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Angiotensin II Type 1 Autoantibody Induced Hypertension during Pregnancy is associated with Renal Endothelial Dysfunction

Marc R. Parrish, D.O.¹, Michael J Ryan, PhD², Porter Glover², Justin Brewer, M.D.¹, Lillian Ray¹, Ralf Dechend, PhD³, James N. Martin Jr, M.D.¹, and Babbette B. Lamarca, PhD^{1,2}

¹Departments of Obstetrics & Gynecology (Division of Maternal Fetal Medicine), Jackson MS, 392162)

²Department of Physiology & Biophysics and the Center for Excellence in Cardiovascular- Renal Research University of Mississippi Medical Center, Jackson MS, 39216

³HELIOS Clinic, Charite, Campus-Buch & Max-Delbrueck Center, Berlin Germany

Abstract

Objective—Previous investigations suggest that agonistic autoantibodies to the angiotensin II type I receptor (AT1-AA) may mediate a hypertensive response through dysregulation of the endothelin-1 system. AT1-AA induced hypertension is attenuated by AT1 receptor and/or Endothelin-1 type A receptor antagonists. This study was undertaken to determine if AT1-AA induced hypertension is associated with renal endothelial dysfunction.

Study Design—We compared the vascular reactivity of renal interlobar arteries from normal pregnant control rats and AT1-AA chronically infused pregnant rats in the presence and absence of endothelin type A (ET_A) receptor antagonism. Renal endothelial function was tested using isolated renal interlobar arteries in a pressure myograph that were exposed to acetylcholine or sodium nitroprusside.

Results—Vasodilatory responses to the endothelial dependent agonist acetylcholine were impaired in AT1-AA rats (74±10%) compared to NP controls (95±5%, p<0.05). In the presence of endothelin type A (ET_A) receptor antagonism, no differences were observed between controls or the AT1-AA treated group in regard to endothelial dependent (acetylcholine) relaxation.

Conclusion—AT1-AA induced hypertension during pregnancy is associated with disparate renal endothelial responses to acetylcholine. The difference in renal vascular responses between AT1-AA and NP rats is abolished by ETA receptor blockade.

Keywords

Hypertension; angiotensin; pregnancy; endothelial dysfunction

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Address correspondence to: Babbette LaMarca, Ph.D Departments of Obstetrics & Gynecology and Physiology Division of Maternal Fetal Medicine University of Mississippi Medical Center 2500 North State Street Jackson, MS 39216-4505 Phone: 601-815-1430 FAX: 601-984-1817 bblamarca@umc.edu.

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Introduction

The initiating event in early onset preeclampsia is postulated to involve reduced placental perfusion that leads to hypertension during pregnancy by mechanisms not yet elucidated.^{1,2} Recent studies have suggested that the production of agonistic autoantibodies to the angiotensin II (AngII) type I receptor (AT1-AA) may be an important link between placental ischemia and hypertension in preeclamptic women.³⁻⁸ The AT1-AA induces signaling in vascular cells including activating protein-1, calcineurin and nuclear factor kappa B activation which are blocked by an AT1 receptor antagonist.^{3,4,7} Recent studies by Zhou *et al.* demonstrate that immunoglobulin isolated from preeclamptic women increased systolic blood pressure and causes renal pathology four days after retro-orbital injection into pregnant mice.^{7,8} This hypertensive response was attenuated by administration of an AT1 receptor antagonist. While these findings suggest that AT1-AAs from preeclamptic women increases blood pressure in pregnant mice, possibly by activation of the AT1 receptor, it remains unclear by what mechanism purified AT1-AA mediates hypertension, renal or vascular function during pregnancy.

We recently reported that the hypertension in response to reductions in uterine perfusion pressure in pregnant rats (RUPP) is associated with circulating levels of the AT1-AA.⁹ Moreover, we found that the increased blood pressure response in RUPP pregnant rats decreased markedly by antagonism of the AT1 receptor. In addition, we previously reported that the hypertension in response to chronic AT1-AA infusion is associated with significant increases in placental and renal expression of preproendothelin and this blood pressure response is attenuated by administration of a selective endothelin type A (ET_A) receptor antagonist.¹⁰ In contrast, ET_A receptor blockade had no significant effect on blood pressure in the normal pregnant animal suggesting a role for ET-1 in mediating AT1-AA induced hypertension^{11,12}. However, there are still a number of unanswered questions that remain in this field of investigation. While AT1-AA causes significant hypertension and renal pathology, the role of the AT1-AA to impaired renal hemodynamics, proteinuria, or renal vascular function during pregnancy is unclear. Therefore, this current investigation was performed in order to evaluate the potential role of the AT1-AA to mediate renal endothelial vascular function during pregnancy.

Materials and Methods

All studies were performed in timed pregnant Sprague Dawley rats purchased from Harlan Inc. (Indianapolis IN). Animals were housed in a temperature controlled room (23°C) with a 12:12 light: dark cycle. All experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi Medical Center.

Rat AT1-AA was isolated and purified from a pregnant female hAogen × male hRen (MDC, Berlin) rat cross that develops hypertension associated with production of the AT1-AA beginning on day 13 of gestation.¹³ The complete details of rat AT1-AA isolation and purification have been previously published.^{3,13} Measurement of arterial pressures in chronically instrumented conscious rats was determined in all groups of pregnant rats at day 19 of gestation as previously described.^{9,10}

Chronic administration of rat AT1-AA

To determine if AT1-AA contributes to renal endothelial dysfunction, purified rat AT1-AA was infused from day 12 to 19 of gestation via mini-osmotic pumps (model 2002, Alzet Scientific Corporation, Palo Alto, CA) into normal pregnant rats (n=5) and normal pregnant

rats orally treated (drinking water) with the ET_A receptor antagonist (ABT-627, 5mg/kg/day) for 5 days (n=3). Normal pregnant rats (n=5) and Normal pregnant rats treated with ET_A receptor antagonist (n=3) alone served as controls. Blood pressure and vascular function was compared among the groups on day 19 of gestation.

Vascular studies

On day 19 of gestation, animals were sacrificed, kidneys were removed, and the renal interlobar arteries were isolated from and prepared for vessel reactivity studies in a pressure myograph as previously described.¹⁴ The interlobar artery was equilibrated at a baseline intraluminal pressure of 75 mmHg. Vasodilation was assessed in vessels that were precontracted with phenylephrine (10^{-5} M). This dose of phenylephrine has previously been shown to induce a sustained vasoconstriction in renal vessels and there was no difference in phenylephrine induced constriction at this concentration.^{14,15} After precontraction, concentration responses to acetylcholine and sodium nitroprusside (10^{-9} to 10^{-4} M) were performed to assess endothelial dependent and smooth muscle dependent vasodilation, respectively. The degree of vessel response to acetylcholine and sodium nitroprusside was determined by measuring the change in the lumen diameter via an electronic caliper with each subsequent exposure to increasing concentrations of these vasodilatory agents. Data are presented as the percent dilation relative to the precontracted inner diameter of the vessel.

Statistics

All data are expressed as mean $\pm$ standard error. Differences between control and experimental groups were analyzed using the student t-test. Differences between multiple groups were analyzed via one way ANOVA with a Student Newman-Keuls Post Hoc test. Associations were considered significant if the p-value was < 0.05.

Results

AT1-AA induced hypertension

As we have previously reported, chronic administration of rat AT1-AA into normal pregnant rats significantly increases blood pressure.¹² In addition, administration of a selective ET_A receptor antagonist for 7 days to normal pregnant control rats and pregnant rats chronically treated with AT1-AA abolished AT1-AA induced hypertension. This study was performed to determine if the AT1-AA induced hypertension was associated with altered renal vascular function and whether differences in renal vascular function are abolished after ETA blockade.

AT1-AA induced renal vascular dysfunction

Renal endothelial function was tested using isolated renal interlobar arteries in a pressure myograph. Vasodilatory responses to the endothelial dependent agonist acetylcholine (10^{-8} M) were lower in AT1-AA rats ($74\pm 10\%$) compared to NP controls ($95\pm 5\%$, $p<0.05$). The response to the smooth muscle dependent agonist sodium nitroprusside was not different (Figure 1). However, in the presence of the ET_A receptor antagonist, vasodilatory responses to the endothelial dependent agonist acetylcholine were not significantly different between the controls and those rats exposed to chronic excess levels of the AT1-AA (Figure 2).

Comment

There is growing evidence to suggest that dysregulation of the tissue-based and circulating reninangiotensin system (RAS) may be involved in the pathophysiology of preeclampsia.¹⁶⁻¹⁹ One mechanism by which this system may exert its effects is through the

excess production of an agonistic autoantibody to the angiotensin II type 1 receptor (AT1-AA).^{17,21} This autoantibody is thought to be a central mediator of several pathways in preeclampsia. However, both the specific mechanisms that lead to excess production and the mechanisms whereby AT1-AA increases blood pressure during pregnancy remain unclear. One proposed mechanism of AT1-AA induced hypertension includes stimulation of the endothelin-1 system, one hallmark of endothelial dysfunction, and a characteristic of preeclampsia. We recently demonstrated that increasing levels of AT1-AA to that observed in preeclamptic women and in placental ischemic rats, led to increased mean arterial pressure (MAP) in pregnant rats via activation of the endothelin system.¹² In the current investigation, we evaluated renal endothelial function during chronic AT1-AA infusion. Vasodilatory responses to the endothelial dependent agonist acetylcholine were reduced in AT1-AA induced hypertensive pregnant rats compared to normal pregnant controls. The response to sodium nitroprusside was not different suggesting normal smooth muscle function. Furthermore, the response to acetylcholine was not different between animals treated with the Endothelin Type A receptor antagonist. The fact that renal vascular function is different between AT1-AA and vehicle treated animals, but not in animals treated with ETA receptor blockers, suggests that one mechanism whereby AT1-AA could contribute to hypertension during pregnancy is via alterations in renal vascular function that are dependent upon the ET-1 pathway.

Our laboratory has been evaluating the role of this autoantibody in a pregnant RUPP rat model. We recently demonstrated that placental ischemic-induced hypertension in pregnant rats is associated with increased circulating levels of the AT1-AA.²² In addition, we demonstrated that chronic elevation of TNF alpha in pregnant rats is associated with increased production of the AT1-AA.⁹ Moreover, we discovered that the hypertension in response to placental ischemia in pregnant rats and in response to chronic infusion of TNF alpha in pregnant rats was markedly attenuated by antagonism of the AT1 receptor. Collectively, these novel findings indicate that placental ischemia and TNF alpha are important stimuli of AT1-AA production during pregnancy and that activation of the AT1 receptor appears to play an important role in the hypertension produced by placental ischemia and TNF alpha in pregnant rats. However, the mechanism(s) by which the autoantibody mediates hypertension has remained undefined.

Although the present study demonstrates that infusion of the AT1-AA is associated with altered renal endothelial function, whether these local vascular changes promote renal hemodynamic impairment that leads to hypertension is not clear. In addition, the stimulus and mechanism whereby the AT1-AA is produced in response to placental ischemia or TNF alpha during pregnancy remains undefined. Experiments designed to inhibit the production of the AT1-AA in pregnant RUPP rats could contribute to our understanding of the pathophysiological role of the autoantibody during pregnancy.

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Figure 1.

Vasodilatory responses to the endothelial dependent agonist acetylcholine (ACh) (10^{-8} M) were impaired in AT1-AA rats ($74 \pm 10\%$) compared to NP controls ($95 \pm 5\%$, $p < 0.05$). The response to sodium nitroprusside (SNP) was not different.

Figure 2.

In the presence of endothelin type A (ET_A) receptor antagonism, no differences were observed between controls or the experimental group in regard to endothelial dependent (ACh=acetylcholine) or smooth muscle dependent (SNP=sodium nitroprusside) relaxation.