COMMENTARY

Is 'pre-eclampsia' simply a response to the side effects of a placental tachykinin?

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Pre-eclampsia (PE) is a pregnancy-specific syndrome that is the principal cause of maternal morbidity and mortality, accounting for almost 15% of pregnancy-associated deaths (American College of Obstetricians and Gynecologists 1996). Hippocrates first described the condition when he wrote in one of his Aphorisms that 'convulsions take place from either repletion or depletion' (Salas 1999). Hippocrates had observed the sudden and unexpected appearance of maternal grand-mal seizures, which occur when PE progresses to eclampsia, the word being derived from the Greek for 'lightning'. It was believed for many centuries that PE was a seizure disorder unique to pregnancy, but during the last 200 years this view of the disease has changed drastically and we now know that it is not only a convulsive disorder. Several new findings have led to this change in opinion. At the turn of the last century, the new ability to measure blood pressure led to demonstration of the association of PE and hypertension (Cook & Briggs 1903); hypertension was often found to precede the development of eclamptic seizures. Young (1927) described placental damage that led to pregnancy-induced toxemia. Later, the involvement of the kidneys was also observed since women who had died from eclampsia also had a unique form of glomerular endotheliosis (Bell 1932). These findings persuaded many to view the syndrome as a hypertensive rather than a seizure disorder (American College of Obstetricians and Gynecologists 1996). This change in classification unfortunately led many researchers to devote attention to the cause of the hypertension, to the exclusion of other facets of the disease (Roberts & Redman 1993). PE is now unanimously viewed as a multi-system disorder, as increases in blood pressure are rarely responsible for multi-organ dysfunction (Friedman *et al.* 1991).

What is the array of complications associated with PE? In its latest document, the National Institutes of Health (2000) define mild PE as including an increase in blood pressure to greater than 140 mmHg systolic or 90 mmHg diastolic in a woman normotensive before her 20th week of pregnancy. Proteinuria is also present with a urinary

excretion of at least 0.3 g protein in a 24 h specimen. Mild PE can develop directly into severe PE over a matter of days or weeks, this advancement being unpredictable in both its onset and its progression. There is a vast diversity of additional symptoms associated with this change (Patrick & Roberts 1999). These can include cerebral oedema (Cunningham & Twickler 2000), neurological manifestations (including headache, confusion, paralysis, coma, visual loss and seizures) (Royburt *et al.* 1991, Thomas 1998), liver capsule distension (Sheehan & Lynch 1973), renal failure (Lindheimer & Katz 1992), pulmonary oedema (Davison 1997), thrombocytopenia (Pritchard *et al.* 1987), coagulopathy (Barron *et al.* 1999), HELLP (haemolysis, elevated liver enzymes, and low platelet count) syndrome (Weinstein 1982) and nausea (Martin *et al.* 1999).

The primary cause of PE has been difficult to elucidate because its symptoms have always presented as a cluster of conditions. Several theories have been advocated and by far the most compelling evidence indicates that the placenta holds the key. The symptoms of PE disappear soon after birth or after pregnancy termination (Palma Gamiz 1998) when the placenta is no longer present. It is also apparent that the presence of a foetus is not necessary as some cases of hydatidiform mole, in which the uterus contains only disordered placental tissue, are complicated by the condition (Scott 1958). Finally, an utero-placental interaction is not required, as abdominal pregnancies can still encounter complications (Shembrey & Noble 1995). However, as PE affects only 5–10% of first pregnancies the presence of a placenta cannot be the sole cause. What then is different in the placentae of women prone to develop PE? Page (1939) first noted that placentae from these women appeared to be poorly perfused and today we know that a consistent feature associated with this poor perfusion is the defective trophoblast invasion of the myometrial portion of the spiral arteries (Pijnenborg *et al.* 1980, 1981). In normal pregnancy, trophoblast invasion of the spiral arteries renders them dilated, flaccid and unresponsive to vasoconstrictive agents (Brosens *et al.*

1970), but if a defective invasion occurs the spiral arteries retain their musculo-elastic properties and responsiveness to vasoactive substances (Lim *et al.* 1997). This is thought to lead to placental ischaemia and eventually to the observed endothelial dysfunction (Roberts *et al.* 1989) with, finally, the clinical syndrome of PE. Many groups have hypothesised that the ischaemic placenta releases an unknown factor(s) into the maternal circulation which then causes the multi-system complications (Higgins & Brennecke 1998, Roberts 1998, Taylor *et al.* 1998, Van Wijk *et al.* 2000). Roberts (1998) predicted that any candidate molecule(s) would not be unique but rather a known molecule(s) that is present in excessive amounts. We believe that any factor capable of causing PE must be able to inflict its effects not only at the utero-placental boundary but also at peripheral sites if it is to cause the diversity of symptoms seen in different patients.

McGuinness (1963) first presented evidence that the PE factor may be a neuropeptide. An intact frog (skin) bioassay was used to demonstrate that there were grossly increased concentrations of 'melanocyte-stimulating hormone (MSH) activity' in extracts of plasma taken from women suffering from PE. Attempts to confirm this observation using an isolated frog skin system *in vitro* (Chadwick & Lowry 1970) were unsuccessful (R. E. Silman & P J Lowry, unpublished observations) and we concluded that this 'PE factor' was activating the intact frog's own neuroendocrine MSH system at a higher level. One potential candidate was corticotrophin-releasing factor (CRF). In its normal hypothalamic role CRF stimulates the release of adrenocorticotrophin/MSH peptides from the pituitary gland; however, it was also found to be secreted by the placenta into the maternal blood in nanomolar concentrations during normal pregnancy. These levels were found to be some 3-fold higher in pregnancy-induced hypertension (PIH) (Campbell *et al.* 1987). However, the presence in blood of a CRF-binding protein (Behan *et al.* 1989, Linton *et al.* 1993) that was effective in neutralising the activity of the placental CRF suggested it did not contribute to the PIH. There was also a discrepancy in the levels of CRF observed (3-fold) and the 'MSH activity' (100-fold) seen by McGuinness (1963). These observations led us to continue our search for the identity of the 'PE neuropeptide'.

We extended our approach by using a range of recently developed molecular biological and bio-informatic tools. We adopted two main strategies: (i) the development of a profile of placental gene expression during the first trimester of pregnancy (Page *et al.* 2000a) and (ii) a systematic bio-informatic database search to identify and reconstruct the full-length cDNA of expressed sequence tags (ESTs) previously uncharacterised in the human placenta. Our searches have led to the creation of a placental database containing more than 2000 known genes, ESTs and uncharacterised genes. From this pool we

Figure 1 Semi-quantitative RT-PCR of the full-length NKB mRNA transcript. NKB mRNA transcripts were amplified from human whole brain, foetal brain, foetal liver, spinal cord and placenta.

identified a number of ESTs showing a high homology with the bovine neurokinin B (NKB) precursor (Kotani *et al.* 1986). We were able to clone the full-length human NKB gene from the placenta and locate its expression by *in situ* hybridisation to the outer syncytiotrophoblasts. These cells are responsible for the secretion of numerous pregnancy-associated polypeptides into the maternal circulation, most notably chorionic gonadotrophin. Using an RIA, NKB concentrations in the plasma of 30 normotensive pregnant women were found to be low or not detected throughout pregnancy, although a proportion exhibited a slight increase at term (less than 1 nmol/l). Four normotensive women between weeks 9 and 14 also had concentrations equivalent to the highest at term. All the eight PE women examined in their third trimester were found to have considerably higher levels of NKB (between 1 and 7 nmol/l). The i.v. infusion of high concentrations of NKB into female unrestrained rats caused a significant, transient, increase in arterial pressure and a gain in uterine wet weight of 37% (Page *et al.* 2000b). In future, it will be of interest to inject NKB into frogs to confirm that it can activate the 'MSH' axis.

The discovery of NKB expression in the placenta and the maternal circulation raises some interesting questions with regard to its role both in normal reproductive physiology and in the pathology of PE. We are the first to demonstrate that this peptide is expressed in the periphery. An extensive distribution search for NKB by Moussaoui *et al.* (1992), using specific RIAs, in the rat, did not reveal NKB in any peripheral tissue examined. Their search, however, did not include the placenta, where we have since found NKB mRNA expression levels in humans to be even greater than in the brain or spinal cord (Fig. 1). We have also examined the expression of the substance P (SP)/neurokinin A (NKA) gene mRNA transcript(s) representing the two other mammalian tachykinins. We found the transcript to be expressed in most tissues

analysed including regions of the central nervous system, although interestingly we failed to detect its expression in the human placenta (results not shown). A recent report by Patacchini *et al.* (2000) states that the physiological significance of the NKB-preferred receptor, NK3, in the peripheral nervous system is uncertain as NKB is either not expressed in peripheral tissues or is present in extremely low amounts compared with those of SP and NKA. Now, perhaps, the observation of NK3 receptors in the circulatory system no longer remains an enigma. The activation of NK3 receptors in the vascular system has been reported to cause the contraction of the hepatic portal vein (Mastrangelo *et al.* 1987), venoconstriction of the mesenteric beds (D'Orleans-Juste *et al.* 1991) and increased heart rate (Thompson *et al.* 1998). It is perfectly feasible to suggest now that during pregnancy the secretion of NKB from the placenta activates these receptors and could be responsible for some of the haemodynamic adaptations observed during gestation. The basic mechanisms underlying haemodynamic alterations in pregnancy are virtually unknown (Thornburg *et al.* 2000). Normally both maternal blood volume and red cell mass increase gradually throughout gestation. Stroke volume and heart rate increase gradually, along with venous compliance and venous blood volume, whereas systolic and diastolic blood pressures decrease (Thornburg *et al.* 2000). These changes coincide with a redistribution of blood flow from some maternal organs to the uterus and placenta (Buelke-Sam *et al.* 1982, Dowell & Kauer 1997). Evidence from the infusion of high doses of NKB into female rats would indicate that NKB might be involved in some of these haemodynamic events. Our data suggest that NKB could be causing vascular changes, not only by increasing maternal blood pressure, but also by shunting blood from organs, including the liver and mesenteric beds, to the uterus and placenta. We cannot yet determine the precise mechanism of action but we speculate that, when the uterus and placenta need a greater supply of blood, the placenta begins to secrete NKB. This secretion into the circulation may activate NK3 receptors on the venous side of the maternal system, causing the blood pressure to increase by the contraction of the large veins of the mesenteric beds and the hepatic portal vein. This, in turn, reduces the blood flow to the liver allowing an increase in blood flow to the uterus. This would represent a novel mechanism whereby increases in maternal blood pressure occur from the venous side of the system. This is in contrast to the usual manner of arterial blood pressure increases that are associated with essential hypertension in males and non-pregnant females. We believe that we are the first to propose this theory, although not the first to make such an observation. Two groups (Stainer *et al.* 1986, Sakai *et al.* 1994) have observed that venous abnormalities involving decreased venous distensibility might contribute to the impaired control of haemodynamics found in PE. Such a mechanism would not need

to be switched on permanently; indeed, most normotensive pregnancies showed low or not detected levels of NKB. Nevertheless, it is apparent that the activation of NK3 receptors by NKB could reduce the blood flow through the liver, satisfying the needs of the uterus and placenta; indeed, in rats (Buelke-Sam *et al.* 1982) and rabbits (Nuwayhid 1979) cardiac output to the liver is consistently reduced throughout gestation. We were particularly interested to note that four women with normotensive pregnancies between weeks 9 and 14 had concentrations of NKB equivalent to the highest values that we found at term. We therefore speculate that an increase in NKB expression occurs at a time when intervillous blood flow to the placenta increases at around 10 to 12 weeks – a period that represents a change in the placental environment from a relative hypoxia to an increase in oxygen tension (Jaffe *et al.* 1997). Low oxygen tension appears to prevent trophoblast differentiation towards an invasive phenotype (Genbacev *et al.* 1996) and optimal placental perfusion requires the controlled invasion of the trophoblast cells deep into the myometrial spiral arteries; the narrow walls of the spiral arteries are replaced with the trophoblasts, rendering them flaccid and distended, and resulting in an increase of blood flow to the placenta. At this critical period, a transient surge in NKB secretion may be required to improve perfusion or increase vascularisation in the newly established placental bed.

What is the role of NKB in the pathogenesis of PE? Two main stages seem to occur in the development of PE. We believe that NKB is the factor that links these two stages. The first stage appears to involve the defective trophoblastic invasion of the spiral arteries, the placenta becomes poorly perfused and ischaemia develops. The suggested maternal risk factors have been extensively reviewed; they include immune maladaptation (Dekker & Sibai 1999), genetic predisposition (Broughton Pipkin 1999), underlying diseases (Dekker 1999) and environmental factors (Neela & Raman 1993). We propose that, if the defective trophoblast invasion does not rectify itself after the 10th to 12th weeks of pregnancy, the placenta will start to secrete NKB into the maternal circulation in ever-increasing amounts; in most normal pregnancies, small surges of NKB may be observed around this period of correction. The extremely high levels of NKB detected in the plasma in third trimester pre-eclampsia identify a group of women in whom the invasion of the trophoblasts was never completed satisfactorily. In this case, the secretion of NKB could begin as early as the 10th week of pregnancy. NKB is, conceivably, the key factor involved in initiation of the second stage of the disease, in which the clinical syndrome of PE develops, as NKB alone could account for many of the diverse symptoms. The stimulation of NK3 receptors could lead to constriction and contraction of the mesenteric and hepatic portal veins, with an increase in blood pressure and potential damage to

Figure 2 Our NKB theory behind the multiple symptoms of PE. In normal pregnancy NKB is found in the brain, spinal cord and placenta (Patient 1). If trophoblast invasion is incomplete, the placenta starts releasing NKB into the maternal circulation. This causes contraction of the mesenteric and the hepatic portal vein and increases the heart rate (Patient 2). If this cannot rectify the problem then the placenta secretes greater amounts of NKB, leading to the host of life-threatening symptoms associated with the clinical manifestation of PE (Patient 3). Drawing by Virginia France.

the kidneys and liver (Fig. 2); furthermore the reduction in blood flow to the liver would result in an accumulation of toxic metabolic products, such as lipid peroxides, which may contribute to endothelial cell damage and dysfunction. In the more severe cases, the concentrations of NKB may be sufficient to stimulate peripheral NK1 receptors; for example, activation of receptors found on platelets (Gecse *et al.* 1996) and neutrophils (Perianin *et al.* 1989, Perretti *et al.* 1993) may contribute to the complications of PE. High circulating NKB concentrations could also be responsible for the cerebral complications of PE; high intravascular concentrations of SP have been shown to dilate cerebral blood vessels via NK1 receptors (Jansen *et al.* 1991, Kobari *et al.* 1996) located to the endothelium (Shimizu *et al.* 1999). Many other potential markers for PE have been reported (Higgins & Brennecke 1998, Roberts 1998, Taylor *et al.* 1998, Wang & Trudinger 1998), although no evidence exists to suggest that any of these are the direct cause of PE. For example, decreased levels of prostacyclin (Bussolino *et al.* 1980), increased levels of thromboxane (Mills *et al.* 1999) and variably reported levels of nitric oxide (Buhimschi *et al.* 1998) could all be secondary to endothelial dysfunction. Low levels of nitric oxide synthetase (Guo *et al.* 1999) and protein C resistance (Leiden V mutation; Spina *et al.* 2000) often appear secondary to genetic predisposition. The increased levels of circulating vascular cell adhesion molecule-1 (Lyll *et al.* 1994) and the variably reported levels of intercellular cell adhesion molecule-1 and E-selectin (Krauss *et al.* 1997, Lyall *et al.* 1994) are likely to represent molecules shed as a result of neutrophil-endothelial interactions.

What of the prospects of developing a diagnostic test? The availability of such a diagnostic tool would be useful

for both diagnosis and basic research, but at the present time no such test exists. A selective marker would have to be one that uniquely predicts and/or specifically accompanies the clinical manifestations and is absent from other hypertensive disorders. Preliminary evidence suggests that NKB may fulfil these criteria, as it was present in high concentrations in the plasma of the eight pre-eclamptic women examined (1–7 nmol/l). NKB plasma levels were low or not detected in most normotensive pregnancies studied and also in those of males and non-pregnant females (Page *et al.* 2000b). We also screened males and non-pregnant females who were receiving medication for essential hypertension, and NKB was again not detected. Much work still needs to be carried out as present assays are laborious and imprecise; therefore the first aim is to design a specific immunoassay that can be performed routinely in the clinical setting. In addition, large longitudinal studies are required to determine the diagnostic value of NKB at various stages of pregnancy. PE diagnosis is often complicated by other disorders such as the development of gestational hypertension and ensuing essential hypertension (Brown & de Swiet 1999); this leads to problems, not only in predicting disease outcome and epidemiology, but also in the selection of cases for scientific study. At the present time, over-diagnosis remains the safest strategy for patients.

Once a reliable diagnosis of PE is available, what treatments can be offered? The availability of potent and selective antagonists for the NK receptors may allow alternative and potentially more effective treatment strategies. Administration of NK3 receptor antagonists could alleviate the effects of high circulating levels of NKB in the plasma of mothers suffering from

hypertension by reducing the vasoconstrictive effects of NKB. Careful management of the blood pressure, however, will also be important, as reducing it too much may not only compromise the welfare of the foetus, but may even lead to higher concentrations of the peptide through compensatory secretion of NKB. In severe cases, high concentrations of NKB could activate NK1 receptors, leading to the other complications associated with PE. Here, a combination of NK3 and NK1 receptor antagonists or a broad-spectrum NK receptor antagonist may be the treatment of choice. To date, many non-specific treatments have been offered to deal with the two main complications of PE – hypertension and seizures. In the first instance, a range of adrenergic-blocking drugs (β -blockers and α - β -blockers) (Magee *et al.* 1999), calcium antagonists (Magee *et al.* 1996), diuretic agents (Collins *et al.* 1985) and angiotensin-converting enzyme inhibitors (Hanssens *et al.* 1991) have been used. Problems encountered include those associated with the use of prazosin – which reduces arterial pressure by dilating both arterial resistance and venous capacitance vessels, thus reducing the blood supply to the uterus and causing a higher incidence of intrauterine deaths (Hall *et al.* 2000). In the case of seizure management, magnesium sulphate (Chien *et al.* 1996) and phenytoin (Lucas & Jordan 1997) have been shown to limit the frequency of the attacks. Also, a number of very general treatments have been suggested for the prevention of PE including aspirin (Christian 1999), calcium supplementation (Ritchie & King 2000) and other dietary supplements (such as vitamins C and E) (Chappell *et al.* 1999). As the direct cause of PE remains undefined, these general treatments can only help to relieve the symptoms whilst the underlying factors triggering the initiation of the disease process remain uncontrolled. NKB antagonists, however, may be able to target not only the hypertension and the seizures, but also other symptoms caused by the direct activation of NK receptors. Nevertheless, the development of an NK antagonist would have to follow strict guidelines and include large multi-centre randomised trials to determine the extent of teratogenicity, foetal growth retardation, miscarriage and any other detrimental physiological effects on both the foetus and mother. However, treatment may be necessary only during the third trimester – a time when many of these effects are receding. With the benefit of an early warning test, the need for very early induction of labour and elective caesarean section may be significantly reduced.

Zweifel (1916) first described PE as 'a disease of theories'. Higgins & Brennecke (1998) re-stated that 'pre-eclampsia . . . was still a disease of theories'. Will the NKB theory be able to supersede previous hypotheses and if it becomes a reality will it offer the potential for the first early predictive test and targeted treatment for this life-threatening disease?

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