Published in final edited form as:

Hypertens Pregnancy. 2010 ; 29(3): 284–293. doi:10.3109/10641950902777739.

Impact of Endothelin A Receptor Antagonist Selectivity in Chronic Nitric Oxide Synthase Inhibition-Induced Fetal Growth Restriction in the Rat

Mark G. Neerhof, D.O.^{1,2}, Sylvia Synowiec, B.S.¹, Saira Khan, M.S.¹, and Larry G. Thaete, Ph.D.^{1,2}

¹ Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, NorthShore University HealthSystem, Evanston, Illinois 60201

² Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

Abstract

Objective—Endothelin receptor A (ET_A) antagonism improves fetal and placental growth and placental perfusion on days 1 and 4, but not day 7 of a 7-day infusion of a nitric oxide synthase (NOS) inhibitor. Our purpose was to evaluate the significance of the degree of ET_A antagonist selectivity on uteroplacental perfusion and fetal growth on day 7 of chronic NOS inhibition.

Methods—Timed-pregnant rats were treated with the NOS inhibitor nitro-L-arginine methyl ester (L-NAME, 2.5 mg/kg/h) with and without one of the following ET_A antagonists or their respective vehicles for 7 days beginning on day 14 of gestation: A-127722 (2,000-fold selective for ET_A over ET_B), FR139317 (8,000-fold ET_A-selective), or ABT-546 (28,000-fold ET_A-selective). Uterine and placental perfusion, as well as fetal and placental weight, was evaluated at the 7th day of treatment (gestation day 21).

Results—L-NAME administration resulted in a significant reduction in uterine and placental perfusion as well as fetal and placental growth. In the setting of NOS inhibition, ET_A antagonism did not improve uterine or placental perfusion or fetal growth after 7 days of infusion irrespective of the degree of selectivity of the antagonist used.

Conclusions—ET_A antagonism, irrespective of the degree of receptor selectivity, does not improve fetal growth or uteroplacental perfusion on day 7 of chronic NOS inhibition.

Keywords

Endothelin antagonism; Fetal growth restriction; Nitric oxide; Perfusion; Rat

INTRODUCTION

Fetal growth restriction (FGR) remains a common cause of perinatal morbidity and mortality. Although there are many potential causes of FGR, the most common identifiable cause is suboptimal uteroplacental perfusion (1). Vasoactive mediators play an important role in the regulation of the vasculature of the uterine placental bed and placenta. Prominent among these mediators are endothelin-1 (ET-1) and nitric oxide (NO) (2–5).

ET-1 is a potent vasoconstrictor and is part of a family of peptides that includes three isoforms, ET-1, ET-2, and ET-3. The endothelins are produced mainly by endothelial or epithelial cells and act primarily as paracrine mediators. Their predominant effect is vasoconstriction, although they also affect cell proliferation, differentiation, and migration, as well as chemokinesis and apoptosis (6,7). Endothelin mediates its effects via two types of receptors. The ET_A receptor has a high affinity for ET-1, is located on smooth muscle cells, and is responsible principally for the vasoconstrictive and proliferative effects of ET-1. The ET_B receptor, located primarily on endothelial cells, binds all three ET isoforms and is principally responsible for vasodilation via nitric oxide, although it can mediate vasoconstriction in some vascular beds (8). ET_B also functions as a clearance receptor to remove excess ET from the circulation (9). During the third trimester of normal pregnancy, administration of an ET_A antagonist alone has no impact on uteroplacental perfusion or on fetal or placental growth in the rat (10,11). Conversely, administration of either an ET_B antagonist or a mixed ET_A/ET_B antagonist results in decreased uteroplacental perfusion and decreased fetal and placental growth, demonstrating the importance of ET antagonist selectivity on these parameters (10,12).

Nitric oxide (NO), a potent vasodilator, is a product of nitric oxide synthase (NOS). NOS inhibition results in vasoconstriction and hypertension (13) that are secondary not only to a decreased production of NO, but also to an upregulation of ET-1. NOS inhibition has been shown to increase ET-1 production in cultured HUVEC cells in vitro, and NOS inhibition in the pregnant rat results in increased circulating ET-1 (14,15). Further, NO decreases the affinity of ET_A for its ligand, displacing bound ET-1 (16–18). With reduced NO in the setting of NOS inhibition, the affinity of ET_A for ET-1 is increased (19).

NOS inhibition in the rat is an established model for the study of FGR. NOS inhibition from day 14 to 21 of the 22-day gestation results in decreased uterine and placental perfusion, decreased fetal and placental growth, and an increase in maternal circulating ET-1 (10,11,15,19–21). In the setting of chronic NOS inhibition, administration of a mixed ET_A/ET_B antagonist does not improve fetal or placental growth or placental perfusion at any time during administration (10). Conversely, administration of the ET_A antagonist A-127722 (2,000-fold selective for ET_A over ET_B) improves uterine and placental perfusion on days 1 and 4 of infusion, but not on day 7 (11). Fetal weights were improved, but not normalized on day 7. Given the known adverse impact that ET_B antagonism has on uteroplacental perfusion and fetal growth (12), we hypothesized that a more highly ET_A-selective antagonist may have a more favorable impact on perfusion and growth. In subsequent preliminary experiments, we found that placental perfusion, but not fetal growth, on day 7 of antagonist infusion was significantly improved by the more highly ET_A-selective antagonist FR139317 (8,000-fold ET_A-selective) compared to A-127722. We therefore undertook to test and compare these two antagonists with an ET_A antagonist having an even greater degree of selectivity, ABT-546 (28,000-fold ET_A-selective) to evaluate the significance of the degree of ET_A antagonist selectivity on uterine and placental perfusion and on fetal and placental growth in the setting of chronic NOS inhibition.

MATERIALS AND METHODS

Nitric Oxide Synthase Inhibitor and Endothelin Receptor Antagonists

The NOS inhibitor, nitro-L-arginine methyl ester (L-NAME), was obtained from Sigma (St. Louis, MO). Results from three ET_A antagonists were compared in this study: A-127722 (provided by Abbott Laboratories, Abbott Park, IL), a nonpeptide ET_A receptor antagonist with 2000-fold selectivity for ET_A compared with ET_B (22), FR139317 (American Peptide, Sunnyvale, CA), a tripeptide with 8000-fold selectivity for ET_A (23), and ABT-546 (Abbott), a nonpeptide ET_A antagonist with 28,000-fold selectivity for ET_A (24). FR139317

and ABT-546 are equally potent while A-127722 has a 10-fold greater potency than the other two antagonists ($IC_{50} = 0.53, 0.49, 0.055$, respectively).

Animals

Female and male Sprague-Dawley rats (Harlan Sprague Dawley, Madison, WI) received a standard laboratory rodent diet (PMI Feeds, St. Louis, MO), water *ad libitum*, and were kept on a 12-hour light/12-hour dark cycle. Animal care and the conduct of all experiments were approved by the Institutional Animal Care and Use Committee. Rats were bred at an age of 11–20 weeks and a weight of 225–250 g. Date of sperm positivity was designated day 0 of a 22-day gestation. Eight groups of 10 timed-pregnant rats were used for this study.

Catheter Implantation

On gestation day 14, in rats receiving FR139317 or vehicle, an indwelling catheter was placed aseptically into the left jugular vein. General anesthesia for this procedure was accomplished with a single intraperitoneal injection of xylazine 8 mg/kg, ketamine 40 mg/kg, and acepromazine 1.3 mg/kg in combination. The catheter was tunneled to the back of the neck, attached to the osmotic pump carrying the ET_A antagonist (or vehicle), and the pump and catheter were placed subcutaneously on the back so that this antagonist was delivered intravenously.

On day 20 of gestation, in all rats used for perfusion studies, indwelling catheters (Intramedic PE10 tubing, OD=0.61mm) were placed aseptically under general anesthesia into the ascending and descending aorta via the right carotid artery, as described previously (11). The descending aortic catheter was used for withdrawal of a reference blood sample to monitor and validate perfusion experiments. The ascending aortic catheter was used for injection of the radioactive microspheres for blood flow determination. The catheters were filled with a solution of 100 U heparin/ml normal saline, they were sealed with sterile stainless steel clips, and then the free ends of the catheters were tunneled to the back of the neck and sutured in place.

Nitric Oxide Synthase Inhibition and ET Receptor Antagonism

On gestation day 14 under general anesthesia (immediately subsequent to the venous catheter placement in FR139317-treated rats), two Alzet osmotic pumps (model 2ML1, output=10 μ l/h, Durect Corp., Palo Alto, CA) were placed subcutaneously on the back of the rat between the scapulae. One pump was used to infuse either L-NAME (2.5 mg/kg/h) or normal saline vehicle. L-NAME infusion continued through gestation day 21 (7 days).

The second pump was used to infuse A-127722 (subcutaneous, 10 mg/kg/day), FR139317 (intravenous, 12 mg/kg/day), ABT-546 (subcutaneous, 20 mg/kg/day), or vehicle (20% ethyl alcohol, 40% propylene glycol, and 0.04 M NaOH in H_2O). These doses of ET_A antagonists were chosen based on titration studies in this and other models of fetal growth restriction (10,25,26). As noted, the vehicle contains ethyl alcohol. Although alcohol can adversely impact fetal growth, the amount of alcohol administered was miniscule, and we have found that fetal weights in vehicle-treated animals are not different from untreated controls (results not shown).

Tissue Blood Flow Determination

On gestation day 21, blood flow to various organs and tissues was determined according to the method of Tuma et al. (27) as described in detail previously (11). These experiments were performed on restrained, unanesthetized animals. Briefly, 100,000 ^{57}Co -labeled microspheres were infused into the ascending aorta. A reference blood sample was collected from the descending aorta at the same rate as the infusion into the ascending aorta. General

anesthesia was then administered and a hysterotomy was performed. Fetal weights were recorded. The placentas were removed and the uterine placental beds were dissected from the remaining uterine free wall. The uterine placental bed is the full thickness of the uterine wall at the sites where each placenta was attached. The uterine free wall is the remaining uterine wall where no placental attachment sites were present. Radioactively labeled microspheres in each tissue were quantified on a gamma counter. Blood flow results were calculated as follows:

$$\text{Tissue perfusion in ml/min/g} = \frac{(\text{reference withdrawal rate in ml/min}) * (\text{tissue cpm})}{(\text{reference cpm}) * (\text{tissue weight in grams})}$$

It should be noted that placental perfusion refers to the maternal perfusion of the placental unit.

Statistical Analyses

Results are presented as mean $\pm$ standard error of the mean (SE). Statistical comparisons among multiple groups were made using an analysis of variance (ANOVA) with *post hoc* Newman-Keuls test or a Kruskal-Wallis nonparametric ANOVA with *post hoc* Dunn's test, as appropriate. All statistical tests were two-tailed and results were considered statistically significant at $p < 0.05$.

RESULTS

Fetal and Placental Growth

Fetal weights were reduced by L-NAME and remained significantly low at 7 days of infusion regardless of which ET_A antagonist was used with L-NAME (Figure 1A). Fetal growth was either unaffected or slightly improved by the various ET_A antagonists when they were administered in the absence of L-NAME. Placental weights were also significantly decreased by L-NAME and were improved by two of three ET_A-selective antagonists (Figure 1B). This improvement in placental weight was not related to either the degree of ET_A selectivity or the potency of the antagonists.

Uterine and Placental Perfusion

Placental perfusion was profoundly decreased by L-NAME. The reduced placental perfusion in response to NOS inhibition was not improved on day 7 by any of the three ET_A antagonists regardless of degree of selectivity (Figure 2A). Uterine wall and uterine placental bed perfusion were largely unaffected by L-NAME or the antagonists, with the exception of a significant decrease in uterine perfusion when the most highly selective antagonist (ABT-546) was present with L-NAME (Figures 2B & 2C). Uterine wall perfusion was moderately increased when A-127722, but not the other antagonists, was used alone.

Fetal growth and uteroplacental perfusion were not different in rats treated with vehicle by either mode, intravenous or subcutaneous, therefore these rats are presented as a single vehicle-treated group.

DISCUSSION

In the setting of chronic NOS inhibition during pregnancy, extremely highly selective ET_A antagonists (e.g., ABT-546) have no additional benefits on perfusion or growth in this model beyond the benefits afforded by antagonists that have a lesser degree of selectivity for ET_A.

over ET_B, but are still considered highly ET_A-selective (e.g., A-127722). We previously demonstrated that in the setting of chronic (7-day) NOS inhibition, an ET antagonist with 2000-fold ET_A selectivity improved placental perfusion and fetal growth on days 1 and 4 of infusion, but not on day 7 (11). Because of the known adverse impact of ET_B antagonism on perfusion and growth, we hypothesized that the use of a more highly ET_A-selective antagonist would have further beneficial effects. That hypothesis was not confirmed by these experiments.

Administration of L-NAME results in decreased placental perfusion and subsequent FGR, as a result of decreased production of NO as well as upregulation of other vasoactive mediators such as ET-1. Because an extremely highly selective ET_A antagonist failed to produce normal fetal growth in this model, we first hypothesized that this may have been a dose effect. However, titration studies failed to demonstrate a beneficial effect at any dose of any of the antagonists utilized in this study. Therefore, we found no evidence that these results were based on a dose effect. We then hypothesized that prolonged administration of L-NAME may have irreversible effects on uterus and/or placenta which may preclude any beneficial effects of therapeutic interventions such as ET_A antagonism. For example, NOS inhibition during gestation has been shown to cause apoptosis in the uterus and placenta in the pregnant rat (28,29). We are currently investigating potential alternative explanations for the lack of efficacy of ET_A antagonism in this setting, including the effects of prolonged NOS inhibition on endothelial integrity and the effects of ET_A antagonism on maternal and fetal nitric oxide metabolism.

Ajne et al evaluated the effects of ET_A antagonism and NOS inhibition on the uteroplacental and renal vasculature in the rat (30). Several of their results are discrepant with what we have observed in this and a previous study (11). First, in sub-acute experiments with awake pregnant rats, they found that ET_A antagonism (BQ-123) increased placental and myometrial blood flow whereas we found perfusion in both unaffected. This may be as a result of their observations being after acute administration of the antagonist, whereas ours were after chronic infusion. More importantly and in agreement with their work, neither Ajne nor we observed detrimental effects on uterine or placental blood flow after administration of an ET_A antagonist alone, either in normal or pathologic circumstances. Second, Ajne et al reported that L-NAME administration did not affect uterine or placental blood flow, whereas we have consistently observed a decrease on days 1, 4, and 7 of infusion (11). This most likely is a result of the dosage administered (2 µg/min acutely versus 2.5 mg/kg/hour chronically – an approximate 14-fold difference in dose/rat/hour), although duration of administration may also be a factor.

The importance of ET receptor subtypes has been demonstrated in the interaction of ET-1 and NO in the regulation of perfusion of other vascular beds. In the rat kidney, the effects of ET-1 are balanced by the endothelial production of NO (31,32). During pregnancy, ET-1 mediates the vasodilation of the kidney through ET_B-mediated NO production (33,34). ET_B antagonism during pregnancy results in vasoconstriction and decreased perfusion of the kidney. Ajne also reported decreased renal blood flow with administration of a mixed ET_A/ET_B antagonist as well as with L-NAME administration during pregnancy in the rat (30,35). In humans, Schmidt et al demonstrated that administration of a NOS inhibitor resulted in a significant reduction in renal plasma flow, and that co-administration of an ET_A antagonist improved renal perfusion (36). These findings are all consistent with what we have observed in the rat placenta and kidney (11).

FGR remains a leading cause of perinatal morbidity and mortality in humans. When FGR is identified close to term, the treatment is relatively straight forward in that delivery is a viable option. When FGR occurs remote from term, delivery may not be a viable option

because the prematurity-related morbidity and mortality compound those of FGR. No treatment modalities are currently available for FGR remote from term. Our results from studies in several animal models of FGR including NOS inhibition as well as chronic hypoxia and ischemia-reperfusion suggest that ET antagonists improve pregnancy outcome in these settings (10,25,37). Endothelin receptor antagonists are currently licensed for treatment of pulmonary arterial hypertension, and are being evaluated for use in other disease states including scleroderma, diabetic nephropathy, and cancer (38). Whether there is an advantage to a mixed antagonist versus an ET_A-selective antagonist in these disorders remains a topic of considerable controversy, and may be dependent on the disease state being evaluated (39). If ET-1 antagonists are to be considered for use during pregnancy, they should be avoided during the first trimester because of the potential for teratogenicity (40,41). Further, the antagonist must be ET_A-selective given the evidence of adverse impact of ET_B antagonism during pregnancy (10,12). However, the results presented herein demonstrate that the degree of ET_A selectivity, among highly selective agents, is of limited significance with respect to the impact of ET_A antagonism on placental perfusion and fetal growth under pathologic circumstances.

Acknowledgments

This work was supported by National Institutes of Health grant HD046968.

References

1. Manning, FA. Principles and Practice. Norwalk: Appleton & Lange; 1995. Fetal Medicine; p. 322
2. Cameron IT, Bacon CR, Collett GP, Davenport AP. Endothelin expression in the uterus. *J Steroid Biochem Mol Biol.* 1995; 53:209–214. [PubMed: 7626456]
3. Stjernquist M, Bodelsson G, Poulsen H. Vasoactive peptides and uterine vessels. *Gynecol Endocrinol.* 1995; 9:165–176. [PubMed: 7502694]
4. Carbillon L, Uzan M, Uzan S. Pregnancy, vascular tone, and maternal hemodynamics: a crucial adaptation. *Obstet and Gynecological Survey.* 2000; 55:574–581.
5. Chang K, Lubo Z. Review article: steroid hormones and uterine vascular adaptation to pregnancy. *Reprod Sci.* 2008; 15:336–348. [PubMed: 18497342]
6. Cervar M, Puerstner P, Kainer F, Desoye G. Endothelin-1 stimulates the proliferation and invasion of first trimester trophoblastic cells in vitro - a possible role in the etiology of pre-eclampsia? *J Invest Med.* 1996; 44:447–453.
7. Shichiri M, Kato H, Marumo F, Hirata Y. Endothelin-1 as an autocrine/paracrine apoptosis survival factor for endothelial cells. *Hypertension.* 1997; 30:1198–1203. [PubMed: 9369276]
8. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature.* 1990; 348:730–732. [PubMed: 2175396]
9. Fukuroda T, Fujikawa T, Ozaki S, Ishikawa K, Yano M, Nishikibe M. Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem Biophys Res Commun.* 1994; 199:1461–1465. [PubMed: 8147891]
10. Thaete LG, Neerhof MG, Silver RK. Differential effects of endothelin A and B receptor antagonism on fetal growth in normal and nitric oxide-deficient rats. *J Soc Gynecol Investig.* 2001; 8:18–23.
11. Thaete LG, Kushner DM, Dewey ER, Neerhof MG. Endothelin and the regulation of uteroplacental perfusion in nitric oxide synthase inhibition-induced fetal growth restriction. *Placenta.* 2005; 26:242–250. [PubMed: 15708126]
12. Madsen KM, Neerhof MG, Wessale JL, Thaete LG. Influence of ET_B receptor antagonism on pregnancy outcome in rats. *J Soc Gynecol Investig.* 2001; 8:239–244.
13. Molnar M, Suto T, Toth T, Hertelendy F. Prolonged blockade of nitric oxide synthesis in gravid rats produces sustained hypertension, proteinuria, thrombocytopenia, and intrauterine growth retardation. *Am J Obstet Gynecol.* 1994; 170:1458–1466. [PubMed: 7909994]

14. Kourembanas S, McQuillan LP, Leung GK, Faller DV. Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. *J Clin Invest.* 1993; 92:99–104. [PubMed: 8326022]
15. Edwards DL, Arora CP, Bui DT, Castro LC. Long-term nitric oxide blockade in the pregnant rat: Effects on blood pressure and plasma levels of endothelin-1. *Am J Obstet Gynecol.* 1996; 175:484–488. [PubMed: 8765273]
16. Goligorsky MS, Tsukahara H, Magazine H, Andersen TT, Malik AB, Bahou WF. Termination of endothelin signaling: Role of nitric oxide. *J Cell Physiol.* 1994; 158:485–494. [PubMed: 8126072]
17. Thompson A, Valeri CR, Lieberthal W. Endothelin receptor A blockade alters hemodynamic response to nitric oxide inhibition in rats. *Am J Physiol.* 1995; 269:H743–H748. (*Heart Circ. Physiol.* 38). [PubMed: 7653640]
18. Maric C, Aldred GP, Antoine AM, Eitle E, Dean RG, Williams DA, Harris PJ, Alcorn D. Actions of endothelin-1 on cultured rat renomedullary interstitial cells are modulated by nitric oxide. *Clin Exp Pharmacol Physiol.* 1999; 26:392–398. [PubMed: 10386227]
19. Neerhof MG, Jilling T, Synowiec S, Khan S, Thaete LG. Altered Endothelin Receptor Binding in Response to Nitric Oxide Synthase Inhibition in the Pregnant Rat. *Reprod Sci.* 2008; 15:366–373. [PubMed: 18325929]
20. Diket AL, Pierce MR, Munshi UK, Voelker CA, Eloby-Childress S, Greenberg SS, Zhang X-J, Clark DA, Miller MJS. Nitric oxide inhibition causes intrauterine growth retardation and hind-limb disruptions in rats. *Am J Obstet Gynecol.* 1994; 171:1243–1250. [PubMed: 7977528]
21. Wight E, Küng CF, Moreau P, Takase H, Lüscher TF. Chronic blockade of nitric oxide-synthase and endothelin receptors during pregnancy in the rat: effect on pregnancy outcome. *J Soc Gynecol Investig.* 1998; 5:132–139.
22. Opgenorth TJ, Adler AL, Calzadilla SV, Chiou WJ, Dayton BD, Dixon DB, Gehrke LJ, Hernandez L, Magnuson SR, et al. Pharmacological characterization of A-127722: An orally active and highly potent ET_A-selective receptor antagonist. *J Pharmacol Exp Ther.* 1996; 276:473–481. [PubMed: 8632312]
23. Sogabe K, Nirei H, Shoubo M, Nomoto A, Ao S, Notsu Y, Ono T. Pharmacological profile of FR139317, a novel, potent endothelin ET_A receptor antagonist. *J Pharmacol Exp Ther.* 1993; 264:1040–1046. [PubMed: 8450448]
24. Wu-Wong JR, Dixon DB, Chiou WJ, Sorensen BK, Liu G, Jae H-S, Tasker A, von Geldern TW, Winn M, et al. Pharmacology of endothelin receptor antagonists ABT-627, ABT-546, A-182086 and A-192621: *in vitro* studies. *Clin Sci.* 2002; 103 (Suppl 48):107S–111S. [PubMed: 12193066]
25. Thaete LG, Neerhof MG, Caplan MS. Endothelin receptor A antagonism prevents hypoxia-induced intrauterine growth restriction in the rat. *Am J Obstet Gynecol.* 1997; 176:73–76. [PubMed: 9024092]
26. Thaete LG, Dewey ER, Neerhof MG. Endothelin and the regulation of uterine and placental perfusion in hypoxia-induced fetal growth restriction. *J Soc Gynecol Investig.* 2004; 11:16–21.
27. Tuma RF, Vasthare US, Irion GL, Wiedeman MP. Considerations in use of microspheres for flow measurements in anesthetized rat. *Am J Physiol.* 1986; 250:H137–H143. [PubMed: 3942232]
28. Miller MJS, Voelker CA, Olister S, Thompson JH, Zhang X-J, Rivera D, Eloby-Childress S, Liu X, Clark DA, et al. Fetal growth retardation in rats may result from apoptosis: role of peroxynitrite. *Free Radical Biol Med.* 1996; 21:619–629. [PubMed: 8891665]
29. Tsukimori K, Komatsu H, Fukushima K, Kaku T, Nakano H, Wake N. Inhibition of nitric oxide synthetase at mid-gestation in rats is associated with increases in arterial pressure, serum tumor necrosis factor-alpha, and placental apoptosis. *Am J Hypertens.* 2008; 21:477–481. [PubMed: 18246055]
30. Ajne G, Nisell H, Jansson T. Effects of blockade of the endothelin receptor A and inhibition of nitric oxide synthesis on uteroplacental and renal blood flow in awake pregnant rats. *Am J Obstet Gynecol.* 2005; 192:295–301. [PubMed: 15672039]
31. Berthold H, Just A, Kirchheim HR, Ehmke H. Interaction between nitric oxide and endogenous vasoconstrictors in control of renal blood flow. *Hypertension.* 1999; 34:1254–1258. [PubMed: 10601127]

32. Kramp R, Fourmanoir P, Caron N. Endothelin resets renal blood flow autoregulatory efficiency during acute blockade of NO in the rat. *Am J Physiol.* 2001; 281:F1132–F1140. *Renal Physiol.*
33. Danielson LA, Kercher LJ, Conrad KP. Impact of gender and endothelin on renal vasodilation and hyperfiltration induced by relaxin in conscious rats. *Am J Physiol.* 2000; 279:R1298–R1304. (Regulatory Integrative Comp. Physiol.).
34. Gandley RE, Conrad KP, McLaughlin MK. Endothelin and nitric oxide mediate reduced myogenic reactivity of small renal arteries from pregnant rats. *Am J Physiol Regulat Integr Comp Physiol.* 2001; 280:R1–R7.
35. Ajne G, Nisell H, Wolff K, Jansson T. The role of endogenous endothelin in the regulation of uteroplacental and renal blood flow during pregnancy in conscious rats. *Placenta.* 2003; 24:813–818. [PubMed: 13129677]
36. Schmidt A, Bayerle-Eder M, Pleiner H, Zeisner C, Wolzt M, Mayer G, Schmetterer L. The renal and systemic hemodynamic effects of a nitric oxide-synthase inhibitor are reversed by a selective endothelin_A receptor antagonist in men. *Nitric Oxide: Biology and Chemistry.* 2001; 5:370–376.
37. Thaete LG, Neerhof MG. Endothelin and platelet-activating factor: Significance in the pathophysiology of ischemia/reperfusion-induced fetal growth restriction in the rat. *Am J Obstet Gynecol.* 2006; 194:1377–1383. [PubMed: 16579936]
38. Dhaun N, Pollock DM, Goddard J, Webb DJ. Selective and mixed endothelin receptor antagonism in cardiovascular disease. *Trends Pharmacol Sci.* 2007; 28:573–579. [PubMed: 17950470]
39. Dhaun N, Goddard J, Webb DJ. The endothelin system and its antagonism in chronic kidney disease. *J Am Soc Nephrol.* 2006; 17:943–955. [PubMed: 16540557]
40. Treinen KA, Loudon C, Dennis MJ, Wier PJ. Developmental toxicity and toxicokinetics of two endothelin receptor antagonists in rats and rabbits. *Teratology.* 1999; 59:51–59. [PubMed: 9988883]
41. Spence S, Anderson C, Cukierski M, Patrick D. Teratogenic effects of the endothelin receptor antagonist L-753,037 in the rat. *Reprod Toxicol.* 1999; 13:15–29. [PubMed: 10080296]

Figure 1.

Fetal and placental weights from pregnant rats in response to L-NAME and three ET_A antagonists. Infusion of the NOS inhibitor, L-NAME (2.5 mg/kg/h), and antagonists (A-127722, 10 mg/kg/day; FR139317, 12 mg/kg/day; ABT-546, 20 mg/kg/day) was for 7 days, gestational days 14–21, and weights were recorded on day 21. **(A)** Fetal weights were decreased by L-NAME and were not improved by any of the ET_A antagonists in combination with L-NAME. The most highly selective antagonist, ABT-546, improved fetal weight when administered alone. **(B)** Placental weights were also decreased by L-NAME but were significantly improved by two of the three ET_A antagonists. Results are presented as mean weight in grams $\pm$ SE; n=10 rats per group; *** p <0.001 by ANOVA compared to vehicle-treated rats.

Figure 2.

Uterine and placental perfusion after 7 days of L-NAME and ET_A antagonist infusion in pregnant rats. The NOS inhibitor, L-NAME (2.5 mg/kg/h), and three antagonists (A-127722, 10 mg/kg/day; FR139317, 12 mg/kg/day; ABT-546, 20 mg/kg/day) were infused for 7 days, gestational days 14–21, and perfusion was measured by injection of radio-labeled microspheres on day 21. **(A)** Placental perfusion was significantly reduced by L-NAME and was not improved by ET_A antagonism. **(B & C)** Perfusion of the uterine placental bed and the uterine free wall were not significantly affected by L-NAME except in combination with ABT-546, the most highly selective ET_A antagonist used. Results are presented as mean

blood flow in ml/min/g $\pm$ SE; n=10 rats per group; * p <0.05, *** p <0.001, by ANOVA compared to vehicle-treated rats.