The nitric oxide pathway in pre-eclampsia: pathophysiological implications

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Pre-eclampsia, one of the most significant health problems in human pregnancy, complicates ~6–8% of all gestations and is the leading cause of fetal growth retardation, infant morbidity and mortality, premature birth and maternal death. Recent research implicates free radicals in the pathophysiology of pre-eclampsia. This review covers the biochemistry of nitric oxide (NO) and possible interactions with other free radicals. Studies in the rat show that pregnancy is associated with enhanced production and responsiveness to NO in both reproductive tissues and blood vessels. Rats infused with NO₂-nitro-L-arginine methyl ester (L-NAME, a NO synthase inhibitor) have been used as an animal model of pre-eclampsia, and the effects of steroid hormones on blood pressure in this model have been tested. Results suggest that pre-eclampsia may be a state of NO deficiency. However, in humans there seem to be contradictions regarding the involvement of NO in maternal adaptation to pregnancy. It is suggested that NO may be one of several systems that act in concert to maintain a symbiotic relationship between mother and fetus. However, the input of each system may be genetically determined.

Key words: endothelium-derived relaxing factor/hypertension/pregnancy/superoxide/toxaemia

Introduction

Pre-eclampsia is considered to be one of the most significant health problems in human pregnancy, complicating ~6–8% of all gestations over 20 weeks (Chesley, 1978). This disease is one of the leading causes of fetal growth retardation, infant morbidity and mortality, premature birth and maternal death (MacGillivray, 1983). Despite continuous advances in research, pre-eclampsia remains a major challenge in both understanding its pathophysiology and management. Our knowledge about the pathophysiology of pre-eclampsia has changed dramatically over the years. For many centuries it was considered a simple convulsive disorder, then a renal or a hypertensive disorder. Today, it is unanimously viewed as a multisystem disorder with vascular dysfunction at its centre.

Classically, pre-eclampsia is defined as the triad of hypertension, proteinuria and pathological oedema during pregnancy. It is a condition that develops more commonly in nulliparous women and regresses postpartum. Haemodynamically, pre-eclampsia is characterized by diminished blood volume with haemoconcentration and consequent rheological changes (Arias, 1975; Klee et al., 1993) and by vasoconstriction with increased peripheral resistance (Lin and Walters, 1979; Mabie et al., 1989).

Pathophysiology of pre-eclampsia

Placenta is the key

During its development, the fetal–placental unit serves as an allograft because it contains paternal antigens foreign to its maternal host. Therefore normal pregnancy is characterized by profound immunological adaptive mechanisms.
Fetal genes will be selected to increase the transfer of nutrients to their fetus, and maternal genes will be selected to limit transfers in excess of some maternal optimum (Haig, 1993). A misalliance of the fetal trophoblast with the maternal tissue has been viewed as a fundamental factor in several theories about the cause of pregnancy induced-hypertensive disorders. This concept is supported by observations such as pre-eclampsia occurs more frequently in first pregnancies (MacGillivray, 1958) or following a change in partner (Feeney and Scott, 1980). However, a gestation has to progress beyond 12 weeks to protect against pre-eclampsia in subsequent pregnancies (Campbell et al., 1985). Studies in inbred communities showed that pre-eclampsia was more common when immunogenetic disparity between mother and fetus was greatest (Need, 1975). Also, women with impaired immune function due to human immunodeficiency virus (HIV) infection were found to have a significantly lower risk of developing pre-eclampsia than matched controls (Saade et al., 1994a). Therefore, understanding the process of placentation with its specific features in the human species and studying alterations in this process is viewed by many investigators as the key to understanding pre-eclampsia.

During normal placentation in humans, the extravillous cytotrophoblastic cells in the early weeks of gestation extensively colonize the decidua and adjacent myometrium of the placental bed. These cytotrophoblasts stream into the spiral bed vessels, destroying and finally replacing the endothelium of the maternal vessels. The process continues by invasion of the arterial walls, where they also destroy the elastic and muscular structure of the maternal vessels. After a rest phase between the 14th and 16th weeks of gestation, there is a second endovascular process, the thick-walled maternal vessels are converted from their origin from the radial vessels. At the end of the process, the thick-walled maternal vessels are converted into conduit utero-placental vessels devoid of a muscular component. These remodelled vessels are able to dilate passively and accommodate the increase in blood flow required for the development of a normal pregnancy and do not respond to humoral or neurogenic stimuli in order to protect the fetus (Harris and Ramsey, 1966; De Wolf et al., 1973; Pijnenborg et al., 1981). The extravillous population of trophoblastic cells therefore plays a key role in establishing the low pressure, high-conductance vascular compartment that assures constant oxygen and nutrient supply to the placenta and fetus. There is a body of evidence to support that failure of this normal process of placentation occurs in women destined to develop pre-eclampsia long before the onset of the clinical syndrome. In these women, a significant portion of the placental bed arteries show a complete absence of endovascular trophoblast (Khong et al., 1986). Moreover, there is a complete failure of the trophoblast to advance into the myometrial portion of the vessels, which results in persistence of their muscular wall and potential for vasoconstriction and restriction of maternal blood flow to the placenta (Brosens, 1964; Gerretsen et al., 1981; Meekins et al., 1994). Other structural abnormalities in the placental bed include the abundance of villous cytotrophoblastic cells (Anderson and McKay, 1966; Redline and Patterson, 1995) and irregular thickening of the villous trophoblastic basement membrane (Fox and Elston, 1978). All this evidence points to an abnormality in trophoblastic invasion which is compensated for by an increase in the number of encroaching cells.

**Fetal/placental hypoxia**

Studies in humans, as well as animal models, suggest that placental and/or fetal hypoxia may be a pathogenetic factor in pre-eclampsia. In vitro experiments on perfused human placental cotyledons show that acute reduction in oxygen tension induces fetoplacental vasoconstriction (Howard et al., 1987). This may be an important mechanism in the local regulation and redistribution of fetoplacental blood flow from hypoxic to normoxic or better perfused regions of placenta, operating in a similar way to that in which hypoxia induces pulmonary vasoconstriction to optimize perfusion and ventilation in the lung. However, whether fetal/placental hypoxia is secondary to impaired trophoblastic invasion and narrow vascular bed, as discussed above, has still to be determined. In humans, the risk of developing pre-eclampsia is increased in asthmatics and in individuals living at high altitudes, an effect presumably attributed to placental hypoxia (Moore et al., 1982; Lehrer et al., 1993). Also, abnormal blood flow in the uterine and arcuate arteries has been reported to precede by several weeks and to predict the development of pre-eclampsia (Harrington et al., 1996). Moreover, in normal pregnancy, a state in which oxygen demands are increased, the oxyhaemoglobin dissociation curve is shifted to the right as compared to non-pregnant women (Pernoll et al., 1975). Paradoxically, in pre-eclamptic patients, the oxyhaemoglobin dissociation curve is shifted to the left, further decreasing oxygen delivery. (Kambam et al., 1986). Another possible scenario is that hypoxia itself induces an impaired cytrotrophoblast function, since it has been demonstrated in in vitro models that lowering oxygen tension in culture media inhibits the differentiation of early gestation human cytotrophoblast (Genbacev et al., 1996).
by altering the expression of the adhesive integrins that restrain invasion (Zhou et al., 1993; Bass et al., 1994). It is most probable that once the process is triggered by the ‘unknown’ causative factor, the feedback loop worsens the condition.

**Evidence of oxidative stress in pre-eclampsia**

Lipid peroxidation is a process that occurs normally at low levels in all cells and tissues. It involves conversion of unsaturated fatty acids to lipid hydroperoxides. This process can be initiated by free radicals, which are unstable molecules that possess an unpaired electron in their outer orbital. Following interaction between the lipid and a free radical, the peroxidation chain becomes self-perpetuating, with the lipid hydroperoxide inducing the formation of more hydroperoxide (Halliwell, 1991). The organism normally has anti-oxidative mechanisms that limit this process. Moreover, low concentrations of lipid peroxides are essential and may act endogenously as intracellular messengers (Slater, 1987). However, under certain circumstances, the protective mechanisms can be overwhelmed, leading to elevated steady-state tissue concentrations of lipid peroxides. An imbalance between the pro-oxidants and the anti-oxidants has been defined as ‘oxidative stress’ (Halliwell, 1996). The major endogenous factors responsible for initiating oxidative stress are formation of superoxide and hydrogen peroxide and induction of pro-oxidant enzymes such as xanthine oxidase and transition metals. Superoxide formation is a continuous phenomenon in aerobic cells exposed to normal oxygen tension. About 1% of oxygen consumption evolves to superoxide mostly due to electron leakage from the electron transport chains, such as those of the mitochondria and the endoplasmic reticulum. Endogenous protective mechanisms against reactive oxygen species are enzymatic superoxide scavengers such as superoxide dismutase, catalase and glutathione peroxidase. In addition, there is an extensive non-enzymatic anti-oxidant network of different lipid- and water-soluble molecules that have in common the ability to scavenge free radicals. β-Carotene and vitamin E are two of the lipid-soluble and ascorbic acid, uric acid and glutathione are some of the water-soluble free radical scavengers (Spatz and Bloom, 1992).

During normal human pregnancy, serum lipid peroxidation products are elevated (Maseki et al., 1981) but are counterbalanced by an increased activity of the anti-oxidant system (Cranfield et al., 1979; Uotila et al., 1991; Wang et al., 1991). Several studies suggest that pre-eclampsia is associated with increased circulating lipid peroxides compared to normal pregnancy (Ishihara, 1978; Wickens et al., 1981; Wang et al., 1991; Hubel et al., 1996a; Loverro et al., 1996). However, there seems to be controversy as to whether circulating anti-oxidant activity is changed in pre-eclampsia. One study found that total serum anti-oxidant activity in pre-eclamptic women is decreased (Davidge et al., 1992), while others found that ascorbic acid, vitamin E and β-carotene are lower only in severe pre-eclampsia as opposed to mild pre-eclampsia, where only a deficiency in reduced ascorbic acid was identified (Mikhail et al., 1994). Loverro et al. (1996) found no differences in circulating anti-oxidant enzyme activity in pre-eclamptic patients compared to normal matched controls. Finally, a few studies even found increased anti-oxidant activity in pre-eclampsia (Uotila et al., 1993, 1994; Poranen et al., 1996; Schiff et al., 1996). However, the view that an imbalance between pro-oxidant and anti-oxidant forces occurs in pre-eclampsia seems unanimous (Hubel et al., 1989; Walsh, 1994; Loverro et al., 1996). Several studies suggest that placental lipid peroxides are increased (Wang et al., 1992; Walsh and Wang, 1995, Poranen et al., 1996) and placental anti-oxidant protective mechanisms decreased in pre-eclampsia (Poranen et al., 1996; Wang and Walsh, 1996), indicating that the placenta may be the source for this imbalance in pro-oxidant/anti-oxidant activity. Whether placental hypoxia is the cause of this imbalance has still to be addressed, since it has been demonstrated that tissue hypoxia promotes lipid peroxidation in rats (Yoshikawa et al., 1982) and increases expression of xanthine oxidase, an enzyme which generates superoxide (Lanzillo et al., 1996). However, most of the literature describes increased reactive oxygen species generation after reperfusion of acutely ischaemic tissue (Granger et al., 1981; Sies and de Groot, 1992). To date, a causative relationship between chronic placental hypoxia similar to that described in placenta in pre-eclamptic women and increased superoxide generation has not been established. Again, another possible scenario is that oxidative stress may be the result rather than the cause of pre-eclampsia, or that the process, once triggered by the ‘unknown’ causative factor, becomes amplified in a vicious circle.

**Role of nitric oxide in pre-eclampsia**

**General mechanism of action of nitric oxide**

In 1980, Furchgott and Zawadski discovered that a diffusible endothelial-derived factor was responsible for the vasodilatation triggered by acetylcholine. This factor, which they named EDRF (endothelium-derived relaxing factor), was found to be highly unstable in vitro, with a half-life of only 2–3 s. In 1987, two independent groups...
suggested that EDRF was nitric oxide (NO), based on their very similar biological properties (Ignarro et al., 1987; Palmer et al., 1987). Like NO, EDRF released from freshly isolated aortic endothelial cells reacted with haemoglobin to yield nitrosohaemoglobin. Moreover, bradykinin caused concentration-dependent release of NO from cells in amounts sufficient to account for the biological activity of EDRF. The relaxations induced by EDRF and NO were inhibited by haemoglobin and enhanced by superoxide dismutase to a similar degree (Palmer et al., 1987). By 1988, two groups had shown that the amino acid L-arginine is essential for generation of NO (Palmer et al., 1988; Schmidt et al., 1988). Today, NO is believed to act as a neurotransmitter, paracrine substance and hormone. NO can also be viewed as an intracellular, as well as intercellular messenger. Although NO is a well-known toxic gas and its role as a biological messenger seems nonphysiological, it appears to be a unique and simple molecule with diverse functions in signal transduction.

As a small hydrophobic gas, NO crosses cell membranes as readily as molecular oxygen and CO₂ without need for channels or receptors. The diffusion coefficient of NO in water at 37°C is higher than for O₂, CO₂ or CO (Wise and Houghton, 1968). The signal period triggered by NO is inherently short because NO decomposes spontaneously by reaction with oxygen as well as with haem proteins.

Most messenger molecules encode information within their shape which is recognized by a specific receptor. NO is the smallest of the biological messenger molecules known, with the exception of CO. Because of its chemical simplicity, its effects must be regulated solely by its concentration and stability. This is also dependent on the spatial proximity of the source and target cells and the short duration of action of NO. Once formed by the family of NO synthase (NOS) enzymes, NO is rapidly inactivated by oxyhaemoglobin in red blood cells and by myoglobin in muscle cells, where it reacts with transition metals which have stable oxidation states differing by one electron. NO is unusual because it reacts with both ferric (Fe³⁺) and ferrous (Fe²⁺) forms of haem iron. The binding of NO to Fe²⁺ is reversible and occurs with very high affinity (10 000 times more than for O₂) (Sharma et al., 1987).

The target for NO is generally the haem moiety of the soluble guanylate cyclase (Murad et al., 1990), which contains protoporphyrin IX with iron in the ferrous form. NO catalyses the formation of guanidine cyclic monophosphate (cGMP), which in turn stimulates different kinases (Connwell and Lincoln, 1989), activates K⁺ channels (Robertson et al., 1993) and lowers cytosolic calcium (Twort and van Breemen, 1988), accounting for most of the physiological effects of NO. Other possible targets for NO besides guanylate cyclase are the soluble ADP-ribosylating enzymes (Brune and Lapetina, 1990) and transcription factors through which NO can directly affect gene transcription (via activation or deactivation) (Kroncke et al., 1994) and mRNA translation (Weiss et al., 1993).

### Synthesis of NO

NOS enzymes generate NO through the conversion of L-arginine to L-citrulline. Three different types of NOS isoforms have been identified and cloned to date: neuronal (nNOS or type I), cytokine-inducible (iNOS or type II) and endothelial (eNOS or type III) (Berdeaux, 1993). The C-terminal portions of all NOS isoforms show homology to NADPH cytochrome P-450 reductase (Bredt et al., 1991). Consensus sequences for NADPH, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) binding sites have also been identified. The sequences are highly conserved across species for each isoform, but overall sequence identity between any two isoforms is modest (Xie et al., 1992). Major differences in regulation as well as in the subcellular location have been described between the NOS isoforms. For instance, eNOS and nNOS, which are constitutive isoforms are also calcium–calmodulin-dependent, while iNOS is usually only identified following exposure to inflammatory cytokines or lipopolysaccarides (Griffith and Stuehr, 1995). Functionally, the most important difference is that constitutive NOS enzymes are low output (picomolar) while iNOS is a high output enzyme which produces large quantities of NO (nanomolar) in short periods.

eNOS was first identified in endothelial cells (Förstermann et al., 1991) and is largely particulate and associated with endothelial cell membranes due to the presence of both a myristoylation (Nishida et al., 1992) and a reversible palmitoylation site (Robinson et al., 1995). Shear-stress is a putative regulator of eNOS function (Marsden et al., 1992), increasing its activity (Lamontagne et al., 1992) and expression (Nishida et al., 1992). The NO synthesized by the endothelial lining in response to the increased blood flow diffuses toward the muscular layer of the vessel, where it activates guanylate cyclase and produces relaxation, thereby regulating the diameter of the vessels to the needs of the tissue.

iNOS induction is primarily regulated at the transcriptional level (Perrela et al., 1994). Consequently, induction of enzyme activity is not as readily reversible as that of constitutive isoforms. In unstimulated cells, expression of iNOS is usually very low or absent. The first agents found to induce expression of iNOS in murine macrophages were lipopolysaccarides and cytokines.
[interleukin-1, interferon-γ (IFN-γ) and tumour necrosis factor-α]. Interestingly, the human iNOS promoter also contains a shear-stress response element similar to that detected on the eNOS gene, the function of which remains to be determined (Fürstermann and Kleinert, 1995).

iNOS induction is also triggered by way of feedback during periods of NO deficiency. Under acute experimental conditions, arginine analogues like N\(^\text{G}\)-nitro-L-arginine methyl ester (L-NAME) inhibit NO synthesis by competitive inhibition of NOS. However, chronic inhibition of NOS with L-NAME triggers a compensatory induction in iNOS gene expression (Miller et al., 1996) and iNOS promoter activation by IFN-γ and lipopolysaccharides (Weisz et al., 1996). Moreover, NOS activity is also inhibited by NO in excess (Buga et al., 1993), most likely through a genomically regulated feedback effect of NO on NOS. Recent studies showed that hypoxia is a coinducer of iNOS in macrophages in the presence of IFN-γ, indicating that decreasing oxygen concentration stimulates both iNOS expression and transcription (Melillo et al., 1995). Moreover, glial cells exposed to hypoxia express iNOS-mRNA and iNOS protein (Kawase et al., 1996).

**Interaction of NO with superoxide. Regulators of NO bioavailability**

Following the discovery of the biology of NO, it became clear that some cells produce not one but two radicals: superoxide and nitric oxide. In 1990, Beckman et al. suggested that these two radicals could combine to form peroxynitrite anion, which is a powerful long-acting oxidant with pronounced deleterious effects through oxidation of a number of biomolecules, including membrane phospholipids, sulphides, thiols, deoxyribose and ascorbate, and through inhibition of mitochondrial electron transport (Radi et al., 1991; Huie and Padmaja, 1993; Pryor and Squadrito, 1995). In addition, peroxynitrite can also combine nitrate and protein-associated or free tyrosine, thereby generating nitrotyrosine (Ischiropoulos et al., 1992), a marker of peroxynitrite action (Beckman and Crow, 1993). The rate constant for the reaction that generates peroxynitrite is larger than those for the reactions between superoxide and superoxide dismutase or NO and haem compounds (Packer and Cadenas, 1995). Therefore, in systems which produce both NO and superoxide, the rate of production of either compound and their ratio is important in predicting the probability of occurrence of each reaction. For example, superoxide can diminish the effect of NO by diverting it to peroxynitrite (Rubanyi and Vanhoutte, 1986; Pryor and Squadrito, 1995). Similarly, NO can capture superoxide and minimize its effect, even to the extent of acting as an anti-oxidant (Rubanyi et al., 1991; Wink et al., 1995). A number of systems have been described in which the presence of NO can alter or even protect against superoxide-derived reactive oxygen species and oxidative damage (Wink et al., 1995; Chang et al., 1996). Recent studies show evidence of increased peroxynitrite production in pre-eclamptic placenta as measured by nitrotyrosine immunostaining mainly in the villous vascular endothelium and less so in the syncytiotrophoblast (Myatt et al., 1996).

There are also other regulators of NO bioavailability that may operate in vivo such as free haemoglobin or transferrin. Although haemoglobin has usually been viewed as a scavenger of NO (Stamler et al., 1992), there is also evidence that haemoglobin can carry a form of NO by S-nitrosylation of its cysteine residues, thus prolonging its half-life while increasing its bioavailability and action on target tissues (Stamler et al., 1991). Moreover, iron ions are themselves free radicals and can take part in electron transfer reactions with molecular oxygen, generating superoxide and hydroxyl radicals and thus being able to initiate and/or amplify the lipid peroxidation chain. These reactions may have pathophysiological significance, since it has been shown that haemoglobin concentration and serum iron are increased and iron-binding capacity is decreased in pre-eclampsia, both parameters returning to normal postpartum (Entman et al., 1987; Hubel et al., 1996a).

**The nitric oxide system in normal pregnancy**

**NO production in reproductive tissues**

Systems for an L-arginine–NO–cyclic guanosine monophosphate (cGMP) pathway have been shown to exist in the rat (Izumi et al., 1993; Yallampalli et al., 1994), guinea pig (Chwalisz et al., 1994), rabbit (Sladek et al., 1993) and human uterus (Buhimschi et al., 1995a). In all these species, the NO system is up-regulated during pregnancy (Conrad et al., 1993; Izumi et al., 1993; Yallampalli et al., 1994; Sladek and Roberts, 1996) and inhibits uterine contractility until term, but not during delivery, suggesting that this intrinsic, autocrine, NO-generating activity in the uterus may contribute to the maintenance of uterine quiescence during pregnancy and its withdrawal prior to term may trigger parturition (Izumi et al., 1993; Yallampalli et al., 1994). Both eNOS and iNOS have been identified in the rat uterus. However, the major isoform responsible for gestational changes in uterine NO production appears to be iNOS (Ali et al., 1995; Buhimschi et al., 1996). Gestational differences in the response of the uterus to NO have also been described, suggesting that the relaxing effect of NO is enhanced during most of gestation as compared to term or preterm labour (Izumi et al., 1993; Natuzzi et al., 1993; Weiner et al., 1996).
NOS activity that is both calcium-dependent and calcium-independent (Buhimschi et al., 1995a; Ramsay et al., 1996) and NOS isoforms have also been identified in the human uterus (Telfer et al., 1995). However, the NO synthetic activity of human myometrium is generally low compared to rat myometrium and is even lower during pregnancy compared to activity in non-pregnant patients or those in labour (Buhimschi et al., 1995a; Ramsay et al., 1996). Moreover, the human myometrium is not relaxed by L-arginine, the substrate for NOS (I.Buhimschi, unpublished observation; Jones and Poston, 1997). However, the human uterus has a much higher and gestational-dependent sensitivity to NO or NO donors (Buhimschi et al., 1997). However, there seems to be some controversy as to which NOS isoforms are present in the human placenta or amnion. Such a source of NO may promote uterine quiescence in a paracrine manner. Our group has shown that iNOS is highly expressed in the rat placenta, is strictly located in the peripheral placental layer (trophospongial cell layer) mainly within the glycogen cells and exhibits a major down-regulation which starts before term (Purcell et al., 1997). However, there seems to be some controversy as to which NOS isoforms are present in the human placenta and whether they are gestationally regulated. Some studies revealed the presence of both calcium-dependent and calcium-independent NOS activities (Morris et al., 1995), the latter being significantly higher during the first trimester than at term (Sooranna et al., 1995; Ramsay et al., 1996). Other studies either failed to identify the presence of iNOS mRNA (Garvey et al., 1994) or could not identify iNOS protein on Western blots (Myatt et al., 1993), despite detecting calcium-independent activity with functional assays. These last two reports (Garvey et al., 1994; Myatt et al. 1993) concluded that the main NOS enzyme in human placenta appears to correspond to the calcium–calmodulin-dependent endothelial isofrom. Moreover, other authors could not demonstrate differences in total placental NOS activity at term, before or during labour (Di Iulio et al., 1996; Thomson et al., 1997). The major drawback of most of the studies on NOS expression in human placental tissue is that the samples were collected at term and compared labouring to non-labouring patients delivered by elective Caesarean section. It is possible that at term iNOS expression may have already been down-regulated to the point of non-detection, as we have observed in our studies in the rat.

Our recent studies suggest that, although iNOS is the major NOS isofrom present in the rat reproductive organs and products of conception (Ali et al., 1995), its gestational regulation differs between the uterus, placenta and cervix, suggesting different roles (Buhimschi et al., 1996; Purcell et al., 1997). Briefly, iNOS expression is high in the pregnant uterus and decreases immediately before labour (on day 22). Opposite changes are seen in the rat cervix. In the placenta, iNOS is abundant during gestation. However, in contrast to the uterus, its expression in the placenta is down-regulated earlier (on day 20).

**NO production in vascular tissues**

There is also evidence that the physiological vascular adaptation to pregnancy (increased blood volume, increased cardiac output and decreased vascular resistance) is accompanied by an increase in endogenous NO production (Nathan et al., 1995; Wiener et al., 1994a) and enhanced responsiveness of the vascular smooth muscle to NO (Izumi et al., 1994; Nelson et al., 1995). The blunted pressor response to agonists during pregnancy may be at least partially mediated by NO (Molnár and Hertelendy, 1992). At any resting tension, isolated vascular rings from pregnant rabbits contract significantly less than those from non-pregnant rabbits (Belfort et al., 1993). In a separate experiment using the same model, we showed that pregnancy is associated with a decrease in the response of isolated aortic rings to serotonin, endothelin and the thromboxane analogue U46619, but not to phenylephrine. Depending on the agent used, this refractoriness was partially or totally reversed by removal of the endothelium or inhibition of NO synthesis respectively (Saade et al., 1994b). Studies in the intact animal have also confirmed the importance of the endothelium in vascular homeostasis in pregnancy. The vasoconstrictor responses to a variety of agonists are decreased in the in-situ blood-perfused mesenteric vessels of pregnant rats, an effect partially reversed by NOS blockade (Chu and Beilin, 1993).

Using isolated human omental artery in organ chambers, it has been demonstrated that the response of isolated omental artery to norepinephrine is decreased during normal pregnancy. This refractoriness to norepinephrine was absent in omental arteries from pre-eclamptic patients, which responded similarly to those from non-pregnant women (Belfort et al., 1995). In a separate experiment, we showed that pregnancy reduced the contractile effect of the thromboxane analogue U46619. This refractoriness was dependent on the presence of an intact endothelium but was not affected by NOS inhibition (Saade et al., 1997c). Finally, in-vitro studies have shown that the endothelium-dependent relaxation of isolated subcutaneous and umbilical arteries is impaired in pre-eclampsia (Pinto et al., 1991; McCarthy...
et al., 1993). Recently, a few in-vivo studies evaluating the role of NO in vascular reactivity in pregnancy or pre-eclampsia have been published. In one study the stable isotope $^{15}$N-L-arginine was infused into normal pregnant volunteers and the rate of production of $^{15}$N-labelled nitrite/nitrate was used to estimate NO production at different stages of pregnancy and postpartum. We found that arginine flux and NO production are increased in early, but not late pregnancy, as compared to postpartum (Goodrum et al., 1996). Other investigators have used the measurement of changes in local blood flow in response to various agents as a method to evaluate the role of NO in human pregnancy in vivo. Iontophoretic administration of acetylcholine and sodium nitroprusside into the inside forearm resulted in marked increase in the skin blood flow measured with laser Doppler. The degree of vasodilatation was not significantly different between non-pregnant, normotensive pregnant and pre-eclamptic women (Eneroth-Grimfors et al., 1993). Ford et al. (1996) measured the change in venous diameter in response to infusions of the L-arginine analogue $N^{2}$-monomethyl-L-arginine (L-NMMA). L-NMMA resulted in venoconstriction in women immediately postpartum but not in the same women 12–16 weeks postpartum or in non-pregnant controls. Measuring the forearm blood flow response to brachial artery infusion of L-NMMA, Anumba et al. (1996) found that normal pregnant women showed an enhanced constrictor response when compared to non-pregnant volunteers, indicating increased basal NO activity in pregnancy. The effect of L-NAME, however, was not different between normal pregnant and pre-eclamptic patients, arguing against a decrease in NO activity in pre-eclampsia. However, these are acute studies that may not duplicate conditions of chronic impairment of the NO system.

In summary, these studies show that normal pregnancy is characterized by a high production and responsiveness to NO both within the maternal reproductive and vascular systems. However, different NOS isoforms are most likely involved in the NO generated by these tissues. Namely, iNOS is probably the isoform responsible for the high amount of NO produced within the uterus and placenta during pregnancy, while eNOS is the isoform involved in the general vasodilatation and blunting of vasoconstrictor responsiveness during pregnancy. This conclusion is supported by a recent study in which L-NAME administration (which inhibits eNOS to a greater extent than iNOS) in pregnant rats resulted in an increase in blood pressure and a decrease in urinary NO metabolites (nitrites and nitrates), in contrast to administration of aminoguanidine, a specific iNOS inhibitor which reduced urinary NO output without affecting arterial blood pressure (Lubarsky et al., 1997).

The nitric oxide system in pre-eclampsia: deficiency or excess?

With the discovery of NO as an endogenous and ubiquitous vasodilator, an attractive hypothesis related to pre-eclampsia emerged: could a decrease in NO production in women with pregnancy-induced hypertension explain the clinical findings?

To date, several groups of investigators have measured the circulating or urinary levels of NO metabolites (nitrites and nitrates) and cGMP in women with pre-eclampsia, considering it to be a reflection of the activity of the NO system. However, the results are contradictory and somewhat confusing. Some investigators have found a decrease in NO metabolites in the serum (Seligman et al., 1994) and urine (Begum et al., 1996; Davidge et al., 1996a), but not in plasma from pre-eclamptic women (Davidge et al., 1996a). No significant differences between nitrites and nitrates in maternal venous blood from women with and without pre-eclampsia have also been reported (Lyall et al., 1995; Boccardo et al., 1996; Silver et al., 1996). Systemic cGMP was found to be either decreased in the urine (Begum et al., 1996) or increased in plasma from pre-eclamptic women compared to control pregnant patients (Lyall et al., 1995). In the fetoplacental circulation (umbilical vein), NO concentration was found to be either unchanged (Boccardo et al., 1996) or increased in pre-eclampsia (Lyall et al., 1995). However, in all studies, normal pregnancy seems to be associated with higher systemic NO metabolites compared to the non-pregnant state in both humans (Boccardo et al., 1996) and animals (Yang et al., 1996).

Some authors have described cytotoxic properties of serum from pre-eclamptic women (Rodgers et al., 1988; Tsukimori et al., 1992). This finding, however, was not confirmed by other groups of investigators (Endresen et al., 1995; Kupferminc et al., 1996; Zammit et al., 1996). Recent studies have also demonstrated that plasma or serum from women with pre-eclampsia contains factors that alter the production of vasoactive mediators such as NO, prostacyclin or endothelin from endothelial cells cultured in vitro. Exposure of human endothelial cells to pre-eclamptic serum or plasma induces an increase in NO generation in vitro (Baker et al., 1995; Davidge et al., 1995, 1996b) and increased NOS expression (Davidge et al., 1995). Prostacyclin production was also increased (Branch et al., 1992; Lim et al., 1995; Davidge et al., 1996b) after 24 h exposure to pre-eclamptic plasma, an effect inhibited by phospholipase A2 antagonist (Lim et al., 1995). This was probably secondary to increased plasma phospholipase A2 activity. The factor stimulating NO production appears to be different from that which
stimulates prostacyclin production. NO generation may be stimulated by lipoprotein or lipoprotein aggregates, while prostacyclin is induced by a small molecular weight aqueous fraction (Davidge et al., 1996b) which is heat and acid labile and activated by mild proteolytic digestion (Groot et al., 1995). Interestingly, increasing the exposure time to 72 h inhibited the prostacyclin production to values lower than those obtained after exposure to plasma from healthy pregnant women (Baker et al., 1996a). Mechanical stress also altered the NO and prostacyclin production from the cultured cells exposed to pre-eclamptic plasma (Baker et al., 1996b).

The effect on endothelin production is also controversial. While Gallery et al. (1995) found that exposure to serum from pre-eclamptic and normal pregnant women had similar effects on endothelin production by endothelial cells, Zammit et al. (1996) found that endothelin production by endothelial cells cultured in vitro was less in cells incubated with serum from pre-eclamptic women than in cells incubated with serum from pregnant normotensive women. One explanation is that the initial endothelial injury in pre-eclampsia triggers compensatory mechanisms which induce cyclooxygenase (COX) and NOS enzymes in order to increase the production of vasodilating mediators. Consequently, tissues from pre-eclamptic patients, when isolated in cultures, will produce less NO after stimulation compared to similar tissues from normal pregnant women (Pinto et al., 1991). This positive feedback loop is probably maintained via circulating factors and might be caused by the lack of responsiveness of the endothelial bed, due to the decreased number of viable cells in pre-eclampsia (Orpana et al., 1996). These compensatory mechanisms may operate efficiently in vivo for an extended period of time and therefore delay the clinical picture of pre-eclampsia. This theory may explain the contradictory results seen in measurements of nitrite and cGMP in serum and urine during pregnancy. However, serum from these ‘borderline’ patients will induce increased production of vasodilating substances (i.e. NO and prostacyclin) and less vasoconstrictors (endothelin) when in contact with normal responsive cells in vitro. Another hypothesis is that, in the context of oxidative stress and enhanced reactive oxygen and lipid peroxide production in pre-eclampsia (see above), NO is diverted away from the guanylate cyclase and consumed in the reaction with superoxide.

**Chronic inhibition of NO production in animals? How close to an experimental model for pre-eclampsia?**

The lack of a naturally occurring model for pre-eclampsia in the laboratory animal has hindered efforts to elucidate the cause of this syndrome. Many attempts have been made to mimic or induce pre-eclampsia in different animal species, including non-human primates. Although there are papers reporting on several models, very little work has been presented on an effective model or on the testing of different therapeutic strategies. Some caution must also be expressed with regard to the reproducibility of these models. A pre-eclampsia-like condition can be produced in 50–83% of pregnant ewes by a 72-h food deprivation. It manifests with maternal hypertension, fetal hypoxia, premature delivery and maternal death due to seizures (Thatcher and Keith, 1986). Similarly, severe toxoaemia can occur either spontaneously or be induced by fasting or an inadequate diet in guinea-pigs during late pregnancy (Wagner, 1976). Whether these conditions are similar to human pre-eclampsia remains unclear, since systematic studies on blood pressure and other symptoms of pre-eclampsia are not available. Pre-eclampsia-like conditions can occur naturally in non-human primates (Stout and Lemmon, 1969), and there is a report of experimentally induced pre-eclampsia in baboons by surgical stricture of the aorta (Cavanagh et al., 1985). However, none of these models has been able to produce consistently the triad of signs associated with human pre-eclampsia.

We and other authors have previously shown that, in pregnant rats, chronic competitive inhibition of NO synthesis during pregnancy with L-arginine analogues causes hypertension, proteinuria and fetal growth retardation without affecting gestational length (Baylis et al., 1992; Yallampalli and Garfield, 1993; Molnár et al., 1994). Glomerular damage (Baylis et al., 1992) and histopathological changes in the placental bed (Oswa, 1996) similar to those in human pre-eclampsia have also been reported. These changes seem to be specific to pregnancy and do not occur in control virgin animals (Molnár et al., 1994). The increased blood pressure and fetal growth retardation can be reversed by simultaneous infusion of L-arginine but not D-arginine (Buhimschi et al., 1995b).

Further studies in our laboratory have shown that chronic NO inhibition in Sprague–Dawley rats results in an initial rise in pressure on the day following the initiation of L-NAME treatment (usually on day 17 or 18 of pregnancy) which persists for 1 day only and then rapidly returns to near the range for the untreated control pregnant rats (Figure 1 left). The day before the onset of labour, the blood pressure starts to increase again and remains elevated throughout the postpartum period. This is in contrast to the sustained blood pressure elevation noted by other investigators in non-pregnant rats treated with L-NAME (Molnár and Hertelendy, 1992). We also performed similar studies
on non-pregnant rats and observed that the blood pressure lowering mechanism after L-NAME infusion is not operative in the absence of pregnancy (Figure 1 right). Shortly after pregnancy termination, the blood pressure in L-NAME-treated animals rose to the same values seen in L-NAME-treated virgin rats. Moreover, the response to L-NAME is independent of the sex of the animal (Figure 1 right).

The compensatory mechanism responsible for this refractoriness is unknown, but it probably represents a fetal and/or placental protective mechanism specific for pregnancy. The refractoriness of the blood pressure to the effects of NOS inhibition seen only in pregnancy resembles that described with other pressor agents (i.e. vasopressin, angiotensin, epinephrine, norepinephrine) in vivo and in vitro using human or animal models (Gant et al., 1987). The compensatory mechanism, however, is confined only to a period of 4–5 days before delivery, as the initiation of L-NAME treatment early in pregnancy (day 11) resulted in sustained hypertension throughout mid gestation that gradually decreased close to term. Postpartum, the blood pressure rose again to levels higher than those seen during mid gestation (Shi et al., 1997).

Another possibility is that chronic NO blockade produced experimentally in rats induces increased expression of iNOS through feedback regulation. The animal may overcome the NO inhibition by overexpression of iNOS, despite continuous infusion of L-NAME. This hypothesis is supported by the finding that chronic L-NAME resulted in induction of the iNOS gene in blood vessels and intestine in rats and guinea pigs, resulting in a compensatory increase or normalization of NO synthesis during sustained administration of L-NAME (Miller et al., 1996). Results from our group show that the rat uterus (Buhimschi et al., 1996) and placenta (Purcell et al., 1997) express high amounts of iNOS during pregnancy. In contrast to the uterine iNOS, which is located in cells that persist throughout pregnancy and labour (i.e. macrophages, mast cells, myometrial and stromal cells; Huang et al., 1995), the placental iNOS is located in the trophospongial cell layer which involutes close to term (Purcell et al., 1997). This trophoblastic, inducible, NO production may contribute through feedback regulation to the compensatory lowering of blood pressure following administration of pressor agents during pregnancy. The compensatory blood pressure lowering system is lost once the placental source of iNOS disappears shortly before delivery. Since the iNOS gene promoter contains a hypoxia-responsive element (Melillo et al., 1995), a similar mechanism may operate in human pre-eclampsia, where induction of iNOS may be in response to low utero-placental flow.

There are differences between strains of rats in the response to NO blockade and reversal with L-arginine, probably due to minor genetic differences in endogenous NO production (Tabrizchi and Triggle, 1992). However, certain rat strains, such as SHR and Dahl/Rapp, exist with major defects in the NO system. SHR rats are considered a model for human essential hypertension. They retain the ability to vasodilate during pregnancy and even become normotensive (Ahokas et al., 1991). Interestingly, exogenous L-arginine does not decrease blood pressure levels in SHR rats (Chen and Sanders, 1991; Matsuoka et al., 1996). There is also evidence for a basal increase in expression and activity of the constitutive (Nava et al., 1995) and inducible NOS that may
be further up-regulated by endotoxin (Wu et al., 1996) or interleukin 1 (Junquero et al., 1993) in these animals. Salt-resistant Dahl is a strain of rats in which a high-salt diet increases NO excretion without changes in blood pressure. In contrast, in salt-sensitive Dahl rats, excess salt intake induces marked hypertension in the absence of increased urinary nitrite (Matsuoka et al., 1996). However, infusion of exogenous L-arginine normalizes blood pressure while increasing nitrite and cGMP excretion (Chen and Sanders, 1991). This effect was suppressed by dexamethasone, an inhibitor of iNOS induction (Chen and Sanders 1993). These data suggest that the L-arginine–NO–cGMP pathway is operational and can overreact when a disequilibrium in the system is generated (for instance in salt-resistant Dahl rats after high salt intake or in SHR during pregnancy). This probably involves increased iNOS expression and activity. The additional NO is able to counterbalance any deficiency in other systems responsible for vasodilation. A genetic defect in the NO pathway or in the iNOS feedback loop (such as in salt-sensitive Dahl rats) results in absence of this compensatory mechanism and a clinical symptom related to the increase in vascular resistance (i.e. hypertension, glomerular damage).

Based on all these studies performed in these different strains of rats, we speculate that various systems act in concert to maintain an adequate peripheral perfusion and compensate for each other in order to maintain equilibrium of the biological system if any become dysfunctional or overwhelmed. It also seems that the inducible NO acts as a final resource or a major buffer for the organism to maintain optimal vascular tone.

Therefore, in the Sprague–Dawley rat with chronic NO inhibition, all the other vasoregulating mechanisms, as well as the feedback loop involving iNOS, are operational. Pregnancy, with its associated increase in endogenous NO (Weiner et al., 1994a; Gregg et al., 1995) and prostacyclin release from the maternal vascular (Magnness and Rosenfeld, 1993) or placental beds and its decrease in thromboxane production (Walsh, 1985), shifts the biological system to a new equilibrium state. This hypothesis might explain why chronic NO blockade in pregnant rats does not result in greater increase in blood pressure compared to non-pregnant rats (Umans et al., 1990). The up-regulation of the other system in the new equilibrium state induced by pregnancy involves already operational feedback loops that can compensate better than in the non-pregnant state. Two or more dysfunctional pathways are probably needed for the system to become unbalanced. This may be the explanation why, in SHR rats, chronic NO blockade does induce a higher increase in blood pressure during pregnancy compared to the non-pregnant state (Ahokas et al., 1991).

In summary, the above arguments suggest that competitive inhibition of NOS in an otherwise normally pregnant organism may not be enough to produce overt pre-eclampsia. However, further studies are needed to study the interaction of NO with other systems in the control of vascular adaptation to pregnancy.

**Steroid hormone modulation in L-NAME-infused rats**

The late gestational period in rats (i.e. days 17–22) is marked by important changes in steroid hormones, such as progesterone withdrawal and rise in oestrogen prior to labour (Puri and Garfield, 1982). Therefore, we tested the hypothesis that steroid hormones (namely oestrogen, progesterone and androgens) modulate refractoriness to L-NAME.

We observed that progesterone derivatives such as progesterone and promegestone (R5020: a ‘pure’ progesterone receptor agonist devoid of any antimineralocorticoid activity) and not the 19-nortestosterone derivatives (e.g. levonorgestrel) decreased blood pressure in L-NAME-treated rats. This hypotensive effect of progestins was evident during pregnancy (Buhimschi et al., 1995b), postpartum (Liao et al., 1996) (Figure 2A) and even in non-pregnant female (Figure 2B) and male rats (Figure 2C). A similar dose of promegestone reduced blood pressure in spontaneously hypertensive animals (Figure 2D).

Treatment with L-NAME during late pregnancy resulted in an ~20% reduction in fetal weight (Figure 3). Promegestone (R5020), at a dose of 0.25 mg/day, markedly improved fetal weights but had little effect on blood pressure (not shown), whereas a dose of 2 mg/day significantly reduced blood pressure (not shown), but surprisingly had a further lowering effect on fetal weight when administered together with L-NAME (Figure 3).

The mechanism by which progesterone agonists reduce blood pressure in L-NAME-treated or SHR rats is not evident. It has been suggested that there is a link between steroid hormones and the NO–cGMP pathway in vascular tissues (Miller et al., 1988). Interestingly, oestrogens, and not progestins, have been mainly implicated in endothelium-dependent relaxation (Miller and Vanhoutte, 1990; Weiner et al., 1994b). Although the effect of progesterone treatment has been previously investigated in vascular tissues, clear-cut conclusions were not made (Miller and Vanhoutte, 1991). Another possibility is that progesterone directly affects vascular tone by acting on calcium sequestration or movement within the muscle cell. Also, involvement of a receptor-activated cAMP mechanism for progesterone-mediated vascular effects has been described (Omar et al., 1995).
Figure 2. Effect of promegestone (R5020: 1.5 mg/kg/day) on systolic blood pressure in rats chronically infused with 150 mg/kg/day L-NAME or saline alone. In pregnant rats (A) the osmotic minipumps were inserted 6 days prior to the start of R5020 or oil injections, on the first day postpartum (pp1). d = day of gestation. Non-pregnant female (B) and normal male (C) rats were infused with L-NAME or saline for 5 days before R5020 or oil was injected. (D) Systolic blood pressure values in male SHR rats after administration of R5020 or oil in daily s.c. injections. Each point in each of the final groups of rats represents mean + SEM for five rats. For abbreviations, see Figure 1.

Figure 3. Average pup weight per rat after s.c. administration of two doses of R5020 (R, 0.25 and 2 mg/day/rat) in controls (saline-infused) and N^G^-nitro-L-arginine methyl ester (L-NAME)-infused animals. L-NAME dissolved in saline solution (LN) at a dose of 150 mg/kg/day was administered s.c. via osmotic minipumps. The pumps were inserted on day 17 of gestation after the first blood pressure measurement. Means were calculated using the average pup weight per rat (4–5 rats in each group, 5–16 pups/rat) within 1 h after delivery. Means with different letters varied significantly (P < 0.001).

These data support the findings that, in spontaneously hypertensive rats, pregnancy itself (high progesterone concentrations) reduces blood pressure to almost normal levels (Akahos et al., 1991). Similarly, the antiprogestin RU486 elevates blood pressure following chronic treatment of normal rats (Kalimi, 1989). In our experimental model with L-NAME-infused rats, RU486 further increased blood pressure in these animals (Liao et al., 1996).

Although the mechanism for fetal weight reduction in L-NAME-treated animals is not clear, we suggest that chronic inhibition of NO release from the placental vessels results in vasoconstriction and reduced fetal perfusion, which indirectly contribute to reduced fetal weight. During pregnancy, both the systemic and the uteroplacental vascular compartments contribute significantly to vascular resistance. Measuring fetal weights only addresses the resistance contributed by the uteroplacental compartment. Therefore, we conclude that, in this model, and by extrapolation also to human pre-eclampsia, these two compartments are differently regulated by various agents or show different sensitivity to steroids and other factors regulating blood pressure. It is possible that the improvement in fetal
Figure 4. Systolic blood pressure in rats during pregnancy (with or without L-NAME infusions) and postpartum after injections of 17β-oestradiol (17β-E2) at a dose of 30 mg/kg/day. The L-NAME or saline osmotic pumps were inserted on day 18. 17β-Oestradiol or vehicle (sesame oil) was administered s.c. daily after the first blood pressure measurements (d17) (A) or from postpartum day 1 (pp1) up to postpartum day 10 (pp10) (B). Each point in each of the final groups of rats represents mean + SEM for 4–5 rats. Points with different letters indicate that the means differed significantly ($P < 0.05$). For abbreviations, see Figure 1.

Figure 5. (A) Systolic blood pressure in rats during pregnancy (with or without L-NAME infusions) and postpartum after injections of testosterone (TST) at a dose of 0.3 mg/kg/day. The L-NAME or saline osmotic pumps were inserted on day 17 (d17) after the first blood pressure measurement. Testosterone or vehicle (sesame oil) was administered s.c. daily from postpartum day 1 (pp1) up to postpartum day 10 (pp10). (B) Systolic blood pressure in rats during pregnancy (with or without L-NAME infusions) and postpartum after injections of dihydrotestosterone (5α-DHT) at a dose of 0.3 mg/kg/day. The L-NAME or saline osmotic pumps were inserted on day 18 (d18) after the first blood pressure measurement. Dihydrotestosterone or vehicle (sesame oil) was administered s.c. daily from postpartum day pp1 up to pp10. Each point in each of the final groups of rats represents mean + SEM for five rats.

Weight by the administration of a low dose of progestin is due to an increase in uteroplacental flow. Increasing the dose of progesterone may decrease the peripheral vascular resistance in the systemic compartment and be followed by a secondary reduction in the placental perfusion through a ‘steal phenomenon’ (Isch and Shumacker, 1975).

17β-Oestradiol (30 mg/kg/day) had no significant effect on blood pressure after NO inhibition in pregnant animals (Buhimschi et al., 1995b) (Figure 4A). Moreover, continuous injection in postpartum animals (Figure 4B) resulted in a further increase in blood pressure in animals that received both L-NAME and oestrogens (Liao et al., 1996). Androgens such as testosterone (Figures 5A) or the more specific compound, dihydrotestosterone (Figures 5B), significantly ($P < 0.05$) reduced blood pressure in postpartum L-NAME-infused rats, although it significantly ($P < 0.05$) increased blood pressure in control postpartum rats.

**Hypothetical model of pre-eclampsia as a disease process**

The complexity of pre-eclampsia as a disease arises from the intricacy of the human body as a biological system and the extremely large number of possible interactions among...
its subsystems and feedback loops. It is possible that pregnancy shifts the equilibrium point of the body to a new stable state. During pathological insult, some adaptive feedback loops may be pushed over their physiological limits, change altogether into positive feedback loops, or trigger counterregulatory mechanisms, with catastrophic consequences for the organism. Hypothetically therefore, four distinct time frames exist in the course of a pregnancy destined to develop pre-eclampsia. The first period includes the time before the presumed causative injury, dysfunction or insufficiency occurs and is characterized by a harmonious relationship between mother and conceptus. The second time frame starts after the dysfunction develops and is characterized by the operation of specific compensatory mechanisms which are fully capable of maintaining this harmonious relationship between mother and fetus without any clinical signs or symptoms detectable on either side. In the third time frame, benign clinical signs become apparent in the mother, reflecting the operating compensatory mechanisms which have been pushed to their limits (Page, 1939; Page and Christianson, 1976). In these circumstances, the symbiotic relationship between the mother and conceptus is still maintained without major costs to either organism. It is in the fourth period where the compensatory mechanisms become either insufficient or overreactive, triggering systems that actually worsen the condition. For example, while the primary aim for the marked increase in the number of cytotrophoblastic cells is improving placentation and fetal oxygenation, thickening of the fetal–maternal interface may worsen the hypoxic state. Moreover, the syncytial abnormalities seen in pre-eclampsia are almost identical to those seen in placental villi maintained in culture under hypoxic conditions (MacLennan et al., 1972). It is also probably during this time-frame that endothelial injury occurs and clinical symptoms such as proteinuria become apparent. The reduced uteroplacental flow also results in fetal growth retardation.

This hypothetical evolution of normal pregnancy and pre-eclampsia as a disease process and the analogy with a system that shifts from the point with the lowest free energy (non-pregnant) to another steady state (normal pregnancy) and possible destabilization towards overt pre-eclampsia is shown in Figure 6.

In the light of this model, the NO pathway is probably a buffer that tries to compensate and adapt the biological system to any new condition which may destabilize it. How much, and in which direction NO needs to shift is probably dependent on the availability of other vasoactive mediators as well as the genetic predisposition of each organism. This may explain the discrepancies between the several clinical studies to date regarding blood and urinary metabolites in
pre-eclamptic women. Similar to the genetic variability between different strains of rats, variability in the human population may account for differences in functionality of the NO system. In some patients, a well operational NO pathway may be able to compensate, whereas in the presence of a genetic dysfunction in the NO system the NO output could be unchanged or even decreased. One could also speculate that the initial injury does not have to be identical in all cases. Therefore, if this hypothesis is true, an increased expression of NOS would be expected at least in some pregnancies which would evolve either without complications or would be accompanied by pre-eclampsia.

Recently, Myatt et al. (1997) showed that patients with pre-eclampsia had a significantly greater eNOS staining of the stem villous endothelium of the placental vessels. Moreover, patients with intrauterine growth retardation but not pre-eclampsia had a much greater increase. It is possible that in the latter group a greater compensatory NOS activity prevented the development of pre-eclampsia symptoms. In light of this hypothesis, the definition of a ‘normal’ NO concentration or ‘appropriate’ NOS expression becomes a serious problem, especially for studies that compare small control with diseased groups of patients. Only longitudinal studies during pregnancy may be able to detect when such compensatory feedback loops become active and either prevent or accelerate, mask or unmask, the occurrence of symptoms the physician is seeking in order to categorize a patient as normal or pre-eclamptic.

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