AT₁ Receptor Heterodimers and Angiotensin II Responsiveness in Preeclampsia

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Preeclampsia is a pregnancy-specific hypertensive disorder with unknown etiology, which affects 5% to 10% of all pregnancies. Increased sensitivity to the vasoconstrictor angiotensin II is a common feature of preeclampsia, although underlying mechanisms are barely understood. Recent data reveal a potential mechanism for the increased angiotensin II responsiveness in preeclampsia: increased levels of heterodimers between the vasopressor receptor AT₁ and the vasodepressor receptor B₂. The receptor heterodimers display increased sensitivity toward angiotensin II and are found in platelets and in omental vessels of preeclamptic women. Moreover, AT₁/B₂ receptor heterodimers are resistant to inactivation by reactive oxygen species, which is elevated in normal and preeclamptic pregnancies. Thus, a major symptom of preeclampsia is the result of complex formation between two G-protein-coupled receptors.

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PREECLAMPSIA IS a pregnancy-specific form of hypertension that complicates 5% to 10% of all pregnancies worldwide. The subsequently developing eclampsia increases both maternal and neonatal morbidity and mortality. In developing countries where prenatal care is not adequate, preeclampsia/eclampsia still accounts for 40% to 80% of maternal deaths, an estimated 50,000 per year. Preeclampsia is a specific form of gestational blood pressure elevation of greater than 140 mm Hg (systolic) or 90 mm Hg (diastolic) together with proteinuria (>300 mg of protein/24 hours) usually occurring after 20 weeks of gestation.¹

Preeclampsia is considered a two-stage disease. The first stage of preeclampsia is abnormal placentation with incomplete remodeling of maternal spiral arteries leading to poor placental perfusion. Reduced placental perfusion does not necessarily lead to preeclampsia, but it is still considered a major cause for the development of the second stage of preeclampsia: the maternal systemic disorder, which manifests by hypertension and proteinuria but which affects perfusion of virtually every organ. The link between the pathophysiology of abnormal placentation and the maternal multi-organ dysfunction is still unclear. Previous studies identified a panoply of different maternal and fetal factors, which seem to interact in a concerted action for the syndrome to develop. Despite the uneasiness concerning the pathogenesis of preeclampsia, a major alteration in the development of preeclampsia preceding the reduction in organ perfusion is the increased responsiveness of the vasculature to a few major pressor reagents such as angiotensin II.²⁻⁴ Interestingly, the sensitivity to endothelin-1, another pressor agent, is not altered.⁵⁻⁶ Because these aberrations manifest before the onset of clinical disease, the increased pressor sensitivity of the vasculature could be a key feature in unraveling the pathogenesis of preeclampsia. The current review focuses on mechanisms accounting for the increased responsiveness of the vasopressor angiotensin II in preeclampsia and attempts to relate such findings to the understanding of the pathogenesis of preeclampsia.

INCREASED ANGIOTENSIN II SENSITIVITY IN PREECLAMPSIA

Preeclampsia is characterized by abnormal vascular function and morphology. These abnormalities are considered important factors underlying the systemic hemodynamic changes in this disorder such as elevated peripheral resistance and blood pressure, and decreased placental blood flow. The abnormal vascular function manifests by enhanced in vivo and in vitro pressor sensitivity to the vasoconstrictor angiotensin II compared with pregnancies not complicated by preeclampsia.²⁻⁴ In addition to the pure phenomenon, the enhanced angiotensin II responsiveness can serve as a marker to discriminate normal pregnancies from...
pregnancies complicated by hypertensive disorders and to identify women at high risk to develop preeclampsia.\(^7\)\(^9\)

There seems to be a selective enhancement of the response to the vasoconstrictor angiotensin II, whereas the responses to endothelin-1 or thrombin are not different between normotensive and preeclamptic vessels,\(^5\)\(^6\)\(^10\) although the latter peptide hormones stimulate similar signalling pathways as the angiotensin II AT\(_1\) receptor. This observation points to specific alterations of the angiotensin II–AT\(_1\) system in preeclampsia. Studies on the angiotensin II responsiveness of platelets of preeclamptic women confirm this concept: although the increase in intracellular free calcium after thrombin stimulation is not significantly different between normotensive nonpregnant, normotensive pregnant and preeclamptic women, there is a specific increase in the calcium response to the vasoconstrictor angiotensin II in platelets of preeclamptic women.\(^10\) Thus, changes in components of the signalling cascades do not seem responsible for the enhanced angiotensin II responsiveness in preeclampsia.

Elucidating the mechanism of the increased angiotensin II responsiveness, much effort focused on alterations of the renin–angiotensin II system. In contrast to normotensive pregnancies, components of the circulating renin–angiotensin–aldosterone system are decreased in preeclampsia such as plasma renin activity, angiotensin I, circulating angiotensin II-converting enzyme (ACE), and circulating angiotensin II levels.\(^11\)\(^17\) However, the decreased activity of the circulating renin–angiotensin II system in preeclampsia does not correlate with changes in the number of AT\(_1\) receptors.\(^18\)\(^20\) Abnormalities of the circulating renin–angiotensin II system could therefore not be causal for the development of the increased angiotensin II sensitivity in preeclampsia.

**INCREASED AT\(_1\)/B\(_2\) RECEPTOR DIMERIZATION IN PREECLAMPSIA**

The previous studies demonstrated that the extracellular angiotensin II system and components of the intracellular signalling cascades do not seem responsible for the enhanced angiotensin II responsiveness in preeclampsia. In light of this knowledge, the AT\(_1\) receptor remains as the only component to mediate the increased angiotensin II responsiveness in preeclampsia. In general, there are very few possibilities to specifically increase the agonist responsiveness of a receptor without affecting other signalling components. Receptor heterodimerization is such a mechanism that modifies signalling of a panoply of different G-protein-coupled receptors specifically at the receptor level.\(^21\)\(^22\) Heterodimerization also modifies AT\(_1\) receptor responsiveness.\(^23\) A specific enhancement of the AT\(_1\) receptor responsiveness occurs when the AT\(_1\) receptor forms dimers with the receptor for the vasodepressor bradykinin, B\(_2\).\(^23\) Interestingly, the appearance of the increased angiotensin II responsiveness in platelets of preeclamptic women correlates with increased levels of AT\(_1\)/B\(_2\) receptor heterodimers.\(^24\) A similar increase in AT\(_1\)/B\(_2\) receptor heterodimerization like in platelets was detected on membranes of omental vessels from preeclamptic women.\(^24\) Omental vessels of preeclamptic patients also display enhanced angiotensin II sensitivity.\(^4\)\(^24\) AT\(_1\)/B\(_2\) receptor heterodimerization on preeclamptic platelets and vessels could be the result of a several-fold increase in cell-surface B\(_2\) receptors, whereas AT\(_1\) receptor levels are not significantly altered.\(^24\) Altogether, the appearance of increased levels of AT\(_1\)/B\(_2\) receptor heterodimers with increased angiotensin II responsiveness correlates with enhanced angiotensin II sensitivity in preeclampsia.

**AT\(_1\)/B\(_2\) RECEPTOR HETERODIMERIZATION AND INCREASED ANGIOTENSIN II SENSITIVITY**

AT\(_1\)/B\(_2\) receptor heterodimers show increased G-protein responsiveness and signalling.\(^23\)\(^24\) The appearance of AT\(_1\)/B\(_2\) receptor heterodimers correlates with increased AT\(_1\) receptor-stimulated G-protein activation and signalling in platelets and vessels of preeclamptic patients.\(^24\) These findings give rise to the conclusion that AT\(_1\)/B\(_2\) receptor heterodimers mediate an enhanced angiotensin II response in preeclampsia. In agreement with such a conclusion is the observation that specific inhibition of AT\(_1\)/B\(_2\) receptor heterodimers on preeclamptic vessels decreases the angiotensin II-stimulated G-protein activation;\(^24\) antibodies shielding a domain of the B\(_2\) receptor, which is required for AT\(_1\)/B\(_2\) receptor heterodimerization and signal enhancement,\(^23\) blocked the increased angiotensin II-stimulated G-protein activation on preeclamptic vessels while not altering the angiotensin II response on normotensive pregnant ves-
This finding is a strong indication that AT$_1$/B$_2$ receptor heterodimers mediate indeed, at least part of, the enhanced angiotensin II responsiveness in preeclampsia (Fig 1).

AT$_1$/B$_2$ RECEPTOR HETERODIMERIZATION REQUIRES AN INCREASE IN B$_2$ RECEPTOR NUMBER

AT$_1$/B$_2$ receptor heterodimers form only in cells with coexpression of AT$_1$/B$_2$ receptors. Therefore, the heterodimerization of AT$_1$ with B$_2$ in preeclampsia requires upregulation of the B$_2$ receptor. Indeed, such an increase in B$_2$ receptor number was detected on platelets and omental vessels of preeclamptic patients, which also displayed increased levels of AT$_1$/B$_2$ receptor heterodimers. A significant induction of B$_2$ receptors can be promoted by each of the major clinical signs of preeclampsia, e.g. uteroplacental ischemia, release of proinflammatory cytokines, decreased kallikrein levels, or sympathetic overactivity. Thus, the increase in AT$_1$/B$_2$ receptor heterodimerization seems specific for preeclampsia and mediates a major clinical symptom of this disorder, the increased angiotensin II sensitivity (Fig 1).

AT$_1$/B$_2$ RECEPTOR HETERODIMERS AND OXIDATIVE STRESS

Normotensive pregnancy and preeclampsia are characterized by a significant rise in circulating markers of oxidative stress. Functionally important cysteines and methionines of different proteins are sensitive to oxidative stress, and AT$_1$ receptors are also blocked by extracellularly applied oxidative stress. Hydrogen peroxide as a form of oxidative stress triggers reversible aggregation of AT$_1$ receptor monomers by intermolecular disulfide bond formation on platelets from normotensive pregnant women. In parallel, oxidative stress also decreases AT$_1$ receptor-stimulated signalling on platelets from normotensive pregnant women. Thus, oxidative stress inactivates the AT$_1$ receptor in normotensive pregnancy by triggering receptor aggregation (Fig 2).

In contrast to AT$_1$ receptor monomers, preformed AT$_1$/B$_2$ receptor heterodimers of preeclamptic women are stabilized by disulfide bonds and reactive oxygen has no effect on AT$_1$/B$_2$ receptor heterodimers. In agreement with this finding, AT$_1$/B$_2$ receptor-stimulated signalling is insensitive to extracellularly applied oxidative stress. Thus, AT$_1$/B$_2$ receptor heterodimerization confers the AT$_1$ receptor...
resistance to inactivation by reactive oxygen species. This resistance of AT1/B2 receptor heterodimers to inactivation maintains angiotensin II signalling in preeclampsia (Fig 2),24 whereas inactivation of AT1 receptor homodimers by oxidative stress correlates with blunted angiotensin II signalling in normotensive pregnancy (Fig 2).2-4

RELATIONSHIP BETWEEN PREECLAMPSIA AND ESSENTIAL HYPERTENSION

Is the pathogenesis of preeclampsia–hypertension related to the pathogenesis of other hypertensive disorders such as essential hypertension? Major features of the AT1 receptor system in preeclampsia–hypertension are strikingly similar with essential hypertension. Essential hypertensive patients (at least a major subgroups thereof) are like preeclamptic patients, also characterized by enhanced angiotensin II responsiveness,32-34 whereas AT1 receptor number is largely unaltered in essential hypertension13,36 and in preeclampsia.18-20 It is therefore tempting to speculate that the dysregulation of the AT1 system in preeclampsia–hypertension and in essential hypertension reflects common pathogenetic features.

Many observations seem to confirm this hypothesis. Preeclampsia and essential hypertension are characterized by the syndrome of endothelial dysfunction,37,38 they are established risk factors for the development of vascular disease in later life,39,40 kallikrein levels of preeclamptic patients41 and of a major subgroup of essential hypertensive patients are decreased42 like is renin secretion,11,12,43 and there is increased activity of the adrenergic system in both hypertensive disorders.44,45 Although this compari-

Fig 2. Reactive oxygen species inactivates AT1 and AT2 receptor monomers and leads to decreased AT1 receptor-stimulated signalling in normotensive pregnancy. In contrast, AT1/B2 receptor heterodimers of preeclamptic patients are resistant to inactivation by oxidative stress, thereby maintaining increased AT1 receptor-stimulated signalling in preeclampsia.

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