Roles of Angiotensin Type 1 and 2 Receptors in Pregnancy-Associated Blood Pressure Change

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**Background:** Activation of the renin-angiotensin system with increased levels of renin and angiotensin (Ang) II in pregnancy has been reported, but the vascular responsiveness to Ang II seems to be decreased, thereby keeping maternal blood pressure (BP) constant. We postulated that the balance of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptor expression, which would exert antagonistic actions on vasoconstriction and cell growth, might control BP in pregnancy.

**Methods:** Using wild type (C57BL/6J), angiotensin type 1a (AT1a) receptor null and AT2 receptor null mice, we examined the changes in BP, expression and localization of AT1 and AT2 receptors in placenta, umbilical cord, and uterus by immunohistochemical staining and urinary albumin measurement during pregnancy.

**Results:** Wild type mice did not show any significant change in BP throughout pregnancy. The BP in AT1a receptor null mice declined significantly in the second trimester of pregnancy, whereas BP in AT2 receptor null mice increased significantly in the third trimester. We did not observe any significant differences in albuminuria, litter size, or body weight of neonates among the three groups. Vascular smooth muscle cells in blood vessels of the umbilical cord and placenta specifically expressed AT2 receptors, which are minimally expressed in adult vessels. In contrast, AT1 receptors were dominantly expressed in the cytotrophoblast and chorionic plate as well as blood vessels in placenta and umbilical cord.

**Conclusions:** Our results suggested that disturbance of the balance of the AT1 and AT2 receptors could trigger pregnancy induced hypertension. Am J Hypertens 2004; 17:684–689 © 2004 American Journal of Hypertension, Ltd.

**Key Words:** Angiotensin II, pregnancy.

Following the development of specific angiotensin (Ang) receptor antagonists, Ang receptors can be classified into several subtypes: the two major subtypes of Ang II designated as type 1 (AT1) and type 2 (AT2) have been cloned. 1-4 Angiotensin II exerts diverse actions on its target tissues, controlling vascular tone, hormone secretion, tissue growth, and neuronal activity via the AT1 receptor. 5 The AT2 receptor is abundantly and widely expressed in fetal tissues. It is present only at low levels in adult tissues; however, it is re-expressed in certain pathologic conditions such as wound injury, myocardial infarction, vascular injury, and ovarian atresia, suggesting that this receptor is closely associated with development, differentiation, and remodeling. 6,7 It has been reported that blood pressure (BP) in AT1a(+/−) mice was reduced by 12 mm Hg and in AT1a(−/−) was reduced by 24 mm Hg compared with that in wild type control mice. 7 In contrast, the vasopressor response to Ang II was enhanced in AT2 receptor null mice, although the baseline BP was normal or increased. 8 These reports suggest that the AT1 and AT2 receptors may exert opposing actions in BP regulation.

Preeclampsia is a hypertensive disorder of pregnancy that occurs in 5% to 7% of human pregnancies and is a major complication of human pregnancies. It is characterized by hypertension, increased peripheral vascular resistance, and proteinuria, although its pathogenesis is still an enigma. During normal pregnancy the renin-angiotensin system is activated, with increased levels of plasma renin and Ang II, whereas maternal BP is maintained normally or is even decreased despite the increase in circulating Ang II. 10,11 The pressor response to Ang II is mainly mediated by way of specific vascular receptors, suggesting that changes in the expression in the vascular AT1 and...
AT2 receptors during pregnancy are involved in the decreased responsiveness to circulating Ang II. Thomas et al.\(^\text{12}\) reported that administration of the Ang II antagonist losartan tended to diminish mean arterial BP and reduced proteinuria in an endotoxin induced preeclampsia rat model. Recently it has been reported that patients with preeclampsia develop agonistic autoantibodies to the AT1 receptor.\(^\text{13}\) Moreover, increased AT1 receptor heterodimers with the bradykinin B2 receptor mediated were found to enhance Ang II responsiveness in preeclampsia.\(^\text{14}\) Burrell et al observed that uterine arteries from pregnant sheep during late gestation contain a large proportion of AT2 receptors (70%), unlike most adult blood vessels, and that the high density of AT2 receptors might contribute to the refractoriness of uterine arteries to Ang II.\(^\text{15}\) McMullen et al.\(^\text{16}\) reported that in uterine arterial rings prepared from pregnant ewes, AT2 receptor stimulation inhibited AT1 receptor mediated vascular contractions. These results led us to postulate that the balance of AT1 and AT2 receptor expression may control BP in pregnancy. To examine these possibilities, we used AT1a receptor null mice and AT2 receptor null mice, which provide unique opportunities to examine the roles of the AT1 and AT2 receptors.

### Methods

#### Animals

For this study, AT1a receptor null mice\(^\text{17}\) were donated by Tanabe Seiyaku Co. (Osaka, Japan), and AT2 receptor null mice\(^\text{8}\) were originally donated by Dr. Victor J. Dzau (Harvard Medical School, Boston, MA). Wild type mice were used as controls. Genetic background of three types of mice was C57BL/6J. These mice were kept in our laboratory. Mice 10 to 15 weeks of age were used in the following experiments. All mice were housed individually in metabolic cages within a specially designed, chronic, rodent, hemodynamic monitoring facility (CL-0305; Clea Japan Inc., Osaka, Japan) and housed at room temperature maintained at 22°C. They were given a standard diet and water ad libitum. The Animal Studies Committee of Ehime University approved the experimental protocol.

#### Mating and BP Measurements

Blood pressure was monitored according to the widely used method of Takimoto et al.\(^\text{18}\) Briefly, mice (\(n = 10\) to 12/group) were first conditioned to the tail-cuff BP apparatus (BP monitor, Muromachi Kikai, Co.) for 5 days. Conditioning was performed daily for 30 min between 9 AM and 12 noon. Subsequently, mice were mated for 12 h. At the conclusion of the cohabitation period, the presence of a plug of semen was confirmed visually or by gentle probing of the vaginal orifice using a blunt cotton tip. The identification of a plug was defined as day 0 of gestation. Mice were placed on a warming plate with temperature preset at 37°C. Five successive BP readings were averaged to establish the BP.

#### Measurements of Urinary Albumin

Mice (\(n = 10\) to 12/group) were housed in individual metabolic cages for urine collection. The urine was collected for 24 h and urinary albumin concentration was determined by enzyme-linked immunosorbent assay using a murine microalbuminuria kit (AlbuwellM; Exocell, Philadelphia, PA).

#### Immunohistochemical Examination

Placenta, umbilical cord, and kidney (\(n = 4\) to 5/group) were obtained immediately after perfusion fixation of the animal before gestation and after 10 and 18 days of gestation. Tissue samples were fixed in 10% formalin and subsequently embedded in paraffin. Sections were prepared and incubated with xylene and were hydrated through several washes in ethanol and water to remove paraffin. Polyclonal rabbit anti-AT1 receptor (Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal rabbit anti-AT2 receptor antibody,\(^\text{19}\) anti-cytokeratin (Santa Cruz Biotechnology) and anti–α–smooth muscle actin (clone 1A4; Sigma Chemical, St. Louis, MO) was used for immunostaining.
Statistical Analysis

All values are expressed as means ± SEM. Analysis of variance with subsequent Bonferroni/Dunnett test was used to determine the significance of differences in multiple comparisons. Values of $P < .05$ were considered statistically significant.

Results

BP Changes in Pregnancy

The changes in BP before and after pregnancy are shown in Fig. 1. Wild type mice did not show any significant change in BP throughout pregnancy (Fig. 1A). The BP in AT1a receptor null mice declined significantly in the second trimester (days 7 to 13) of pregnancy (Fig. 1B), whereas BP in AT2 receptor null mice increased significantly in the third trimester (days 14 to 20) of pregnancy (Fig. 1C). These changes in BP returned to the levels before pregnancy after delivery in each mouse strain. We did not observe any significant differences in albuminuria, litter size, or body weight of neonates among the three groups.

Expression of AT1 and AT2 Receptors

In the placenta and umbilical cord, AT1 receptors were dominantly expressed in the cytotrophoblast and chorionic plate, which express cytokeratin, as well as in blood vessels, which express $\alpha$-smooth muscle actin, in both the second and third trimesters (Figs. 2 to 6, upper panels, and Fig. 7). Vascular smooth muscle cells in blood vessels of

FIG. 2. Expression of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors, cytokeratin, and $\alpha$-smooth muscle (SM) actin in placenta from wild type mice at 10 days of gestation. Paraffin embedded sections were stained as described in Methods. Representative immunohistochemical staining of four to five independent experiments is shown (magnification ×50). Arrows indicate positive staining in cytotrophoblast for AT1 receptor and in the placental artery for AT2 receptor.

FIG. 3. Expression of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors, cytokeratin and $\alpha$-smooth muscle (SM) actin at maternal side of placenta from wild type mice at 18 days of gestation. Paraffin embedded sections were stained as described in Methods. Representative immunohistochemical staining of four to five independent experiments is shown (magnification ×100). Arrows indicate positive staining in cytotrophoblast for AT1 receptor and cytokeratin.
the umbilical cord and placenta specifically expressed AT2 receptors throughout pregnancy (Figs. 2 to 6, lower and middle panels, and Fig. 7), which are minimally expressed in adult vessels. No apparent changes in AT1 and AT2 receptor expression were found in the uterus before and after pregnancy. In the maternal kidney, there were no significant changes in AT1 and AT2 receptor expression before and after pregnancy. With immunohistochemical staining, AT1 receptor expression in the AT2 null mice and AT2 receptor expression in the AT1a receptor null mice did not appear to be different from those in the wild type mice (data not shown).

**Discussion**

Recent evidence has revealed that the functions of the AT1 and AT2 receptors are mutually antagonistic in BP regulation. We demonstrated in this study that the BP of AT2 receptor null mice increased significantly in the third trimester of pregnancy, whereas that of AT1a receptor null mice declined significantly in the second trimester. Although the pathogenesis of preeclampsia is not well understood, compelling evidence has implicated the placenta as a central culprit in the disease. The disease can occur even in the absence of a fetus, and removing the placenta cures preeclampsia. After delivery and removal of the placenta, these changes in BP return to the levels before pregnancy. In humans, the placenta has been considered to possess a local renin-angiotensin system, which may play a physiologic role in the regulation of uteroplacental blood circulation. In normal pregnancy, the systemic vasculature becomes refractory to the effects of Ang II as early as week 20 of gestation. The uteroplacental vasculature in pregnant women is more refractory than the systemic vasculature, and women with preeclampsia are more sensitive to infused Ang II than are normal pregnant women. Changes in the production of such placental Ang II as well as the expression of the AT1 and AT2 receptors during pregnancy could be important in the physiologic and pathophysiologic aspects of BP regulation in pregnancy.

We examined the localization of AT1 and AT2 receptor expression in the placenta during pregnancy. We observed that vascular smooth muscle cells in blood vessels of the

**FIG. 4.** Expression of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors, cytokeratin, and α-smooth muscle (SM) actin at maternal side of placenta from wild type mice at 18 days of gestation. Representative immunohistochemical staining of four to five independent experiments is shown (magnification ×200).

**FIG. 5.** Expression of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors, cytokeratin, and α-smooth muscle (SM) actin at fetal side of placenta from wild type mice at 18 days of gestation. Representative immunohistochemical staining of four to five independent experiments is shown (magnification ×100). Arrows indicate positive staining in chorionic plate (left) and placental artery (right) for AT1 and AT2 receptors.
umbilical cord and placenta specifically expressed AT2 receptors in both the second and third trimesters. On the other hand, AT1 receptors were dominantly expressed in the cytotrophoblast and chorionic plate as well as blood vessels in the placenta and umbilical cord. In contrast, there were no detectable changes in AT1 and AT2 receptor expression in the maternal kidney before and after pregnancy. These results suggest that AT2 receptor expression in blood vessels in the placenta and umbilical cord is one of the important factors regulating BP during pregnancy. Recently, AbdAlla et al.\textsuperscript{22} reported that the AT2 receptor binds directly to the AT1 receptor, thereby antagonizing the function of the AT1 receptor. These investigators also reported that AT1 receptor specific antagonism of the AT2 receptor was independent of AT2 receptor activation and signaling and that it was effective in different cells, supporting the notion that the AT2 receptor plays critical roles in BP regulation by antagonizing the AT1 receptor.

It is possible that AT1 receptors in the syncytiotrophoblasts play a pathophysiologic role in patients with preeclampsia.\textsuperscript{23} The increased expression of AT1 receptor protein was predominantly localized to the syncytiotrophoblast, which is responsible for the secretion of numerous pregnancy associated polypeptides into the maternal circulation. Recent in vitro studies using human trophoblastic cells have demonstrated that Ang II stimulated human placental lactogen and specific β1-glycoprotein secretion through the AT1 receptor.\textsuperscript{24} One peptide, neurokinin B, has recently been reported to be excessively secreted during the third trimester of pregnancy; this peptide may contribute to the pathogenesis of preeclampsia.\textsuperscript{25}

**FIG. 6.** Expression of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors, cytokeratin, and α-smooth muscle (SM) actin at fetal side of placenta from wild type mice at 18 days of gestation. Representative immunohistochemical staining of four to five independent experiments is shown (magnification ×200).

**FIG. 7.** Expression of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors, and α-smooth muscle actin in umbilical cord from wild type mice. Samples were obtained from wild type mice at 18 days of pregnancy. Paraffin-embedded sections were stained as described in Methods. Representative immunohistochemical staining of four to five independent experiments is shown (magnification ×100). Arrows indicate positive staining in umbilical artery and vein.
although the roles of Ang II in neurokinin B production and secretion have not been well defined.

Taken together, our results suggest that AT1 and AT2 receptors in the umbilical cord and placenta may contribute at least partially to pregnancy induced maternal BP changes by exerting opposing actions in BP regulation. It is possible that disturbance of the expression of the AT1 or AT2 receptors could trigger pregnancy induced hypertension. More detailed analysis of the roles of changes in AT1 and AT2 receptor expression would help to further our understanding of the pathophysioologic mechanism of pregnancy induced hypertension.

However, as we did not observe an increase in albuminuria or a change in litter size or body weight of neonates among wild type AT1 receptor null and AT2 receptor null mice, it would seem that additional factors are necessary to act together with Ang II to induce preeclampsia. More detailed identification of the roles of these pregnancy associated factors as well as the crosstalk of Ang II with these factors will provide further understanding of the pathogenesis of pregnancy induced hypertension.

References


