Endothelin mediates renal vasodilation and hyperfiltration during pregnancy in chronically instrumented conscious rats

Kirk P. Conrad, Robin E. Gandley, Satoshi Nakanishi, and Lee A. Danielson. Endothelin mediates renal vasodilation and hyperfiltration during pregnancy in chronically instrumented conscious rats. Am. J. Physiol. 276 (Renal Physiol. 45): F767–F776, 1999.—Profound vasodilation of the kidneys and other nonreproductive organs transpires during early pregnancy. Because nitric oxide (NO) was found to mediate renal vasodilation and hyperfiltration in conscious pregnant rats, and endogenous endothelin (ET) was suggested to be vasodilatory in the renal circulation of nonpregnant rats, we tested whether endothelin mediates the NO-dependent changes in the renal circulation during pregnancy. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured in conscious pregnant and virgin rats before and during infusion of 30 µg/min RES-701-1 (a selective ETB receptor subtype antagonist). Baseline GFR and ERPF were significantly increased by 35% in gravid rats relative to virgin controls. During infusion of RES-701-1, the pregnant rats responded more robustly, showing a greater decline in both GFR and ERPF such that renal function converged in the two groups of rats. ERPF also converged in pregnant and virgin rats during infusion of SB-209760, a nonselective ETA/B receptor subtype antagonist. Combined infusion of N-nitro-L-arginine methyl ester [L-NAME, an NO synthase (NOS) inhibitor] and RES-701-1 reduced GFR and ERPF to levels comparable to those reached with either agent given alone, suggesting inhibition of a common vasodilatory pathway. RES-701-1 and SB-209760 significantly lowered the cGMP content of small renal arteries from gravid and virgin rats in vitro, strengthening the link between the renal endothelial ETB receptor subtype and NO. Importantly, we showed that RES-701-1 is not a direct inhibitor of NOS. We conclude that endothelin mediates the NO-dependent changes in the renal circulation of conscious rats during pregnancy.

nitric oxide; N-nitro-L-arginine methyl ester; guanosine 3′,5′-cyclic monophosphate; endothelin receptors; RES-701-1; SB-209760; glomerular filtration; renal circulation; small renal arteries

VASODILATION of nonreproductive organs is one of the earliest maternal adaptations to occur in normal pregnancy, leading to a marked decline in total peripheral vascular resistance. The kidneys make a major contribution to this fall in peripheral resistance: a nadir in renal vascular resistance and a reciprocal peak in renal blood flow and glomerular filtration rate (GFR) are reached by the end of the first trimester (8, 13). Both renal blood flow and glomerular filtration increase by 40–80% above nonpregnant values (8, 13). Insight into the hormonal signals and molecular mechanisms of this vasodilatory response to pregnancy may be particularly critical, because in preeclampsia (a commonly occurring hypertensive disease peculiar to human pregnancy) both renal and systemic vasodilation are compromised (13).

Endothelin-derived relaxing factors have been hypothesized to be important mediators of peripheral vasodilation in pregnancy. Although the prostaglandins are unlikely to play a major role (1, 3, 4, 9, 11, 26, 38, 41, 47), other endothelium-derived vasodilators may be involved. In this regard, enhanced biosynthesis of both NO (12) and its second messenger, cGMP (7, 14), was shown to be increased in gravid rats. Although the tissue sources of this increased NO and cGMP production during pregnancy were not delineated, in light of the marked vasodilation of the maternal circulation, a vascular contribution seemed likely. Thus a role for NO in the changes of pregnancy was tested, and acute blockade of NOS, the vasodilating prostaglandins were recruited to maintain the renal circulation during pregnancy in a compensatory fashion (17).

The mechanisms underlying altered renal NO activity during pregnancy are unknown. Conceivably, NOS mass, activity, or cellular targeting could be modified. A variety of agonists including endothelin (ET) interact with their respective endothelial receptors to increase cytosolic calcium, thereby stimulating NOS activity. Although endothelin is more commonly viewed as a potent vasoconstrictor by interacting with both ETA and ETB receptor subtypes on vascular smooth muscle (48), endothelin was also shown to produce transient hypotensive responses (18, 50) and to increase intracellular calcium and NO synthesis by interacting with an ETB receptor subtype on endothelial cells (20, 21, 27, 42, 45, 51). Furthermore, disruption of the ET-1 gene in heterozygous mice produced elevated blood pressure (31, 46), and blockade of the ETB receptor subtype in rats produced renal vasoconstriction (19, 24, 25, 35, 40), suggesting a vasodilatory role for endogenous endothelin.

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An objective of our current work is to delineate the upstream signals mediating NO-dependent renal vasodilation and hyperfiltration in pregnancy. Because endogenous endothelin apparently contributes in a major way to the maintenance of low renal vascular resistance in the nonpregnant condition (24, 25), we reasoned that this mechanism may be exploited and enhanced during pregnancy, thereby mediating gestational renal vasodilation and hyperfiltration. On the basis of studies using pharmacological antagonists of the ET$_B$ receptor subtype in chronically instrumented conscious virgin and midterm pregnant rats, our results support the concept that endogenous endothelin is an important factor in the renal vasodilation and hyperfiltration of pregnancy. We also provide evidence that the renal vasodilatory action of endogenous endothelin in gravid rats is mediated via the NO/cGMP pathway, a conclusion drawn from in vivo experiments involving the simultaneous blockade of the ET$_B$ receptor subtype and NOS and from in vitro studies dealing with ET$_B$ receptor subtype blockade and cGMP production by small renal arteries.

**MATERIALS AND METHODS**

**Animal Preparation**

Female Long-Evans rats aged 12–14 wk were purchased from Harlan Sprague Dawley (Frederick, MD) and fed ProLab RMH 2000 diet containing 0.48% sodium (PMI Feeds, St. Louis, MO). The rats were habituated to a Plexiglas restraining cage on at least five different occasions before surgical preparation, with each training session lasting several hours. These experimental cages were especially designed to accommodate the swollen abdomen of gravid rats and to allow sufficient room for grooming of the face and front paws while preventing the rat from turning around. Thus accurate timed urine collections were possible via the chronically implanted bladder catheter. Rats that failed to habituate to the cage (<5%) were eliminated from the study.

The details of the surgical procedures have been previously described (6, 9, 16, 17). In brief, with ketamine (6.0 mg/100 g body wt) and pentobarbital sodium (2.1 mg/100 g body wt) anesthesia, Tygon catheters were implanted in the abdominal aorta and inferior vena cava via the femoral artery and vein, respectively. A cannula of Silastic-covered stainless steel was sewn into the urinary bladder with a pursestring suture and exteriorized through the ventral abdominal wall. The bladder catheter was always plugged except during experiments, so that the rats routinely voided through the urethra. All surgical procedures were conducted using aseptic technique. The rats were then allowed a minimum of 10–14 days of recovery. They were next randomly divided into two groups. One group, destined to become pregnant, was housed with male rats. The other group was not mated and served as a control. Identical procedures were followed except that the vehicle for RES-701-1 was infused at the same flow rate instead of the drug. In one of the virgin rats and three of the pregnant rats, two experiments were performed at least 48 h apart, and the results were averaged. The details of our methodologies for the measurement of renal function and mean arterial pressure in conscious rats have been previously published (6, 9, 16, 17).

**Experimental Protocols**

Administration of RES-701-1 (6 virgin and 6 pregnant rats). Renal function and mean arterial pressure were assessed in aged-matched virgin control and midegestation rats. The renal clearances of inulin and p-aminohippurate were used to measure GFR and effective renal plasma flow (ERPF), respectively. The preparation of the inulin and p-aminohippurate solution was previously described (6, 9, 16, 17). Three 30-min baseline urine collections were obtained with midpoint blood samples. For the experimental period, RES-701-1, a selective ET$_B$ receptor subtype antagonist purified from the broth of Streptomyces sp. (44, 48), was infused at 30 µg/min, and five 40-min urine collections with midpoint blood samples were made. This drug was prepared in dilute 0.02% sodium carbonate solution containing 5% dextrose at 37°C (25). In previous work by Gellai and colleagues (25), different infusion rates of RES-701-1 were evaluated, and renal vasoconstriction was greatest at the 30 µg/min dose in conscious male rats (25). In one of the virgin rats, two experiments were performed 48 h apart, and the results averaged. The details of our methodologies for the measurement of renal function and mean arterial pressure in conscious rats have been previously published (6, 9, 16, 17).

**Administration of SB-209670 (6 virgin and 6 pregnant rats).** The experimental procedures were the same as described above except that SB-209670, a mixed ET$_A$/B receptor antagonist (39, 48), was infused during the experimental period at 9 µg/min. Previous work, again by Gellai and coworkers (25), demonstrated that this dose of SB-209670 significantly attenuated both the transient systemic vasodilation and subsequent vasoconstriction of sarafotoxin S6c in conscious rats. In one of the virgin and pregnant rats, two experiments were performed at least 48 h apart, and the results were averaged. The preparation of SB-209670 for infusion was identical to RES-701-1.

**Administration of L-NAME and the vehicle for RES-701-1, or L-NAME and RES-701-1 (7 pregnant rats).** After the three 30-min baseline renal clearances were obtained, an infusion of N-nitro-L-arginine methyl ester (L-NAME) HCl was begun at 2 µg/min. We previously evaluated a dose response for L-NAME and showed that this infusion rate produced marked renal vasoconstriction in conscious pregnant rats with little or no perturbation of mean arterial pressure (16). A 1-h equilibration with L-NAME was allowed to achieve adequate blockade of renal NOS before an infusion of RES-701-1 at 30 µg/min or of the vehicle for RES-701-1 was instigated. Five 40-min renal clearances were then obtained. The vehicle for RES-701-1 was administered with L-NAME on one day, and after at least 48 h of rest, the same rat was administered RES-701-1 and L-NAME.

**cGMP content of small renal arteries (11 virgin and 11 pregnant rats).** Five renal interlobar arteries of ~100–150 µm diameter were dissected from one of the kidneys of virgin and midterm pregnant rats. After a 30-min equilibration,
tion in 1.0 ml Krebs-Ringer bicarbonate buffer containing 20 mmol/l HEPES and 9% dextran (36) saturated with 5% CO2-10% O2-balance N2 in a gently shaking water bath at 37°C. Vessels were incubated either with the appropriate vehicle, 0.1 mmol/l L-NAME, 10 µmol/l RES-701-1, 10 µmol/l SB-209670, 10 µmol/l BQ-123, or 0.1 mmol/l sodium nitroprusside and 0.1 mmol/l IBMX. BQ-123 was brought up in 0.9% NaCl, and L-NAME was prepared in distilled water. Sodium nitroprusside and IBMX were made in distilled water and 0.9% NaCl, respectively. Because RES-701-1 specifically inhibited 125I-ET-1 binding to ETB receptors in the rat kidney with an IC50 value of 0.6 µmol/l (43), a concentration of 10 µmol/l for RES-701-1 was chosen. We used similar concentrations of the other ET receptor antagonists for the sake of consistency. BQ-123 is a selective ETA receptor subtype antagonist (48). Vessels were first incubated for 10 min with either RES-701-1, SB-209670, L-NAME, or the appropriate vehicle for these agents. Then, IBMX was added to inhibit phosphodiesterases, and 10 min later the vessel was snap frozen in liquid nitrogen and stored at -80°C until extraction of cGMP. In the case of sodium nitroprusside, IBMX was added first, followed by sodium nitroprusside.

To extract cGMP, each vessel was mechanically shaken for 8 s in a small capped vial containing a steel ball bearing which had been chilled in liquid nitrogen (WIG-L-BUG; Crescent Dental, Lyons, IL). This technique pulverized the frozen tissue. Then, 0.25 ml of ice-cold 0.6 N perchloric acid containing ~1,500 cpm/ml of [3H]cGMP was added, and with rapid shaking by hand for 1 min, the cGMP was extracted from the tissue powder. After centrifugation to pellet the protein, the supernatant was neutralized with 5.0 N KOH. After another centrifugation to remove the precipitated KHClO4, the volume was restored to 0.25 ml gravimetrically, and the radioactivity of 75 µl was counted in duplicate to assess procedural losses. The vessel protein was assessed by the technique of Lowry et al. (32), and cGMP was quantitated by specific radioimmunoassay as previously described (14, 49).

RESULTS

The ETB receptor subtype antagonist, RES-701-1, elicited an overall increase in mean arterial pressure of 25–30 mmHg (P < 0.0001 by ANOVA) that was comparable in pregnant and virgin rats (Fig. 1A, top). In contrast, the effect of RES-701-1 on the GFR in the two groups of rats was markedly different (P < 0.0001 by ANOVA; Fig. 1B, top). Whereas GFR was inconsistently influenced by RES-701-1 in the virgin rats, it was significantly reduced in the gravid animals (P < 0.001 by ANOVA). Because the GFR of the pregnant rats was initially greater by 30–40% (P < 0.0001 vs. virgin rats), the GFR converged in the two groups of animals during the infusion of RES-701-1. The ERPF was also initially greater in the gravid rats, again by 30–40% (P < 0.0001 by ANOVA).
vs. virgin controls; Fig. 1C, top). RES-701-1 reduced ERPF in both groups of rats (both \( P < 0.001 \) by ANOVA); however, the ET\(_B\) receptor subtype antagonist attenuated ERPF in the pregnant animals to a greater degree, such that during the infusion of RES-701-1, ERPF became equal in the two groups of rats. When the vehicle for RES-701-1 was infused instead of the antagonist, mean arterial pressure, GFR, and ERPF were reasonably stable over time (Fig. 1, A–C, bottom). In particular, the gravid rats consistently demonstrated renal vasodilatation and hyperfiltration throughout these time control experiments relative to the virgin control animals (\( P < 0.0001 \) by ANOVA).

Similar experiments were conducted using the mixed ET\(_{A/B}\) receptor antagonist, SB-209670. At baseline, the gravid rats again showed significantly higher values for ERPF compared with the virgin animals (\( P < 0.001 \)). SB-209670 significantly reduced ERPF in both groups of rats (Fig. 2) but more so in the pregnant animals, such that ERPF converged in the two groups. GFR was also initially greater in the pregnant rats (2,992 ± 65 vs. 2,443 ± 75 \( \mu \)l/min, \( P < 0.05 \)). GFR was significantly decreased in the gravid rats to 2,547 ± 65 \( \mu \)l/min by SB-209670 (\( P < 0.005 \) vs. baseline). However, it was also diminished in the virgin rats to 2,089 ± 63 \( \mu \)l/min (\( P < 0.05 \) vs. baseline). During infusion of SB-209670, mean arterial pressure declined slightly but significantly in both groups of rats by ~6 mmHg.

To test the hypothesis that the vasoconstrictive effect of ET\(_B\) receptor subtype blockade in the renal circulation of gravid rats is mediated through reduced NO, we evaluated the combined administration of \( \text{L-NAME} \) and RES-701-1. We reasoned that failure to achieve either additivity or synergism with the combination relative to the infusion of the two agents separately would be consistent with the hypothesis. The data for the time control and RES-701-1 protocols were presented above (Fig. 1) and condensed for presentation in Fig. 3 to facilitate comparisons. No further reduction in either GFR (Fig. 3B) or ERPF (Fig. 3C) was noted with the concomitant administration of \( \text{L-NAME} \) and RES-701-1 compared with either agent given individually. If the combined treatment of \( \text{L-NAME} \) and RES-701-1 was additive, then GFR and ERPF should have been reduced to 67% and 47% of baseline, respectively. Instead, the combined administration only lowered GFR and ERPF, respectively, to 84% and 69% of baseline, levels not significantly different from those observed when either agent was administered alone. Based on our previous work (16), we deliberately chose a low dose of \( \text{L-NAME} \) of 2 \( \mu \)g/min, so that mean arterial pressure would only be mildly perturbed (Fig. 3A); thus a nonsignificant increase of 9 ± 3% was observed. For RES-701-1 alone and for \( \text{L-NAME} \) plus RES-701-1, increases in mean arterial pressure of 17 ± 2% and 27 ± 5% were observed, respectively. Thus, in contrast to the data for GFR and ERPF, the combined action of \( \text{L-NAME} \) and RES-701-1 was additive with respect to mean arterial pressure, compared with either agent administered alone.

Using another approach to address the hypothesis that the renal vasoconstrictive effect of ET\(_B\) receptor subtype blockade is mediated through reduced NO, we tested whether RES-701-1 and SB-209670 would lower cGMP production of small renal arteries in vitro (Fig. 4). As expected, \( \text{L-NAME} \) markedly reduced the cGMP content of small renal arteries to 20–25% of control levels (\( P < 0.0001 \)). Using vessels isolated from the same kidneys and tested concurrently under identical experimental conditions, we found that both RES-701-1 and SB-209670 consistently reduced the cGMP content to 50–75% of control levels (\( P < 0.05 \)). In contrast, BQ-123 did not significantly affect cGMP content: for the vessels from three virgin rats, average control and experimental values of 10.66 ± 1.50 and 10.89 ± 0.25 pmol/mg protein were obtained, and for the vessels from three midgestation rats, average control and experimental values of 9.04 ± 0.65 and 7.57 ± 0.97 pmol/mg protein were observed (both \( P = \) nonsignificant). As expected, sodium nitroprusside significantly increased the cGMP content of the small vessels from 10.66 ± 1.50 to 16.87 ± 0.92 pmol/mg and 9.04 ± 0.65 to 17.07 pmol/mg protein for the virgin and gravid rats, respectively (both \( P < 0.005 \)).

To exclude the possibility, albeit a remote one, that RES-701-1 exerts renal vasoconstriction in vivo and reduces vessel cGMP in vitro by directly inhibiting NOS, we tested whether the antagonist would reduce the calcium-dependent NOS activity present in human villous placental homogenates. As expected, \( \text{L-NAME} \) virtually eliminated all of the NOS activity, whereas neither RES-701-1 or its vehicle significantly affected the enzyme activity (Table 1).

**DISCUSSION**

The objective of the present investigation was to identify the signal(s) regulating NO activity in the
kidney during pregnancy, which mediates the gestational changes in the renal circulation. Albeit paradoxical in light of its reputation as the most potent vasoconstrictor discovered to date (50), there were several compelling reasons to consider endothelin. First, endothelin was shown to produce transient hypotensive responses in vivo (18, 50) and to increase intracellular calcium and/or stimulate NO in cultured endothelial cells from several species (21, 27, 45, 51) and in isolated rat glomeruli (20, 42) and kidneys from rats (28), dogs (5, 34), rabbits (22), and fetal sheep (2). The hypotensive responses, as well as the increased intracellular calcium levels and NO production, were mediated by an ETB receptor subtype located on the endothelium (Fig. 5). Second, the disruption of the ET-1 gene in heterozygous mice did not reduce, but rather elevated, blood
endothelin in the vasodilatory changes of the renal circulation during pregnancy that are NO dependent. Unlike all of the other nonselective ET_{A/B} or selective ET_{B} receptor subtype antagonists, RES-701-1 not only blocked the transient systemic vasodilatory effects of exogenous ET-1 (or of the ET_{B}-selective analog, sarafotoxin S6c; Ref. 29) but also potentiated (rather than inhibited) the subsequent systemic and renal vasoconstrictive responses (25). Thus RES-701-1 was apparently more selective for the “vasodilator” endothelial ET_{B} receptor subtype relative to the “vasoconstrictor” vascular smooth muscle ET_{B} receptor subtype (23–25; Fig. 5). Furthermore, by administering RES-701-1 alone to conscious male rats, Gellai and coworkers (24, 25) demonstrated profound renal vasoconstriction comparable in magnitude to that observed with the infusion of the NOS inhibitor, L-NAME. These results suggested a major physiological role for endogenous endothelin and the endothelium ET_{B} Receptor subtype in maintaining the low vascular resistance of the renal circulation presumably via the NO pathway. The renal vasoconstriction elicited by either RES-701-1 or L-NAME was greatly attenuated by the nonselective ET_{A/B} receptor antagonist, SB-209670, but not by the selective ET_{A} receptor subtype antagonist, BQ-123, suggesting that blockade of the endothelial ET_{B} receptor subtype by RES-701-1 or of NO by L-NAME allowed for unopposed action of endogenous endothelin on the “vasoconstrictor” vascular smooth muscle ET_{B} (but not the ET_{A}) receptor subtype (23–25). Because NO normally restrains endothelin production by the endothelium (30; Fig. 5), the loss of this negative feedback mechanism during L-NAME and RES-701-1 administration may have contributed to the profound renal vasoconstriction observed with infusion of these agents. Thus, under normal conditions, the endothelial and vascular smooth muscle ET_{B} receptor(s) oppose each other, with one mediating vasodilation presumably through NO and the other mediating vasoconstriction, respectively. Parenthetically, selective ET_{A} receptor subtype antagonists did not significantly affect the renal circulation in normal rats, suggesting little or no role for this endothelin receptor subtype (25, 35, 40).

The current study corroborated and extended previous work (25), insofar as we observed significant renal vasoconstriction in conscious female rats administered RES-701-1. A new finding, however, was that RES-701-1 provoked even greater renal vasoconstriction in conscious pregnant rats, such that the gestational increases in ERPF were reversed (Fig. 1). Although GFR was inconsistently affected in the virgin rats by RES-701-1, it was significantly reduced in the gravid animals, such that the gestational hyperfiltration was also reversed during RES-701-1 administration. In fact, the present findings using RES-701-1 were remarkably similar to our earlier work using the NOS inhibitors in pregnancy (16). Taken together, the results implicate endogenous endothelin in the regulation of NO-dependent renal circulatory changes during pregnancy.

Table 1. Endothelin ET_{B} receptor antagonist, RES-701-1, does not affect human placental villous NOS activity in vitro

<table>
<thead>
<tr>
<th>Placenta Number</th>
<th>NOS Activity, pmol citrulline·mg (^{-1})·40 min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>102.9</td>
</tr>
<tr>
<td>2</td>
<td>182.2</td>
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<tr>
<td>3</td>
<td>118.6</td>
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<tr>
<td>Mean ± SE</td>
<td>134.6 ± 24.3</td>
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Vehicle for RES-701-1 was a 0.02% sodium carbonate + 5% dextrose solution. L-NAME, N\(^{-}\)nitro-L-arginine methyl ester. Each enzyme activity measurement represents the mean of duplicate or quadruplicate determinations. For details of the NOS assay, see Refs. 10 and 15.
The equalization of renal function in gravid and virgin rats during administration of RES-701-1 was unlikely to be simply a nonspecific consequence of renal vasoconstriction. We previously showed that infusion of another vasoconstrictor, angiotensin II, actually magnified the initial differences in both GFR and ERPF between pregnant and virgin rats, mainly because the former demonstrated resistance to the renal pressor action of the hormone (16). Furthermore, during chronic blockade of NOS, renal vasoconstriction was observed in both pregnant and virgin rats, but renal function declined in parallel such that renal vasodilation and hyperfiltration persisted in the gravid rats relative to the virgin control animals (17).

We attempted to corroborate our findings obtained with RES-701-1 using the nonselective ET_A/B receptor antagonist, SB-209670, but admittedly we only had partial success (Fig. 2). Overall, we observed a more modest renal vasoconstrictive response to SB-209670 in comparison to either RES-701-1 or the NOS inhibitors. We actually anticipated less reduction in ERPF because of the nonselective nature of SB-209670, which, unlike RES-701-1, blocks both the “vasoconstrictor” ET_B receptor subtype located on the vascular smooth muscle, as well as the “vasodilator” ET_B receptor subtype on the endothelium, with the two effects thus opposing each other (23–25). The nonselective nature of this endothelin receptor antagonist was underscored by a small, albeit consistent reduction in blood pressure (see RESULTS), rather than the robust increase observed with RES-701-1 (Fig. 1). Nevertheless, there was a convergence of ERPF in gravid and virgin rats during infusion of SB-209670, analogous to the results with RES-701-1 and our previous work with l-NNAME and N^G-monomethyl-L-arginine (NMA) (compare Fig. 2 with Fig. 1 and Ref. 16). GFR, however, remained increased in the gravid rats relative to virgin controls, not so much because SB-209670 failed to reduce GFR in the pregnant rats, but rather because the drug also decreased GFR in the virgin controls almost in parallel. This unexpected finding for the virgin rats was not previously observed during infusion of RES-701-1, l-NNAME, or NMA (Fig. 1 and Ref. 16). We are currently unable to explain it, especially in light of the data showing that endogenous ET_A receptor activity in the renal circulation of both male and female rats is negligible (25, 40).

Although the work of Gellai et al. (23–25) implied a link between endogenous endothelin and NO in the regulation of low vascular tone in the renal circulation of conscious male rats, this relationship was never directly tested. In the present work, we provide experimental evidence for this linkage. When infused together, l-NNAME and RES-701-1 elicited decrements of GFR and ERPF in conscious pregnant rats that were comparable to those observed when either agent was administered alone (Fig. 3). Stated differently, the combined effect of l-NNAME and RES-701-1 was not even close to being additive, which suggested that they were both blocking the same vasodilatory mechanism, i.e., the NO/cGMP pathway. In contrast, the effect of coadministration of l-NNAME and RES-701-1 on blood pressure was additive, or nearly so, which most likely stems from partial blockade of NOS in the systemic circulation by the low dose of l-NNAME infused. We deliberately selected a “subpressor” dose, thereby circumventing large perturbations in blood pressure that can obfuscate the interpretation of data gathered on the renal circulation. Yet, this dose of l-NNAME provoked significant renal vasoconstriction (Ref. 16 and Fig. 3).

We further reasoned that, if RES-701-1 produced renal vasoconstriction by inhibiting tonic production of NO, then the drug should reduce cGMP content of small renal arteries in vitro. For these experiments, therefore, we measured vascular cGMP as a surrogate

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**Fig. 5.** Proposed scheme for the physiological role of endogenous endothelin in maintaining low renal vascular tone via the endothelial ET_B receptor subtype and NO. Endothelial ET_B receptor subtype is sensitive to pharmacological inhibition by both RES-701-1 and the nonselective ET_A/B receptor antagonist, SB-209670. On the other hand, the ET_A receptor subtype located on the vascular smooth muscle is relatively insensitive to RES-701-1. The endothelin-NO/cGMP vasodilatory pathway is exaggerated during pregnancy, thereby accounting for gestational renal vasodilation and hyperfiltration (see text). NOS, nitric oxide synthase. [Modified from Gellai et al. (24).]
for NO activity (33, 49). The results supported the overall hypothesis, because RES-701-1 reduced the cGMP content of small renal arteries from both gravid and virgin rats (Fig. 4). As we anticipated, this reduction was considerably less than that observed for L-NNAME, because there are undoubtedly other factors produced by the vessel wall, in addition to endothelin, capable of stimulating NO and cGMP production (33). Nevertheless, vascular cGMP was consistently reduced by RES-701-1 (with only one exception), and comparable results were obtained using the nonselective ETA/B receptor antagonist, SB-209670, but not with the specific ETA-selective antagonist, BQ-123 (Fig. 4 and results). Despite the comparable reduction of vascular cGMP in vitro, the renal vasoconstriction produced by RES-701-1 was more marked than that of SB-209670 (see Figs. 1 and 2). One explanation for this apparent discrepancy is that SB-209670 simultaneously blocks both the vasodilatory ETB receptor on the endothelium and the vasoconstrictive ETA receptor on the vascular smooth muscle, whereas RES-701-1 is more selective for the endothelial receptor subtype (23–25).

Because of the remarkable similarity between the results obtained for RES-701-1 and the NOS inhibitors on the renal circulation in conscious pregnant and virgin rats in vivo, and on cGMP production by small renal arteries in vitro, it was important to exclude the possibility, albeit remote, that RES-701-1 was a direct inhibitor of NOS. Using the human placenta as a rich source of calcium-dependent, endothelial isoform of NOS as initially reported by Conrad et al. (15) and Myatt et al. (37), we found that RES-701-1 did not affect enzyme activity, whereas, as expected, L-NNAME virtually abolished NOS activity (Table 1). Thus RES-701-1 is not a direct inhibitor of NOS, rather RES-701-1 mimics the L-arginine analogs in blocking renal vasodilation and hyperfiltration in conscious pregnant rats and reducing cGMP content of small renal arteries in vitro by antagonizing the endothelial ETB receptor subtype.

In summary, we demonstrated that the ETB receptor subtype antagonist, RES-701-1, inhibits renal vasodilation and hyperfiltration in chronically instrumented conscious pregnant rats, analogous to our earlier findings using NOS inhibitors. Furthermore, these results using RES-701-1 were partly substantiated by the nonselective ETA/B receptor antagonist, SB-209670, insofar as ERPF was equalized in conscious pregnant and virgin rats receiving this agent, too. Based on additional studies evaluating the combined infusion of L-NNAME and RES-701-1 on the renal circulation of gravid rats in vivo, as well as the impact of RES-701-1 and SB-209670 on the cGMP content of small renal arteries in vitro, we suggest that the endothelial ETB receptor subtype mediates gestational renal vasodilation and hyperfiltration through the NO/cGMP pathway. Last, using homogenates of human villous placenta as a rich source of calcium-dependent endothelial NOS, we proved that RES-701-1 is not a direct inhibitor of the enzyme. Further investigation is ongoing to determine which component(s) of the endothelin-NO/cGMP vasodilatory pathway is altered in the renal circulation during pregnancy. Whether this vasodilatory mechanism is also exploited or recruited de novo in the renal circulation of gravid women is currently unknown. Given the dual nature of endothelin, it is tempting to speculate that the vasodilatory component may be compromised during preeclampsia, thereby contributing to the “endothelial dysfunction” and impairment of renal function in the disease.

NOTE ADDED IN PROOF

We have recently shown that the present results in vivo are fully recapitulated by the dynamic and complex integrative behavior of myogenic reactivity in small renal arteries in vitro. That is, endothelin and nitric oxide mediate the reduced myogenic reactivity of small renal arteries from midterm pregnant rats (R. E. Gandley, K. P. Conrad, and M. K. McLaughlin, FASEB J. 12: A99, 1998).

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REFERENCES


ENDOTHELIN AND RENAL FUNCTION IN CONSCIOUS PREGNANT RATS


