Chapter 2

Gasotransmitters: a solution for the therapeutical dilemma in preeclampsia?

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Preeclampsia complicates 2-8% of all pregnancies and is a major contributor to maternal mortality worldwide. The only therapy is delivery, often before term.\(^1\) Nitric oxide, carbon monoxide and hydrogen sulfide are gasotransmitters that regulate vascular development, vascular tone and affect antioxidant status.\(^2\) Abnormalities in gasotransmitter signaling and production are linked to hypertension, atherosclerosis and inflammation.\(^2\) Drugs that enhance gasotransmitter signaling have proven therapeutic potential in the clinical and experimental setting.

Gasotransmitters are involved in the vascular adaptations of normal pregnancy,\(^3-7\) and experimental studies have shown that abnormal production is associated with preeclampsia.\(^8-12\) The aim of this review is to give an overview of the role of gasotransmitters in the physiology of pregnancy, and relate their aberrant production to preeclampsia. Specific emphasis will be put on and overview their therapeutic potential for preeclampsia.

**Preeclampsia**

Preeclampsia (PE) is defined by hypertension and proteinuria during the second half of gestation. While the exact cause of PE is unknown, the placenta and/or the maternal inflammatory response play a key role in its pathogenesis.\(^1,13\) PE is often described as a two-stage disease. During placentation in normal pregnancy, trophoblasts invade into the endometrium and spiral arteries, resulting in spiral artery dilation.\(^1\) In PE, trophoblast invasion is incomplete, resulting in inadequate spiral artery remodeling and placental hypoperfusion; this is stage 1 of the disease.\(^1\) Subsequently, the oxygen deprived placenta produces factors that enter the maternal circulation, causing maternal endothelial dysfunction and maternal immune response activation, leading to the signs of preeclampsia; this is stage 2 of the disease.\(^3\)

Some of the factors produced by the placenta are anti-angiogenic factors, such as soluble FMS-like tyrosine kinase receptor 1 (sFlt1).\(^14\) They are (partly) responsible for the maternal syndrome in PE since increased sFlt1 is associated with reduced levels of its circulating pro-angiogenic ligands, placental growth factor (PlGF) and vascular endothelial growth factor (VEGF).\(^14\) Additionally, preeclamptic women have less transforming growth factor-β (TGF-β) caused by increased placental production of soluble TGF-β co-receptor endoglin (sEng).\(^15\) Alterations in (anti-)angiogenic factors are thought to lead to endothelial dysfunction and, consequently hypertension and proteinuria.\(^14,15\)
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The systemic inflammatory response is activated during normal pregnancy and exaggerated in PE. An improper activated inflammatory response is linked to abnormal trophoblast invasion, endothelial cell damage and renal dysfunction. Several pro-inflammatory factors, like tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are released by the preeclamptic placenta. In rat models, triggering of the inflammatory system (e.g. by LPS and TNF-α) leads to hypertension and other features of PE.

**Gasotransmitters in pregnancy**

**Nitric oxide**

Nitric oxide (NO) is a potent vasodilator formed by the conversion of L-arginine to L-citrulline by one of the three NO synthase (NOS, figure 1) isoforms. NO mainly acts through activation of its second messenger, cyclic guanosine monophosphate (cGMP). Endothelial NOS (eNOS) is involved in regulation of peripheral vascular resistance during pregnancy. The biosynthesis of NO increases along with gestation. This is reflected by increasing amounts of NO metabolite (nitrite/nitrate) levels in maternal plasma. cGMP is also increased in plasma and urine, indicating an increased bioactivity of NO during pregnancy. In addition, plasma asymmetric dimethylarginine (ADMA), a competitive inhibitor of NOS, is reduced.

In humans, eNOS and inducible NOS (iNOS) proteins are expressed by the fetal endothelium and villous trophoblasts. iNOS is also expressed by Hofbauer cells (macrophages) (figure 2). In the placental bed, iNOS and eNOS are expressed by interstitial and endovascular cytotrophoblasts. NO might be involved in trophoblast invasion and spiral artery remodeling, since the ability of trophoblasts to remodel the spiral arteries relates to local NO production by interstitial trophoblasts. In addition, placentae from eNOS deficient mice have spiral arteries with a retained smooth muscle cell layer. NO may also be involved in fetal vascular function in the placenta, since it is able to vasodilate feto-placental vasculature in an isolated human placental cotyledon.

**Carbon monoxide**

Carbon monoxide (CO) is a gaseous vasodilator synthesized during the conversion of heme to biliverdin, by the enzymes heme oxygenase-1 and -2 (HO-1 and HO-2) (figure 1). CO protects against ischemia-reperfusion injury and inhibits inflammatory
During pregnancy, the HO/CO system is thought to maintain maternal systemic vascular tone. In pregnant mice, increased HO-activity decreases peripheral vascular resistance, while pregnant mice heterozygous knocked-out for the HO-1 gene demonstrated increased diastolic blood pressure.

Figure 1 - Schematic overview of the enzymatic production of NO, H₂S, and CO, and agents able to increase functionality or availability of gasotransmitters with potential therapeutic effects in preeclampsia

NO (left panel) is produced by three enzymes, neuronal NO synthase (nNOS), inducible NO synthase (iNOS) and endothelial NO synthase (eNOS) from L-arginine. Nitrate and nitrite are metabolites of NO production. H₂S (middle panel) is produced by cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3MST) from homocysteine, cystathionine and L-cysteine. Thiosulfate, sulfate and sulfite are metabolites of H₂S. The right panel shows the enzymatic production of CO, formed from heme by heme-oxygenase-1 (HO-1) and -2 (HO-2). ADMA, asymmetric dimethylarginine; DDAH, dimethylarginine dimethylaminohydrolase; GTN, gliceryl trinitrate; GTP, guanosine triphosphate; GC, guanyl cyclase; cGMP, cyclish guanosine monophosphate; PDE-5, phosphodiesterase type-5; PPAR, peroxisome proliferator-activated receptors.
The enzymes are heterogeneously distributed throughout the human placenta. Healthy term placenta (left panel). Preeclamptic placenta (right panel), cytotrophoblast invasion is shallow and fails to enter the maternal myometrium, resulting in smaller and high-resistant spiral arteries. eNOS, endothelial NO synthase; iNOS, inducible NO synthase; HO-1, heme oxygenase-1; HO-2, heme oxygenase-2; CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase.

In the placenta, HO-1 and -2 are expressed in fetal endothelium and villous trophoblasts while in the placental bed both enzymes are expressed by interstitial and endovascular trophoblasts (figure 2). HO-1 is also expressed by leucocytes and other cells in...
the decidua. In isolated human placenta, CO decreases villous vascular tone and HO-inhibition causes dose-dependent constriction of the feto-placental vasculature. In vitro, HO was found to stimulate trophoblast invasion.

Hydrogen sulfide

Hydrogen sulfide (H$_2$S) is produced by cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase from L-cysteine (figure 1). H$_2$S mediates vascular resistance and systemic blood pressure, has anti-inflammatory effects, promotes angiogenesis and protects against ischemia-reperfusion injury. A precursor in the H$_2$S production pathway is homocysteine, converted by CBS into cystathionine.

In human placentae, CBS is expressed by the fetal endothelium, Hofbauer cells and villous trophoblasts, while CSE is expressed by the fetal endothelium and vascular smooth muscle cells (figure 2). In the placenta, these enzymes are active (both rat and human) and able to produce H$_2$S. The catalytic activity of placental CBS was also shown by incubation of human placental explants with increasing homocysteine concentrations, leading to elevated CBS activity and increased cysteine in vitro. In the human fetoplacental circulation, H$_2$S mediates vasodilatation in vitro via K$_{ATP}$ channels and by interaction with NO. CBS deficiency causes impaired decidualization in mice.

**Gasotransmitters in preeclampsia**

Table 1 summarizes alterations and actions of the gasotransmitters and precursors in the two stages of PE both in the placenta as well as in the systemic circulation. The presence of alterations before the onset of PE, i.e. in stage one of the disease, implies that alterations may be primarily and not due to the onset of PE (table 1).

Nitric oxide

A recent meta-analysis showed that genetic variations in the eNOS gene contribute to an increased risk for PE. Data on circulating NO in PE are inconsistent (reviewed by ). The severity of the disease or gestational age at sampling might explain these variations. However, concentrations of cGMP are consistently lower in PE, indicating decreased NO bioactivity. This is in line with increasing plasma ADMA during and before the onset of PE as compared with healthy pregnancy.
<table>
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<th>Table 1 - Overview of alterations of gasotransmitters and related pathways in preeclampsia</th>
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<td><strong>Trophoblast Studies</strong></td>
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<td><strong>Maternal circulation</strong></td>
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| NO | Stimulates trophoblast invasion* | ADMA | Increased
d | eNOS | Increased genetic variations |
| CO | Stimulates trophoblast invasion† | HO-1 | Decreased | HO-1 | Inconsistent data |
| H₂S | PAG (inhibition of CSE) reduces trophoblast invasion | Homocysteine | Increased | CBS | Inconsistent data |
| | | | | CSE | Inconsistent data |

*animal study, †in vitro trophoblasts. NO, nitric oxide; CO, carbon monoxide; H₂S, hydrogen sulfide; PAG, DL-propargylglycine; ADMA, asymmetric dimethylarginine; eNOS, endothelial NO synthase; iNOS, inducible NO synthase; cGMP, guanosine monophosphate; HO-1, heme oxygenase-1; HO-2, heme oxygenase-2; CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase.
The placental protein expression of iNOS and eNOS is not different in PE versus control.22,23,37 However, the total activity of the enzymes is decreased in preeclamptic placentae.38

The modulating effect of NO on the angiogenic balance might be important during PE. NO production in primary human trophoblasts increased PlGF and VEGF and decreased sFlt1 mRNA expression, resulting in an enhanced pro-angiogenic environment in vitro.39 In addition, lack of eNOS aggravates the sFlt1 induced PE-phenotype in mice.40 Since NO production is impaired during PE, the pathway may be insufficient to counteract pathological events like the anti-angiogenic state.

Carbon monoxide
In the circulation of preeclamptic women, HO-1 enzyme levels are elevated.12 On the other hand, CO concentrations in end-tidal breath of preeclamptic women are lower compared to controls.41 The difference might be explained by decreased HO-1 activity during PE. Increased CO consumption, reflected by HO-1 induction and lower secretion of CO by breathing, can also explain this difference.

Placental gene and protein expression of HO-2 is unaltered during PE.11,42,43 However, HO-1 expression is affected by PE: during the first trimester, i.e. in stage 1 of the disease, HO-1 gene expression in chorionic villi is lower in PE.44 However, during PE, data on placental HO-1 expression and activity are conflicting.11,42,43,45 In decidual leucocytes and other decidual cells, HO-1 expression is increased.12 Since the decidua is one of the major sources of oxidative and inflammatory stress products in PE, increased decidual expression of the cytoprotective protein HO-1 might act as a protective mechanism during PE.12

The HO/CO-system might influence the angiogenic balance in PE. Mice heterozygous for the HO-1 gene show morphological changes in the placenta similar to PE and elevated diastolic blood pressure and plasma sFlt1.28,46 The relation between the CO/HO system and sFlt1 was further shown in in vitro experiments: overexpression of HO-1 in endothelial cells decreased the production of sFlt1 and sEng, whereas HO-1 knockdown potentiates sFlt1 and sEng release from both endothelial cells and placental villous explants.47

Hydrogen sulfide
First reports on the H2S pathway showed increased amounts of plasma homocysteine during and before the onset of PE.48 This could be due to decreased CBS or CSE activity in the placenta.10,33,49 However, studies on villous placental expression of CBS and CSE
in PE, are conflicting.10,33 While two reports showed decreased CSE mRNA and protein levels in placentae of PE patients,33,49 we found no differences in CSE expression, but a decreased CBS expression.10 Differences in severity of the disease may play a role in these different results. Interestingly, miRNA-21, which down-regulates CSE, is elevated in the abnormal Doppler placentae.33 In vitro experiments with human placentae show that H₂S production increases under hypoxic conditions.6

A potential role for these enzymes in the pathophysiology of PE have been shown by in vitro experiments, in which down regulation of CSE was associated with alterations in angiogenic factors (increased sFlt1 and sEng and decreased PlGF).49 while pregnant CBS-deficient mice exhibit hyperhomocysteinemia which is associated with blunted endothelial-dependent vessel relaxation.50 Another pathophysiological role for decreased H₂S during PE might be its ability to alter the angiogenic balance. H₂S increases angiogenesis by up regulation of VEGF and by direct action on the VEGFR2 receptor.51,52 Also, H₂S-stimulated ischemic vascular growth is dependent on augmented expression and activity of VEGF.53 H₂S also has an anti-inflammatory potential,27 and decreased H₂S might be involved in the activated inflammatory response in PE. H₂S is able to down regulate several pro-inflammatory cytokines, like IL-6 and TNF-α and modulates leukocyte adhesion and infiltration.54 Finally, H₂S is a potent anti-oxidant and a scavenger of reactive oxygen species which are abundantly present during PE.54

Gasotransmitters in preeclampsia – a therapeutic potential?

During PE, the functionality of gasotransmitters is aberrant. Therefore, factors able to increase functionality or availability of gasotransmitters may be beneficial as therapeutic agents in (figure 1).

Nitric oxide

Drugs that enhance NO availability are widely available. In 2007, a Cochrane review concluded that there was insufficient evidence to use the NO-donor glyceryl trinitrate (GTN) and L-arginine, to prevent or treat PE.55 However, more recent studies, showed that these drugs might have therapeutic potential in PE. In vitro data showed that GTN protects chorionic villi from ischemia-reperfusion injury, while it inhibits release of sFlt1
and sEng.\textsuperscript{56,57} Although this drug is applicable during pregnancy, the severe headaches that are reported avert its use.\textsuperscript{55} L-arginine attenuates hypertension in rat models with reduced uterine perfusion pressure and sFlt1-overexpression.\textsuperscript{58,59} In human pregnancy, L-arginine did not lower blood pressure in women with pre-existent hypertension, but supplementation resulted in less need for additional anti-hypertensive medication.\textsuperscript{60} The combination of L-arginine and anti-oxidant vitamins (C and E) prolonged the latency to develop PE in a high-risk population.\textsuperscript{61}

Phosphodiesterase type-5 (PDE-5) inhibitors are novel in the PE field and potentiate the effects of NO by preventing cGMP degradation. In the L-NAME rat model for PE, Sildenafil supplementation from day 7 of gestation decreased fetal mortality, lowered (but not normalized) blood pressure and reduced albuminuria, sFlt1 and sEng.\textsuperscript{62,63} When administered from day 0 of gestation, Sildenafil prevented hypertension and proteinuria,\textsuperscript{64} suggesting that timing of treatment in pregnancy is important. This therapeutic potential of Sildenafil clearly calls for an RCT in which Sildenafil is administered in a high-risk population before onset of PE.

Since increased concentrations of ADMA can be measured before clinical manifestations of PE, agents decreasing ADMA could have therapeutic potential.\textsuperscript{9} ADMA levels can be reduced, by agents enhancing activity or expression of dimethylarginine dimethylaminohydrolase (DDAH). Pravastatin increases DDAH \textit{in vitro}.\textsuperscript{65} Indeed, in mice with sFlt1 induced PE, pravastatin increased vascular eNOS and ameliorated hypertension, proteinuria and glomerular endotheliosis.\textsuperscript{55,55} Moreover, pravastatin was able to antagonize sFlt1 by up regulation of PlGF.\textsuperscript{65} The first clinical trial with pravastatin in women with PE (Statins to Ameliorate early onset PE; StAmP trial) is currently being executed.

Interestingly, the therapeutic effect of NO its metabolite nitrate is recently described. It was shown that nitrite protects against ischemic renal injury and that nitrite in beetroot attenuates human hypertension.\textsuperscript{67,68} Therefore, studying the effect of nitrite in PE would be of high value.

Being widely available and suitable for clinical use during pregnancy, the therapeutic potential of NO-based agents appears promising. However, evidence is based on animal experiments, results of RCTs are lacking.
Carbon monoxide
Agents able to increase HO-expression, and consequently CO production, might be beneficial in PE. This is in line with the well-known effect of smoking in pregnancy: the incidence of PE in smokers is reduced by 32%. This may be partly attributed to increased CO. Induction of HO-1, or delivery of its bioactive metabolites CO and bilirubin, downregulated sFlt1 and reduced oxidative stress in placental explants in vitro and attenuated hypertension in preeclamptic rats.

As mentioned above, pravastatin influences the ADMA/NOS/NO pathway during pregnancy. Statins can also exert anti-inflammatory and anti-proliferative effects through HO-1 induction. Simvastatin improves symptoms of PE by up regulation of HO-1 and down regulation of sFlt1 expression in healthy term placental explants.

Another compound capable of inducing HO-1 is rosiglitazone, a peroxisome proliferator-activated receptor (PPAR)-agonist. PPARs play a role in trophoblast differentiation, and reduced placental expression of PPAR is described in PE. In models of cardiovascular disease, activation of PPAR restores vascular structure and endothelial function. Its beneficial effects are mediated by up regulation of HO-1. In experimental PE, the PPAR-agonist rosiglitazone ameliorates hypertension and endothelial dysfunction in a HO-1 dependent pathway.

In conclusion, agents capable of HO induction are beneficial as therapeutic agents in experimental PE. Statins and PPAR-agonists are HO inducing agents and ameliorate hypertension, restore endothelial function and lower sFlt1 in experimental PE. Unfortunately, human data are lacking.

Hydrogen sulfide
$H_2S$-based therapies are emerging in the field of cardiovascular diseases and $H_2S$-releasing compounds are developed for clinical use. These compounds can be divided into two groups, sulfide salts and synthetic $H_2S$ donors. Sulfide salts include sodium hydrosulfide (NaHS) and sodium sulfide (Na$_2$S). Intravenous administration of Na$_2$S in humans increased $H_2S$ levels, and the compound is now tested in phase II trials for cardial ischemia-reperfusion injury (clinicaltrials.gov). Synthetic $H_2S$ donors mainly consist of cysteine analogues, like S-allyl cysteine (SAC), S-propyl cysteine (SPC) and n-acetyl cysteine (NAC), which all increase $H_2S$ production and decrease ischemia-reperfusion injury in a rat models. Although beneficial effects of SAC on placental oxidative stress were shown in vitro, oral NAC administration to women with PE did not alter maternal and fetal
outcomes. A recent experimental study showed that PE symptoms (including alterations in angiogenic factors) in mice, induced by CSE-inhibition by DL-propargylglycine, were restored by a slow-releasing H$_2$S compound (GYY4137). Taking together, H$_2$S-releasing compounds are available and clinically tested. However, too few studies on their therapeutic potential during PE were performed to draw any conclusions.

Conclusions

NO, CO and H$_2$S have a high potential for therapeutic intervention in PE. Their shared vasoactive properties, anti-inflammatory functions, ROS scavenging capacities and angiogenic potential are shown to be cardinal during pregnancy and in the development of PE. Clinical trials showing definite therapeutical potential and safety of gasotransmitters in PE are warranted. We firmly believe that these volatile substances could well be a solution for the continuing therapeutical dilemma of PE.
References


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