, neuronal and blood vessel-derived NO, locally synthesized NO may be directly involved in the physiology of the reproductive organs.

The presence of NOS (e.g. in human glandular epithelium, endometrial stroma and myometrial blood vessels) implies that NO plays a local role in the control of uterine function (Telfer *et al.*, 1995). It is possible that NO augments the vasodilatory effects of the uterine vascular bed in a paracrine fashion and acts as an alternate inhibitor of endometrial platelet aggregation via activation of soluble guanylyl cyclase and cGMP genera-

tion. Moreover, local synthesis of NO within the uterus may be important for regulating myometrial activity, i.e. spontaneous contraction and relaxation of the uterus.

Buhimschi *et al.* (1996) demonstrated that the rat cervix expressed all three isoforms of NOS (iNOS, nNOS, eNOS), whereas the uterus expressed only iNOS and eNOS, but not nNOS. Furthermore, they demonstrated that expression of iNOS was increased in the cervix, and decreased in the uterus, during labour and preterm labour. Moreover, nNOS, which was not expressed in the uterus during gestation, increased in the cervix during labour. In contrast to nNOS and iNOS, no significant changes were observed in eNOS expression during labour at term. These findings suggest that NOS activity in the uterus and cervix is differentially regulated during labour and may be involved in the connective tissue remodelling that occurs during cervical ripening. The physiological and biological relevance of NO in pregnancy and labour can be deduced from the finding that inhibition of NO synthesis by administration of N^G -nitro-L-arginine methyl ester (L-NAME, which competes with L-arginine and inhibits NO synthesis) prolonged the duration of delivery and decreased the cervical extensibility. These findings not only suggest the importance of NO synthesis in the uterus and cervix during labour and pregnancy, but also point towards the roles of the various isoforms of NOS in regulating these effects.

NO also seems to be involved in pre-eclamptic conditions and pregnancy-related hypertension. Evidence for the role of NO in pre-eclampsia comes from the studies of Buhimshi *et al.* (1996) who demonstrated that administration of L-NAME to pregnant rats resulted in pre-eclampsia-like conditions. Moreover, co-administration of rats with L-NAME and L-arginine reversed both the increase in blood pressure and the decrease in fetal weight that occurred with L-NAME.

Additionally, a recent study by Yallampalli *et al.* (1996) demonstrated that calcitonin gene-related peptide reduced fetal mortality and reversed hypertension in L-NAME-induced pre-eclampsia. Taken together, these findings suggest that vasoconstriction caused by decreased synthesis of NO within the vasculature can lead to conditions such as pre-eclampsia. Hence, in a clinical setting, not only agents which induce vasodilatation via NO generation but also agents which induce vasodilation independently of NO generation may be therapeutic.

ically important in preventing pre-eclampsia-associated disorders.

Myatt *et al.* (1996) recently demonstrated that expression of eNOS and hence NO synthesis was increased in the fetal-placental vasculature obtained from patients having pre-eclampsia with or without intrauterine growth restriction. The authors speculate that the increase in eNOS expression in the fetal-placental vasculature of pre-eclamptic subjects may be an adaptive response to increased resistance, poor perfusion and hypoxia. Indeed, stress and hypoxia are known to induce eNOS activity. Moreover, an increase in NO synthesis as a compensatory mechanism to reduce blood pressure has been shown to occur in rats with experimentally-induced hypertension (Dubey *et al.* 1996), as well as in spontaneously hypertensive rats (Arnal *et al.* 1993).

In light of the findings that L-NAME induces pre-eclampsia-like conditions (Buhimschi *et al.*, 1996), whereas increased eNOS activity was observed in the placental-fetal microcirculation in patients with pre-eclampsia (Myatt *et al.*, 1966), how do we explain the role of NO synthesis this condition? Most of the emphasis has been focused on the vasodilatory effects of NO and little is known about its effects on the growth of smooth muscle cells in the placental-fetal microcirculation. Abnormal growth of smooth muscle cells contributes to the remodelling process associated with the vasoocclusive disorders observed in the placental-fetal microvessels. Since NO has been shown to differentially regulate smooth muscle cell growth, i.e. inhibit as well as induce growth, the regulatory effects of NO on placental-fetal microvascular smooth muscle cells may be important in pre-eclampsia and need to be investigated.

NO could also play an important role in the fertilization process via autocrine and paracrine mechanisms. We have provided both biochemical and immunohistochemical evidence for the presence of iNOS and eNOS in the human oviducts (Rosselli *et al.*, 1996). The oviduct plays a key role in the reproductive process by regulating the transport of gametes and embryos as well as by providing a specific micro-environment for both the fertilization process and the primary stages of embryonic development. Hence, local synthesis of NOS could also play a paracrine role in this tissue.

NO has also been shown to regulate sperm motility. Low concentrations of NO have been shown to enhance sperm motility (Lewis *et al.*, 1996), whereas high concentrations of NO decrease sperm motility (Rosselli *et al.*, 1995). The opposing effects of NO on sperm motility provide a good example of the differential effects of NO (low and high concentrations) on a single biological system. Under physiological conditions, low amounts of NO are generated which can neutralize free radicals and thereby prevent the O₂-mediated reduction of sperm motility. However, excessive generation of NO under pathological conditions such as infection or endometriosis may be toxic and could reduce sperm motility by contributing to the formation of peroxynitrite, a highly toxic anion of peroxidation. Indeed, in semen collected from different subjects, a positive correlation was observed between the concentration of NO and the percentage of immotile spermatozoa. Hence the concentration of NO could define the level of sperm

motility and viability which is hence dependent on the amount of NO generated, i.e. low (physiological) or high (pathological conditions).

A recent study by Lewis *et al.* (1966) provides evidence for the presence of eNOS and bNOS in human spermatozoa and their regulation of sperm motility in an autocrine fashion. Moreover, these authors demonstrated that, in comparison with normozoospermic samples, the expression of eNOS and the amounts of NO generated were lower in asthenozoospermic samples, suggesting that decreased endogenous NO may influence sperm motility and hence fertilization. However, in contrast to these findings, Schaad *et al.* (1966) found no evidence for the presence of NOS activity in human spermatozoa. It is possible that these differences are due to the procedures used to assay NO synthesis and further studies will be required to confirm whether spermatozoa contain NOS and whether endogenous sperm-derived NO regulates sperm motility.

Schaad *et al.* (1996) also reported that human seminal plasma inhibited NOS activity in the brain and characterized the factor involved as a heat-stable non-competitive inhibitor of bNOS. The physiological relevance of the presence of such an endogenous NO inhibitor is unclear. Considering the immunogenic properties of spermatozoa in the female reproductive tract, it is possible that spermatozoa in the female reproductive tract activate iNOS and the subsequent generation of large amounts of NO could prove toxic to the spermatozoa and lead to infertility. Hence, the presence of an endogenous NO inhibitor in the seminal plasma could play a physiological role in inhibiting NOS activity (iNOS), and hence maintaining NO at low concentrations to prevent toxic damage to spermatozoa and surrounding cells. Moreover, considering that low amounts of free radicals seem to be important in modulating sperm motility and, therefore, inducing sperm capacitation, such an endogenous NO inhibitor could also regulate the capacitation process.

Our group (Rosselli *et al.*, 1994) first reported that NO increased with follicular development and these increases were correlated with the concentration of oestradiol. Anteby *et al.* (1996) identified increased NO concentrations in follicular fluid which were positively correlated with the follicular volume and oestradiol concentrations. This observation implies a functional relationship between the NO pathway and folliculogenesis. Cell-specific localization of NOS has also been demonstrated in the thecal and stromal cells of the rat ovary during follicular development, ovulation and luteal formation (Zackrisson *et al.*, 1996). It is feasible that an abundance of eNOS in stromal and thecal cells during ovulation increases ovarian blood flow and is involved in the ovulation process. The role of NO in ovulation is further supported by the findings that in rats infused with N^G-methyl-L-arginine, an inhibitor of NO synthesis, the rate of ovulation was significantly reduced (Shukovski *et al.*, 1994).

Direct evidence of the role of non-vascular NO in regulating the ovulation process comes from the findings that if blood flow were reduced to same extent as after NO inhibition, the ovulation rate was not suppressed to the same extent as occurred when NO synthesis in perfused rat ovary was blocked.

Since NO is an important regulator of the vascular remodelling process, it may be speculated that NO is also an important mediator of vascular changes and tissue remodelling during the ovulatory and luteinization process. Moreover, NO may be involved in an autocrine/paracrine regulation of the developing follicle and have a direct effect on granulosa cells, thecal cells or on the developing oocytes. Therefore, NO in the follicular microenvironment may also be involved in the atretic process by inducing cell death via paracrine mechanisms.

Based on the finding that oestradiol induces NOS mRNA expression in different tissues (Wiener *et al.*, 1994) and the positive correlation between circulating values of NO₂/NO₃ and oestradiol concentrations in ovulating patients (Rosselli *et al.*, 1994), NO must be considered as an important general endocrinological regulator of the ovulatory process. Ovarian dysfunction is associated with cardiovascular disease and decreased endothelium-derived NO synthesis, oestrogen substitution has cardioprotective effects and circulating values of NO₂/NO₃ are increased in post-menopausal women substituted with oestrogen (Rosselli *et al.*, 1995). These clinical findings suggest that NO is a molecule that is in part controlled by the reproductive system and is implicated in sexual cardiovascular dimorphism and hence may be of clinical relevance.

Moreover, NO seems to be involved in the release of luteinizing hormone-releasing hormone (LHRH) (McCann and Rettori, 1996), and may therefore indirectly control sexual behaviour and steroidogenesis. It is likely that the molecule follows the classic cascade of events of the hypothalamus–hypophysis axis and may therefore be a crucial molecule in the reproduction process.

Based on the above findings, it is clear that the biology/physiology of the reproductive system is regulated by NO generated locally by different cells in the reproductive organs as well as by blood vessels, nerves, and the immune system. Moreover, NO may play a role in several disorders associated with the reproductive system. The obvious question is: how can we apply these findings in a clinical setting? It is possible that NO donors and agents that increase the basal generation of NO may prevent pre-eclampsia and pregnancy-related hypertension. Moreover, inhibitors of NO synthesis may prevent infertility caused by NO toxicity to spermatozoa under pathological conditions. In conclusion, future research must aim to elucidate the exact and detailed role of NO in the different pathways of the reproductive system and determine whether modulators of NO synthesis can be of clinical importance to the reproductive process.

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