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# GPCRs as potential therapeutic targets in preeclampsia

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## Abstract

Preeclampsia is an important obstetric complication that arises in 5% of women after the 20th week of gestation, for which there is no specific therapy and no cure. Although much of the recent investigation in this field has focused on soluble forms of the angiogenic membrane receptor tyrosine kinase Flt1 and the transforming growth factor β co-receptor Endoglin, there is significant clinical potential for several GPCR targets and their agonists or antagonists in preeclampsia. In this review, we discuss several of the most promising candidates in this category, including calcitonin receptor-like receptor / receptor activity modifying protein 1 complexes, the angiotensin AT1, 2 and Mas receptors, and the relaxin receptor RXFP1. We also address some of the controversies surrounding the roles and therapeutic potential of these GPCRs and their (ant)agonists in preeclampsia.

### Keywords

preeclampsia

## Introduction

Normal human pregnancy is characterized by a massive decrease in systemic vascular resistance (SVR), leading to an enhancement of cardiac output (CO) and intravascular volume by at least 40% above pre-pregnant levels [1, 2]. Much of this adaptive change, which peaks near the end of the first or beginning of the second trimester, antedates the accelerated growth of the fetus and placenta in the second half of gestation (reviewed in [3]). The anticipatory nature of normal maternal cardiovascular adaptations in pregnancy is reflected by the fact that they are also observed, albeit to a lesser degree, during the luteal phase of the menstrual cycle [4, 5]. These adaptations are essential for optimal fetal development and normal obstetrical outcomes [6-10].

Preeclampsia - defined clinically by the presence of new onset hypertension and proteinuria after the 20th week of gestation - is a potentially life-threatening pregnancy complication, accompanied by vascular abnormalities including endothelial dysfunction, systemic vasoconstriction and reduced organ perfusion, as well as activation of platelets and the



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coagulation cascade [11]. The origins of the condition are thought to lie in the placenta, wherein impaired uterine spiral artery modification during early pregnancy leads to inadequate perfusion, resulting in the release of factor(s) that may conspire with a susceptible maternal constitution (e.g., vulnerable endothelium) to cause clinical disease. Expectant management of the symptoms includes administration of anti-hypertensive agents and magnesium sulphate for seizure prophylaxis (i.e., preventing progression to eclampsia), and iatrogenic delivery of the fetus(es) and placenta(e). Despite decades of research, there are no specific treatments for preeclampsia.

Arguably the most promising recent development in this area was the 2003 discovery of elevated anti-angiogenic proteins, sFlt1 and sEng, in the circulation of preeclamptic patients [12]. These soluble forms of vascular endothelial growth factor receptor 1 (VEGFR1), a receptor tyrosine kinase (RTK), and the transforming growth factor β (TGFβ) co-receptor endoglin, respectively, are thought to sequester their endogenous ligands, both of which are essential for endothelial function.

Thus, the membrane-bound VEGFR1/VEGFR2 and TGFβ (co-)receptors and their respective soluble forms are important therapeutic targets in preeclampsia [13-15].

However, several G protein-coupled receptor (GPCR) targets also hold great potential for amelioration of disease symptoms and safe prolongation of gestation (the ultimate goal of all preeclampsia drugs) in the desease; these will be the subject of this review. There are undoubtedly many candidate GPCR targets that could be considered, but we present those, which have been linked to the cardiovascular changes in either normal or preeclamptic pregnancy as reported in the literature (**Table**). It is worth noting that although GPCRs comprise the greatest proportion of targets for drugs in current medicinal use (~40%), this represents only 10% of the total number of GPCRs that could theoretically be exploited for therapeutic benefit [16, 17]. Because the maternal syndrome of preeclampsia converges on the vascular system, the most promising targets are vasoactive agents and their cognate GPCRs. Due to space limitations, we will mostly focus on GPCRs and their agonists or antagonists as therapeutic candidates, and less on their potential roles in disease causation or as biomarkers.

## CRLR, calcitonin gene-related peptide and adrenomedullin

The calcitonin receptor-like receptor (CRLR) GPCR mediates transmembrane signalling for three non-allelic vasodilatory peptides: calcitonin gene-related peptide (CGRP), adrenomedullin, and intermedin [18]. Specificity is achieved in this system by combination with one of three receptor activity modifying proteins (RAMPs). Co-expression of CRLR and RAMP1 yields a CGRP receptor, whereas combination with RAMP2 or RAMP3 produces an adrenomedullin receptor, and intermedin is able to bind any of the three receptor complexes [18]. Although intermedin is expressed in the placenta and has a putative role in implantation [19], less is known about this peptide and there is no specific literature with regard to preeclampsia; it will therefore not be further discussed here.

CRLR and RAMP1 are expressed in maternal and fetoplacental vessels in both endothelial and vascular smooth muscle compartments [20]; CGRP stimulation of the latter is thought to directly induce vasodilation via the second messenger cAMP, although endothelium-derived nitric oxide (NO) may also play a role in some vessels (reviewed in [21]). Indeed, CGRP is reportedly the most potent endogenous vasodilator known [21]. Historically, CGRP is known as a neuropeptide, expressed predominantly in dorsal root ganglia and released from sensory nerve terminals impinging upon peripheral blood vessels [22]. Circulating levels of CGRP increase during the first trimester in women and are maintained until term [23],

consistent with a potential role in maternal cardiovascular adaptations. In rats, CGRP is increased at term, but to our knowledge, the time course of change during gestation has not been reported [24]. Yallampalli and colleagues demonstrated that CGRP administration decreases hypertension and fetal growth restriction and resorption in the L-$N^G$-nitroarginine methyl ester (L-NAME) infusion model of preeclampsia in pregnant rats, and that infusion of $CGRP_{8-37}$, a CGRP antagonist, results in further elevation of arterial pressure (AP) in this setting, suggesting a compensatory role for endogenous CGRP in experimental preeclampsia (reviewed in [25]). Although $CGRP_{8-37}$ had no effect on AP in control pregnant rats, SVR and CO were not measured in this study, so whether endogenous CGRP contributes to vasodilation of normal pregnancy remains unknown. Interestingly, the depressor effect of CGRP in L-NAME–infused rats is progesterone-dependent [25].

More recently, it was shown that CRLR and RAMP1 mRNA and protein levels as well as CGRP binding sites are decreased in fetoplacental vessels and placental villous tissue from women with preeclampsia [26]. This was associated with decreased sensitivity to the vasodilatory stimulus of exogenous CGRP in umbilical and chorionic arteries [26]. Furthermore, circulating CGRP levels and placental production of the vasodilator are reportedly decreased in preeclamptic patients [27]. The angiogenic properties of the peptide have also recently been demonstrated [28]. Collectively, these data suggest that deficient placental production and activity of CGRP could result in relative fetoplacental vasoconstriction and impaired fetoplacental vascular development, leading to decreased fetoplacental blood flow in preeclampsia. Hence, activation of CGRP signalling in the placenta and peripheral vasculature may improve fetoplacental blood flow and maternal symptoms, respectively, in preeclamptic women.

Adrenomedullin is expressed in diverse tissues including endothelium and vascular smooth muscle, uterus and placenta during gestation [29], but its role in normal and pathological pregnancy is unclear. Circulating adrenomedullin is increased in pregnant women at term, but this rise does not begin until after the first trimester, arguing against a prominent role for adrenomedullin in the early adaptive change of the maternal cardiovascular system [30-32]. There is little consensus in the literature as to whether plasma levels are further increased, decreased, or not different in women with preeclampsia vs normotensive pregnant women (reviewed in [33]). In rats, plasma adrenomedullin increases progressively as gestation proceeds [34]. Infusion of adrenomedullin decreased AP, most likely in part via dilation of mesenteric arteries, and this effect was more pronounced in pregnant compared to non-pregnant rats [35]. In late pregnant rats, Makino et al. determined that adrenomedullin ameliorates L-NAME-induced hypertension and fetal mortality [36]. Witlin and colleagues similarly observed a beneficial hypotensive action in this model at mid-gestation, but in this case the effect was transient (lasting 24 – 48 hrs) [37]. Moreover, these authors further showed that adrenomedullin actually exacerbated the negative effects of L-NAME on fetal growth and mortality, as well as placental weight and litter size [37], suggesting that adrenomedullin may have adverse consequences on the fetus in the absence of fully-functional NO systems. Thus, whether adrenomedullin would be salutary or harmful in preeclampsia is unclear.

Homozygous deletion of adrenomedullin is embryonic lethal in mice [38], with a phenotype of extreme hydrops fetalis, and maternal genetic haploinsufficiency results in aberrant implantation, placentation and fetal growth restriction [39]. Fetal adrenomedullin production is also absolutely required for normal fetal growth [39]. Moreover, infusion of an adrenomedullin antagonist from gestational day 14 in rats decreased fetal growth, placental size, and fetal vessel development, and induced necrosis of the placenta and fetal membranes as well as fetal edema, with a modest maternal hypertensive effect [40]. The same treatment earlier in gestation (from day 8) also resulted in decreased placental weight

and fetal growth restriction in association with activation of mitochondrial apoptotic pathways in uterine and placental tissues [41]. Most recently, pregnancy and estrogen administration were shown to potentiate adrenomedullin-induced dilation of isolated rat uterine arteries, whereas progesterone attenuated it [42]. This was associated with increased mRNA levels of the AM2 receptor (CRLR + RAMP3). Clearly, adrenomedullin is essential for normal implantation, placental development and probably uteroplacental blood flow (and therefore may have an etiologic role in preeclampsia), but its therapeutic usefulness in the clinical stage of disease is uncertain.

## The angiotensin system

Angiotensin II (AngII) signalling is mediated by AT1 and AT2 GPCRs that in general have opposite effects on the systemic vasculature and kidneys: the former promotes vasoconstriction and sodium retention, whereas the latter induces vasodilation and natriuresis [43]. In women, the typical pressor response to AngII is blunted as early as the $10^{th}$ week of gestation [44], in part because of refractoriness to AngII-induced vasoconstriction. This occurs in the face of increased circulating levels of AngII relative to the non-pregnant state [45]. In contrast, AngII hypersensitivity is a well- documented phenomenon in preeclamptic women that may contribute to their hypertension [44]. It follows then that inhibition of aberrant AngII responses via AT1 may reduce blood pressure and alleviate preeclampsia disease symptoms. However, AT1 antagonists are contraindicated in pregnancy due to adverse fetal effects. Nonetheless, there are several other angiotensin system components potentially at play in preeclampsia that present therapeutic targets.

### The AT2 receptor

In addition to functionally opposing AT1 activity, AT2 may also directly antagonize AT1 via heterodimerization [46]. In AT2-knockout mice or mice treated with a specific AT2 blocker, midgestational decreases in AP are abolished, suggesting that AT2 has a role in normal maternal cardiovascular adaptation in this species [47, 48]. Other investigators showed that AT2 deficiency in mice was associated with hypertension during late gestation, whereas ablation of the *AT1a* gene (mice have two AT1 genes; *AT1a* and *AT1b*) resulted in hypotension in both non-pregnant and pregnant states [49]. In spontaneously hypertensive rats, renal AT1 and AT2 receptors were decreased and increased, respectively, on day 20 of gestation, in association with markedly decreased AP, suggesting that AT2 may contribute to the lowering of AP in these rats [50]. In uterine arteries isolated from mid- (but not late- or non-) pregnant mice, the contractile response to AngII was augmented by AT2 receptor blockade, suggesting that the AT2 receptor mitigates AngII-induced uterine artery constriction at this gestational time point [51]. These studies suggest that specific AT2 agonists might be used to ameliorate hypertension in preeclampsia. However, one possible caveat is an opposing effect in the placental circulation: an *in vitro* study using placental vascular smooth muscle cells (VSMC) suggested that AT2 mediates VSMC contraction in response to preeclamptic placenta-conditioned media [52]. Therefore, pharmacological activation of AT2 signalling may restrict placental blood flow specifically; clearly an undesirable outcome in preeclampsia.

### AT1-B2 receptor heterodimers

A 2001 study showed that surface AT1 – bradykinin receptor 2 (B2) heterodimers are enhanced on platelets and omental vessels from preeclamptic women, in association with increased B2 expression, and that this results in increased signalling through the AT1 receptor [53]. Thus, enhanced AT1-B2 heterodimer formation may be one mechanism of AngII hypersensitivity in preeclampsia. However, a more recent study did not find any

evidence for spatial or functional interaction between AT1 and B2 in several different cell lines [54]. This casts some doubt on the significance of the AT1-B2 heterodimer in preeclampsia and its potential as a therapeutic target.

## The Mas Receptor

The octapeptide AngII may be further processed by angiotensin converting enzyme 2 to Angiotensin 1-7 (Ang(1-7)), which can also derive directly from Angiotensin I via neutral endopeptidase or prolyl endopeptidase. In 2003, the cognate receptor for Ang(1-7) was identified as the GPCR Mas [55]. Because Ang(1-7) possesses vasodilatory attributes, it was investigated during the menstrual cycle, normal pregnancy and preeclampsia. During the menstrual cycle, urinary excretion of Ang(1-7) was reported to be constant, but progressively increased throughout gestation [56]. Since most of the increase occurred after the first trimester, the timing suggests a potential role for Ang(1-7) in the “maintenance”, but not “developmental” phase of hemodynamic changes in pregnancy [57].

Plasma concentrations of AngII and Ang(1-7) were comparable in the non-pregnant state, ~ 20 pg/ml [58]. Although both were increased in normal third trimester pregnancy, the rise in AngII exceeded that of Ang(1-7) reaching levels of ~ 60 and ~ 30 pg/ml, respectively. These results showing that the ratio of Ang II to Ang(1-7) virtually doubled in the third trimester compared to the non-pregnant state is not supportive of a net vasodilatory influence.

In preeclampsia, plasma Ang II and Ang(1-7) were reduced compared to normal third trimester pregnancy, but the ratio of the two peptides remained unchanged, ~ 2:1 [58]. On the one hand, finding of comparable ratios of Ang II to Ang(1-7) in preeclampsia and normal third trimester pregnancy is not supportive of a net vasoconstrictory role in the former. On the other, in comparison to the non-pregnant condition, there was a rise in plasma Ang II to ~ 35 pg/ml and fall in Ang(1-7) to ~ 15 pg/ml in preeclampsia, which could manifest relative vasoconstriction. These studies highlight the difficulty of ascribing functional outcomes to correlative observations.

The role of Ang(1-7) and the Mas receptor is an exciting area of investigation. In this regard, it may be revealing to test whether administration of a specific Mas inhibitor impacts either the developmental or maintenance phases of maternal renal and systemic vasodilation in conscious gravid animal models. The markedly potentiated vasodilatory influence of Ang(1-7) in preconstricted mesenteric arteries from late gravid rats suggests an important vasodilatory role for the peptide hormone [59]. Furthermore, whether the refractory renal and systemic vasoconstrictory action of Ang II in conscious gravid animals administered a Mas antagonist might be restored to the robust levels observed in non-pregnant animals similarly administered a Mas antagonist could be informative.

## AT1-activating autoantibodies

Recently, there has been a surge of interest in the role of AT1-activating autoantibodies (AT1-AA) in preeclampsia, following the discovery of elevated serum AT1-AA in women with the disease [60]. Granger and colleagues have shown in the pregnant reduced uterine perfusion pressure (RUPP) rat model of preeclampsia that administration of losartan or depletion of B-lymphocytes (and hence the ability to generate autoantibodies) attenuates hypertension, suggesting an AT1-AA–mediated mechanism [61, 62]. Direct administration of AT1-AA (purified from the serum of late gestation female human angiotensinogen-overexpressing rats mated with male human reninoverexpressing rats) from d12 of gestation causes hypertension in rats on d19, an effect that is blocked by losartan administration from d14 [63]. AT1-AA–induced hypertension is associated with increased placental production of reactive oxygen species (but no change in placental NADPH oxidase subunit expression)

[64]. Accordingly, co-administration of the superoxide dismutase mimetic tempol attenuated AT1-AA–induced hypertension [64]. Interestingly, the hypertensive effect of AT1-AA in pregnant rats was abolished by administration of the endothelin A receptor antagonist ABT-627, suggesting that endothelin mediates hypertension in response to AT1-AA. This was supported by evidence that preproendothelin transcripts were increased in the renal cortex and placenta by AT1-AA infusion, an effect that could also be blocked by the AT1 antagonist losartan [63]. AT1-AA–induced hypertension in pregnant rats is also associated with increased circulating sFlt1 and sEng, which is again blocked by losartan co-administration [65]. However, it is not clear from these studies whether appropriate controls (e.g., the purified IgG fraction from normal pregnant rats at the same dilution) were used, or whether the difference in AP measured on d19 reflects hypertension above pre-pregnant levels or a failure of the decline in AP typically seen in late gestation rats [66]. Moreover, AT1-AAs alone have not been effective in inducing hypertension in pregnant rats in all studies [67], although the different AT1-AA source (immunized rabbits) could contribute to this discrepancy.

In pregnant mice, adoptive transfer of human AT1-AA (purified from the serum of preeclamptic women) results in increased circulating TNFα, and the resultant preeclampsia-like symptoms are at least partly mediated by this cytokine, because they were attenuated by TNFα neutralizing antibodies [68]. Further studies in this model, which uses IgG from normotensive pregnant women as a control, have suggested a role for IL-6 as an intermediary between TNFα and endothelin-1, downstream of AT1-AA mediated AT1 receptor activation [69]. Circulating sFlt1 and sEng are also increased in AT1-AA infused pregnant mice, an effect that is again blocked by TNFα antibodies [68]. Conversely, it has been shown that TNFα administration to pregnant rats results in increased circulating AT1-AA, and that AT1-AA partly mediates the resultant hypertension, because it is abrogated by co-administration of losartan [61]. TNFα–induced sFlt1 is also abolished by losartan, suggesting that AT1-AA is responsible for the elevation of sFlt1 in this model. Although these data are exciting, the precise interaction among these factors and the sequence of events leading to hypertension in these animal models remain to be clarified.

Another potential difficulty in this field is that the assessment of AT1-AA activity is not straightforward, historically relying on an indirect measure of neonatal rat cardiomyocyte beat frequency *in vitro*. This has made it difficult to verify AT1-AA seroprevalence in large numbers of patients. One recent study estimated that 71% of preeclamptic women harboured AT1-AA (vs. 19% of normal pregnant women), but this varied from 50 - 88% between early- and late-onset disease, respectively (n=31/group) [70]. A newer assay, utilising CHO cells expressing the rat AT1 receptor and a 4×NFAT-luciferase reporter construct, was used to determine AT1-AA seroprevalence among 37 preeclamptic women at >95% [71]. However, a larger study using the same assay (as well as a modified assay in HEK-293 cells) failed to detect serum AT1-AA in a population of 426 Mexican-Mestizo patients with preeclampsia, or in control subjects (n=99) [72]. Although ethnicity may be a factor in AT1-AA seroprevalence, the controversy over whether circulating AT1-AA is elevated in the majority of preeclamptic patients remains resolved. There is also considerable debate regarding the specificity of AT1-AA for preeclampsia [73-75]: for example, AT1-AA is also elevated in normotensive IUGR, suggesting perhaps (in line with RUPP model data) that increased circulating AT1-AA is secondary to uteroplacental insufficiency and hypoxia [75].

Very recentlyy, Jensen et al. demonstrated that circulating $CD19^{+}CD5^{+}$ B1-a B cells are increased in preeclamptic women, possibly due to elevated hCG levels, and that these cells produce AT1-AA in the presence of preeclamptic serum [76]. However, one potential cautionary note is that the investigators apparently did not show whether normal pregnant serum or hCG can induce AT1-AA production in $CD19^{+}CD5^{+}$ cells. Moreover, although

conditioned media from villous explants or serum prepared from preeclamptic women increased the levels of $CD19^{+}CD5^{+}$ cells *in vitro* associated with the higher hCG concentrations in these biological fluids, it would have been interesting to determine whether hCG immunoneutralization reversed the phenomenon, thereby supporting a causal role for hCG. If the pathogenic role of AT1-AA can be confirmed, it is possible that preeclamptic hypertension may be alleviated by immunoadsorption of AT1-AA or administration of the 7-amino acid epitope peptide. However, earlier studies demonstrated that plasmapheresis in preeclamptic women did not ameliorate disease symptoms, suggesting that circulating immunoglobulins were not causative [77] (of course, it is possible that the damage has already been done, so to speak, by the time of presentation, or that AT1-AA - AT1 receptor binding is long-lived). Furthermore, to our knowledge, there are no prospective studies demonstrating that circulating AT1-AAs are increased before clinical symptoms emerge, a postulate that has been fulfilled by other candidate pathogenic factors (e.g., sFlt1), and which would strengthen the case for a causal role of AT1-AA. In summary, excellent support is being marshalled for the pathogenic relevance of AT1-AA and its potential as a therapeutic target in preeclampsia, but controversy remains and further work is needed.

## RXFP1 and relaxin

Relaxin is a 6kD peptide hormone that emanates primarily from the corpus luteum. It induces dilation of renal and systemic arteries via a sustained and a more recently discovered rapid mechanism that involves endothelial NOS, Akt, PI3 kinase, and $G\alpha_{i/o}$ coupling to the primary GPCR, RXFP1 [78]. Circulating relaxin peaks in pregnant women around the end of the first or beginning of the second trimester [79]. Notably, this profile is consonant with the peak systemic and renal vascular adaptations in pregnancy (*vide supra*); in fact, this was one reason for initially investigating relaxin as a candidate factor responsible for these adaptations. Indeed, ablation of circulating relaxin by ovariectomy or administration of neutralizing antibodies to pregnant rats from d8 of gestation completely abolishes the increased stroke volume (SV), CO, and global arterial compliance (ACg), as well as decreased SVR and plasma osmolality ($P_{osm}$), normally observed at mid-pregnancy in this species (reviewed in [80, 81]). This treatment also abolishes mid-gestational increases in effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), and the reduction in renal vascular resistance (RVR). Conversely, administration of relaxin increases SV, CO, and ACg, and decreases SVR and $P_{osm}$. Via the sustained mechanism alluded to above, relaxin also induces renal vasodilation and hyperfiltration [80, 81]. That these effects are observed in both intact and ovariectomized female as well as male rats indicates that relaxin-induced vasodilation is sex steroid-independent. Human studies suggest that these effects are at least partly conserved, insofar as pregnant women who conceive through in vitro fertilisation with donor eggs (and who therefore lack a corpus luteum) exhibit subdued changes in creatinine clearance (an indirect measure of GFR) and $P_{osm}$ in the first trimester [82]. Therefore, relaxin appears to contribute to the systemic cardiovascular and renal adaptations in early pregnancy; whereas other hormones such as adrenomedullin, Ang(1-7) or placental growth factor may maintain the physiological hyperdynamic circulation later in pregnancy (more consistent with their circulating profiles).

The sustained mechanism of relaxin-induced vasodilation has been explored in detail. *In vivo*, increased ERPF/GFR and decreased RVR in response to relaxin infusion in rats was blocked by administration of L-NAME [83] and the specific $ET_B$ receptor antagonist, RES-701-1 [84], suggesting that endothelin and NO (synthase) mediate relaxin-induced vasodilation. These findings mirrored prior observations in normal pregnant rats [85, 86]. Inhibitors of the gelatinases MMP-2 and MMP-9, but not of endothelin converting enzyme, also prevented renal vasodilation and hyperfiltration in response to relaxin infusion in rats

[87]. The resultant working model from these studies is that relaxin stimulates renal vascular gelatinases, which cleave Big Endothelin to $ET_{1-32}$, [88] causing activation of the endothelial $ET_B$ receptor, NO production, and ultimately, vasodilation.

The phenomenon of myogenic constriction has also been exploited to study the sustained mechanism of relaxin-induced vasodilation in isolated arteries *ex vivo*. In normal vessels, this manifests as smooth muscle contraction and vascular constriction in response to a stepwise increase in intraluminal pressure, such that vessel diameter before and after the pressure jump is not significantly different. In contrast, vessels isolated from mid-term pregnant [87, 89] or relaxin-infused rats [90], or first isolated from non-pregnant rats, mice or humans and then incubated with relaxin *in vitro* [91], exhibit a phenotype of blunted myogenic constriction, such that arteries remain dilated after the pressure jump (i.e., diameter is significantly greater compared to baseline). Endothelial removal abolishes the effect in arteries isolated form mid-term pregnant or relaxin-infused rats [89-91]. *Ex vivo* studies have also revealed that relaxin regulates the passive mechanics of rat small renal arteries; this structural modification may contribute to increased ACg in response to relaxin infusion [92, 93].

In accordance with whole-animals studies, small renal arteries isolated from relaxin-infused nonpregnant rats or midterm pregnant rats and subsequently treated with NOS inhibitors or RES-701-1 [90], or with gelatinase inhibitors [87], exhibited robust myogenic constriction (i.e., the dilatory effect of relaxin was blocked). Similar observations were made when small renal arteries isolated from non-pregnant rats and mice, and subcutaneous arteries from humans were incubated *ex vivo* with relaxin and NOS/$ET_B$/MMP2/9 inhibitors [91]. Analysis of isolated arteries from relaxin-infused $ET_B$ receptor-deficient rats strongly suggested that MMPs act upstream of endothelin, because myogenic constriction was robust despite increased vascular gelatinase activity [87]. *In vitro*, using NO production by human endothelial cells as an endpoint, we have recently observed that relaxin-induced NO is again dependent on gelatinase(s), the $ET_B$ (but not $ET_A$) receptor and NOS (unpublished data). Hence, there is excellent congruency between the observed mechanisms of relaxin action *in vivo*, *ex vivo* and *in vitro*.

Most recently, we have shown that the angiogenic growth factors VEGF and PlGF are crucial intermediates in relaxin-induced vasodilation [91]. VEGF RTK inhibition with SU5416 and/or antibody-mediated VEGF/PlGF neutralization blocked the increases in ERPF and GFR in response to relaxin infusion in virgin rats, and prevented relaxin-induced dilation of isolated rat and mouse small renal as well as human subcutaneous arteries (without compromising endothelial function). Analysis of arterial gelatinase activity from relaxin/SU5416 treated rats did not, however, support the hypothesis that relaxin increases vascular MMPs via angiogenic growth factors. Instead, angiogenic growth factors may play a permissive role, e.g., by maintaining optimal levels of endothelial RXFP1, $ET_B$ or preproET expression (necessary intermediates for relaxin-induced NO production and hence vasodilation). *In vitro*, SU5416 also blocks relaxin-induced NO production from endothelial cells (unpublished data), again highlighting the consistency between *in vivo*, *ex vivo* and *in vitro* experimental paradigms with respect to relaxin's sustained vasodilatory mechanisms.

Because relaxin contributes to the systemic cardiovascular and renal adaptations of pregnancy through vasodilation and modification of arterial structure, it is reasonable to propose that it may be a useful therapeutic in preeclampsia. Additionally, relaxin was recently shown to mobilize and activate bone marrow-derived angiogenic cells [94]. Insofar as these cells are reduced in the circulation of preeclamptic women [95], thus compromising endothelial repair, relaxin therapy may be salutary on this basis as well. Like pregnancy, relaxin attenuates renal vasoconstriction (i.e., decreased ERPF) in rats chronically

administered angiotensin II [83, 96, 97], and increases CO and ACg, and decreases SVR, in models of AngII-induced hypertension and spontaneously hypertensive rats [98]. Thus, relaxin could potentially ameliorate aberrant AT1-AA activity in preeclampsia. Furthermore, the sustained mechanism of relaxin-induced vasodilation involves angiogenic growth factors, which, if upregulated by relaxin locally in the vascular wall, could mitigate the effects of elevated circulating sFlt1.

Unlike other vasodilators (e.g., CGRP and adrenomedullin), relaxin does not markedly reduce blood pressure in normotensive subjects. The reduction in afterload produced by decreased SVR is accompanied by a reciprocal elevation in CO, primarily through increased stroke volume, and to a lesser extent, heart rate. Therefore, one would not necessarily expect relaxin infusion to ameliorate hypertension in preeclamptic women. Blood pressure could instead be controlled by standard anti-hypertensive drugs [99]. Accordingly, placental perfusion would not be compromised by hypotension during relaxin administration. Interestingly, we have recently shown in a pilot study that a subset of women with plasma relaxin concentrations of < 500 pg/ml in the first trimester are at high risk for developing preeclampsia [100], raising the possibility of an etiological role for relaxin deficiency in the disease and further supporting the idea that relaxin or other RXFP1 agonists are viable therapeutic candidates in preeclampsia. Although provocative, the finding of an inverse relationship between low plasma relaxin concentration in the first trimester and subsequent preeclampsia needs to be corroborated in larger cohorts of patients.

## Perspectives and Conclusions

A recent search of the NIH website www.clinicaltrials.gov using the term “preeclampsia” in the “medical condition” field revealed 36 open interventional studies, of which 8 are specific investigations of GPCR agonists. However, most of these trials actually do not relate to preeclampsia or only peripherally so, e.g., interventions for postpartum bleeding, hypotension during spinal anesthesia, abnormal cardiovascular and autonomic reactivity in formerly preeclamptic women, and labor duration and obstetric complications in term pregnancies. Only 3 studies target hypertension in preeclamptic women, and all are investigations of calcium channel blockers and/or α2-adrenergic agonists / α1- and β-adrenergic antagonists. This search also reveals 39 completed, suspended or terminated studies, of which only 3 involved the use of GPCR agonists to prevent or treat preeclampsia/eclampsia (again predominantly α2-adrenergic agonists / α1- and β-adrenergic antagonists). Clearly then, the field is ripe for investigation of novel GPCR-based therapies in preeclampsia. An audit in January 2011 revealed that $17M was allocated to preeclampsia research by the NIH, a small fraction of the total NIH budget. Perhaps now would be an opportune time for the National Institutes of Health and non-governmental organizations to request grant applications for pre-clinical studies of therapeutics in preeclampsia. Undoubtedly, the submissions would include proposals to use specific CRLR/RAMP1, AT2, Mas or RXFP1 agonists, and perhaps other GPCRs and their agonists or antagonists. These investigations should uncover promising candidates to carry forward into clinical trials and on to the bedside.

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## Table

### GPCRs as potential therapeutic targets in preeclampsia

Circulating levels of the endogenous ligands of these vasodilatory GPCRs are elevated in normal pregnancy (some in association with the development phase of the maternal cardiovascular adaptations in the first trimester, others later in gestation), and most are reportedly decreased in preeclamptic patients. Administration of the endogenous ligands or of specific agonists during the clinical phase of preeclampsia may alleviate disease symptoms through systemic and/or uteroplacental vasodilation, as well as other mechanisms (see text for details and citations).

| GPCR | Endogenous ligand | Normal pregnancy | Preeclampsia |
|---|---|---|---|
| CRLR/RAMP1 | CGRP | ↑ | ↓ |
| CRLR/RAMP2 | Adrenomedullin | ↑ | ? |
| AT2 | AngII | ↑ | ↓ |
| Mas | Ang(1-7) | ↑ | ↓ |
| RXFP1 | Relaxin | ↑ | ↓ * |

* In the first trimester in a subset of patients who go on to develop preeclampsia