Endothelin and nitric oxide mediate reduced myogenic reactivity of small renal arteries from pregnant rats

ROBIN E. GANDLEY,1 KIRK P. CONRAD,1,2 AND MARGARET K. McLAUGHLIN1,2

Departments of 1Obstetrics, Gynecology and Reproductive Sciences, and 2Cell Biology and Physiology, University of Pittsburgh and Magee-Womens Research Institute, Pittsburgh, Pennsylvania 15213

Received 6 March 2000; accepted in final form 25 August 2000

Gandley, Robin E., Kirk P. Conrad, and Margaret K. McLaughlin. Endothelin and nitric oxide mediate reduced myogenic reactivity of small renal arteries from pregnant rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1–R7, 2001.—We tested the hypothesis that endothelin acting through the endothelial ETB receptor subtype and the nitric oxide (NO) pathway accounts for reduced myogenic reactivity of the renal resistance vasculature during pregnancy. Small renal arteries (100–200 μm) were isolated from virgin and midterm pregnant rats when gestational renal hyperfiltration and vasodilatation are maximal in this species. Myogenic reactivity (the adjustment of arterial diameter in response to a change in transmural pressure) was assessed with a pressurized myograph system. A rapid increase in transmural pressure from 60 to 80 mmHg resulted in a 2.4% diameter increase in vessels from virgin compared with an 8.1% increase in arteries from midgestation rats (n = 8 each, P < 0.05). Thus myogenic reactivity is markedly reduced during pregnancy. Incubation with the NO synthase inhibitors, an ETB receptor subtype antagonist (RES-701–1), the nonselective ETA receptor blocker (SB-209670), or endothelial removal abrogated the reduced myogenic reactivity of vessels from gravid rats without affecting myogenic reactivity in arteries from virgin animals. Thus the endothelium mediates the reduced myogenic reactivity of small renal arteries of midgestation rats most likely through the ETB receptor subtype and NO pathway.

MYOGENIC REACTIVITY IS DEFINED as the active response of an artery (either constriction or dilation) to a rapid change in transmural pressure. The blood vessel behavior is an integrative process that depends on the endothelium, vascular smooth muscle, and extracellular matrix (9, 10, 25, 27). Arterial wall composition (10), vascular wall tension (14, 17, 29), and many vasoactive pathways (12, 13, 22) are some factors that influence the degree of myogenic reactivity observed in a variety of vascular beds. Arteries that are subjected to a rapid increase in transmural pressure respond with an active constriction if they possess myogenic reactivity. This phenomenon can be assessed in small renal arteries in vitro using a pressurized arteriograph

(11). In this way, circulating factors and other confounding influences encountered in vivo are circumvented.

Human pregnancy induces critical changes in renal hemodynamics that are also observed in the gravid rat. Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) increase in early pregnancy and peak at midgestation as a result of a reduction in renal vascular resistance. There are many potential mechanisms for these changes in the kidney circulation during pregnancy, including altered endothelial and vascular smooth muscle function. Changes in the endothelium and vascular smooth muscle can influence myogenic reactivity. In the present work, we evaluated the myogenic reactivity of small renal arteries from midterm pregnant and virgin rats, because it is a dynamic and complex integrative behavior of blood vessels that approximates the in vivo state. We hypothesized that, in response to increases of luminal pressure, myogenic reactivity would be reduced in vessels from the gravid rats.

Using chronically instrumented rats, Danielson and Conrad (6) demonstrated that nitric oxide mediates the profound vasodilation of the kidney during pregnancy via an endothelial ETB receptor (5). Assuming that myogenic reactivity is reduced in small renal arteries from pregnant rats as proposed above, another hypothesis we set out to test was whether this phenomenon is also mediated by nitric oxide (NO) and the endothelial ETB receptor. If so, the physiological importance of this vasodilatory pathway in pregnancy would be further supported and strengthened.

MATERIALS AND METHODS

Animal Model

Virgin Long-Evans rats (Harlan Sprague Dawley, Frederick, MD) were housed and bred in the Magee-Womens Research Institute animal facility. The presence of sperm in a vaginal lavage was used to document day zero of pregnancy (term = 22 days). Pregnant rats were studied at 13–15 days gestation; age-matched virgins were used as control animals.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: R. E. Gandley, Magee-Womens Research Institute, 204 Craft Ave., Pittsburgh, PA 15213 (E-mail: gandleyr+pitt.edu).
Rats were killed with an intraperitoneal injection of 50 mg/kg body wt methohexital sodium (Brevital, Lily), and the left kidneys were immediately removed and placed in cold HEPES-buffered physiological saline solution (PSS). Small renal arteries were exposed by carefully dissecting renal tissue overlaying them. Typically, the main renal artery divided into three branches in each half of the kidney. These further bifurcated to form two smaller arteries that gave rise to the arcuate arteries. Segments of these smaller arterial branches were dissected free from surrounding medullary tissue (100–200 μm unpressurized inner diameter). These vessels were transferred to a pressurized arteriograph (Living Systems, Burlington VT) and mounted on two microcannulas suspended inside the chamber. Residual blood was flushed from the lumen, and the distal cannula was occluded to prevent flow. The proximal cannula was attached to a flow-through pressure transducer and pressure servo-control unit. This system allowed the intraluminal pressure to be maintained and controlled. A video dimension analyzing system (Living Systems) processed a selected vidicon line to provide a selected arterial diameter and wall thickness measurements. Both the pressure and dimensional parameters were calibrated at the beginning of each experiment. Further description of this system is reported elsewhere (11).

The arteriograph chamber was maintained at a temperature of 36.5 ± 0.5°C and a pH of 7.38 ± 0.2. The arteries were equilibrated at 60 mmHg for 30 min. The intraluminal pressure was slowly increased to 100 mmHg and returned to 60 mmHg (1–2 min total). Fifteen minutes of additional equilibration was allowed before the beginning of experimentation.

In the studies of arteries without endothelium (n = 7 virgin; n = 6 pregnant), the endothelium was removed by passing air through the lumen of the artery mounted in the arteriograph (7, 20). Endothelial removal was verified by the response of precostrected arteries to the addition of methacholine (an endothelium-dependent relaxant). All arteries denuded of endothelium relaxed <10% on addition of methacholine (data not shown).

Myogenic Protocol

Myogenic reactivity is a dynamic and complex integrative vascular behavior that can be assessed in small renal arteries using a pressurized arteriograph. This vascular behavior was determined using an approach similar to that of MacPherson et al. (21). Equivalent tone was first established in all vessels by constricting them to 75% of their baseline diameter and wall thickness measurements. These parameters were normalized for wall thickness and, therefore, characterize the stiffness of the arteries. These parameters were then used to evaluate baseline myogenic reactivity, the maneuver was repeated in the presence of one of the following treatments.

NO synthase (NOS) was inhibited in arteries by a 20-min treatment with 0.25 mmol/l N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME, n = 8 rats per group) or 0.1 mmol/l L\textsuperscript{G}-monomethyl-L-arginine (L-NMA, n = 4 rats per group). Nitro-L-arginine methyl ester (L-NAME), 0.25 mmol/l, the inactive enantiomer, was also tested in arteries from four pregnant animals after a 20-min treatment. The inhibition of NOS with L-NMA was reversed using L-arginine (L-Arg, 2.5 mmol/l).

Blockade of the ET\textsubscript{A} receptor subtype was achieved by a 15-min treatment with RES-701–1 (Kyowa Hakko Kogyo Japan) in five arteries per group or the nonselective ET\textsubscript{A/B} receptor antagonist SB-209670 (n = 4/group), both at 10 μmol/l. Because RES-701–1 specifically inhibited 125I-labeled ET-1 binding to ET\textsubscript{A} receptors in the rat kidney with an IC\textsubscript{50} value of 0.6 μmol/l (28), a concentration of 10 μmol/l for RES-701–1 was chosen. The selective ET\textsubscript{A} receptor subtype antagonist BQ-123 was also used at 10 μmol/l (15 min) on arteries from four pregnant rats (31). The vehicle for RES-701–1 (0.02% Na\textsubscript{2}CO\textsubscript{3}, 5% dextrose) was used in the time control experiments (n = 4 rats per group).

The NO donor, sodium nitroprusside (SNP, 1 μmol/l) was given to arteries from four virgin rats before preconstriction to examine the effect of exogenous nitric oxide on the myogenic response of renal arteries.

At the end of the experiments, the arteries were rinsed in calcium-free PSS and then exposed to 1×10\textsuperscript{-4} mol/l papavereine and 1×10\textsuperscript{-4} mol/l EGTA for at least 10 min, after which passive diameter measurements were made at pressures from 5 to 150 mmHg in the continued presence of papavereine.

Drugs and solutions. The following pharmacological agents were used: phenylephrine, methacholine, papavereine, SNP, EGTA, L-NAME, L-NMA, D-NAME, and L-Arg (Sigma, St. Louis, MO) prepared in distilled water with final dilutions in PSS. RES-701–1, a selective ET\textsubscript{A} receptor antagonist, and SB-209670, the mixed ET\textsubscript{A/B} receptor antagonist, were prepared in dilute 0.02% sodium carbonate solution containing 5% dextrose at 37°C (5, 7). SB-209670 and BQ-123, the specific ET\textsubscript{A} receptor antagonist, were generous gifts from M. Gellai, SmithKline Beecham, King of Prussia, PA. BQ-123 was prepared in 0.9% NaCl. HEPES-buffered salt solution (PSS) contained the following concentrations (mmol/l) of solutes: 119 NaCl, 4.7 KCl, 1.18 KH\textsubscript{2}PO\textsubscript{4}, 1.17 MgSO\textsubscript{4}, 2.5 CaCl\textsubscript{2}, 5.5 glucose, and 10 HEPES.

Calculations. The percent change in arterial diameter after a 20- or 40-mmHg pressure step was calculated using the following equation: %change in diameter = (D\textsubscript{i} – D\textsubscript{o})/D\textsubscript{i} × 100, where D\textsubscript{i} = the initial internal diameter at 60 mmHg, and D\textsubscript{o} = the final internal diameter at the higher pressure in PSS.

The circumferential stress-strain relationship was calculated to further describe the passive mechanical properties of these arteries. These parameters were normalized for wall thickness and, therefore, characterize the stiffness of the components that comprise the vascular wall. Circumferential stress describes the force exerted on the vascular wall per unit of tissue and was calculated by the following equation: stress = (P × D) / 2T, where P is the transmural pressure in millinewtons per square millimeter (1 mmHg = 0.133 mN/mm2). D is diameter, and T is wall thickness. Circumferential stress represents the response of an artery to the force or intraluminal pressure it experiences. Strain was calculated as (D\textsubscript{i} – D\textsubscript{o})/D\textsubscript{o}, where D\textsubscript{o} is the initial diameter at a pressure of 5 mmHg, and D\textsubscript{i} is the diameter at the new pressure. With the use of least-squares analysis, each stress-strain relationship was fitted to the exponential curve described by the equation y = ae\textsuperscript{bx}, where y is stress, a is the initial stress at the initial diameter, x is strain, and b is the rate constant for the stress-strain curve. The rate constant b, which is calculated
for each artery, was used to compare the elastic stiffness between groups.

**Statistical analysis.** Data are expressed as the means ± SE. All data were first analyzed by one- or two-factor ANOVA. If significant main effects or interactions were observed, then individual group means were compared with the level of significance for each test adjusted by the Bonferroni method to account for multiple comparisons or by orthogonal contrasts. A P value of <0.05 was considered to be significant.

**RESULTS**

Small renal arteries of equivalent size were removed from virgin and midterm pregnant rats (250–350 μm internal diameter when pressurized to 60 mmHg). Midterm pregnant rats were used because ERPF and GFR are maximal in this species at this time. The passive mechanical properties of the vessels from the two cohorts of rats were not significantly different. Specifically, rate constants for the stress-strain comparison for the arteries from virgin animals was 6.75 ± 0.40, $r^2 = 0.96$ versus $6.86 ± 0.69$, $r^2 = 0.97$ at midgestation ($P > 0.05$; not significant).

The phenylephrine sensitivity of the small renal arteries from five virgin and midgestation rats was evaluated, using a cumulative dose response (Fig. 1). No significant difference in response to the adrenergic agonist phenylephrine was found ($EC_{50}$ virgin 10.1 ± 2.5 × 10⁻⁸ mol/l vs. pregnant 9.4 ± 0.9 × 10⁻⁸ mol/l). The percent constriction to a low dose of phenylephrine (5 × 10⁻⁸ mol/l) from the initial diameter was not significantly different between the vessels obtained from the virgin rats, 9.2 ± 2.5% ($n = 18$), and those from the midgestation pregnant rats, 8.2 ± 1.7% ($n = 17$). Furthermore, treatment with l-NAME, RES-701–1, or SB-209670 caused no significant change in the response to 5 × 10⁻⁸ mol/l phenylephrine between the groups or treatments (data not shown).

There was a minimal amount of basal tone in these arteries before the phenylephrine treatment [2.8 ± 0.9% in virgins ($n = 18$) vs. 2.1 ± 0.4% in midterm arteries ($n = 17$)]. Basal tone was unchanged with all treatments, with the exception of RES-701–1, which resulted in a mean increase in tone of 12% with no significant difference between groups ($n = 5$/group).

Phenylephrine was used to establish an equivalent amount of starting tone (25% constriction of the initial diameter of the vessel at 60 mmHg). Then a rapidly applied pressure step of 20 mmHg was imposed (Fig. 2). The initial response (dashed line) of a representative artery from a virgin and midgestation rat is overlaid with the response of the same arteries after inhibition of nitric oxide (NO) synthase (NOS) for 15 min using 0.25 mmol/l L-NAME (solid line). The increase in pressure initially expanded the arteries, which then underwent active constriction (i.e., myogenic reactivity), maintaining a stable diameter at 80 mmHg within ~2 min. The initial response of arteries used in L-NAME, L-NMA, RES-701–1, and SB-209670 protocols ($n = 21$/group) had a mean increase in diameter (from 60 to 80 mmHg ) of 6.4 ± 1.8 μm for arteries from virgin animals and 14.5 ± 1.4 μm for arteries from pregnant animals. The lack of active constriction observed in the artery from the midterm
reduced (*Pmyogenic reactivity of vessels from midpregnant rats was again
virgin and midterm pregnant rats were used.

bars. L-NAME significantly increased the myogenic reactivity of
20-mmHg increase in intraluminal pressure was 8.1
vessels from midterm pregnant and virgin rats after a
was completely restored in the same vessel after treat-
(dashed line). Note, however, that myogenic reactivity
pregnant rat indicates a loss of myogenic reactivity
the 20-mmHg step in pressure relative to arteries from virgin rats
a greater percent increase in steady-state diameter in response to
arteries from midgestation and virgin rats. The lack of myogenic
Fig. 3. A: comparison of myogenic reactivity between small renal
vessels from the two groups of rats (Table 1). Moreover, similar
inhibitor, L-NMA, i.e., the inhibitor restored the re-
duced myogenic reactivity of the small arteries from
gravid rats to virgin levels (Table 1). The effect of
L-NMA in arteries from four midterm rats was re-
versed by coincubation with L-Arg, i.e., increasing the
change in diameter from 2.5 ± 1.2% with L-NMA to
6.0 ± 1.4% with L-Arg. In contrast, D-NAME (the
inactive enantiomer of L-NMA) was without effect
(Table 1).

Myogenic reactivity was also reduced in arteries
from virgin rats exposed to exogenous NO from the NO
generator SNP. The initial response of this group of
arteries (n = 4) was a 3.3 ± 2.5% decrease in diameter
in response to an increase in pressure of 20 mmHg. In

pregnant rat indicates a loss of myogenic reactivity
(dashed line). Note, however, that myogenic reactivity
was completely restored in the same vessel after treatment
with L-NAME.

On average, the new steady-state diameter of the
vessels from midterm pregnant and virgin rats after a
20-mmHg increase in intraluminal pressure was 8.1 ±
1.3 and 2.4 ± 1.3% greater than the initial diameter,
respectively (P < 0.005, n = 8 rats each, Fig. 3A). Thus
the arteries from gravid rats demonstrated a signifi-
cant loss of myogenic reactivity. After NOS inhibition
with L-NAME, the myogenic reactivity was restored to
virgin levels (3.6 ± 1.5 vs. 3.1 ± 0.8% for arteries from
the gravid and virgin rats, respectively). A comparable
response to L-NAME treatment was also observed in
the midgestation arteries using a 40-mmHg step in
pressure. The initial response to a 40-mmHg increase
in pressure was a 2.5 ± 1.0% increase in diameter in
arteries from virgin rats and a 10.5 ± 1.0% (P < 0.002)
increase in arteries from the midterm rats. After inhibi-
tion of NOS (using L-NAME), the response was un-
changed in arteries from virgin rats, 2.5 ± 1.6%, and
significantly reduced to 4.6 ± 1.7% (P < 0.02) in arter-
ies from midterm rats. In time control experiments,
the myogenic reactivity was stable in the small renal ar-
teries from the two groups of rats (Table 1). Moreover,
similar findings were obtained using another NOS
inhibitor, L-NMA, i.e., the inhibitor restored the re-
duced myogenic reactivity of the small arteries from
gravid rats to virgin levels (Table 1). The effect of
L-NMA in arteries from four midterm rats was re-
versed by coincubation with L-Arg, i.e., increasing the
change in diameter from 2.5 ± 1.2% with L-NMA to
6.0 ± 1.4% with L-Arg. In contrast, D-NAME (the
inactive enantiomer of L-NMA) was without effect
(Table 1).

Reduction in myogenic reactivity was observed again
for small renal arteries from another group of pregnant
rats (Fig. 3B). Analogous to the findings with NOS
inhibition, blockade of the ETB receptor subtype with
RES-701–1 also restored the myogenic reactivity. Sim-
ilar results were obtained with the nonselective ETA/B
receptor antagonist SB-209670 (Fig. 4A). In contrast,
BQ-123 (a selective ETA receptor antagonist) was with-
out effect (Table 1).

Myogenic reactivity was also reduced in arteries
from virgin rats exposed to exogenous NO from the NO
generator SNP. The initial response of this group of
arteries (n = 4) was a 3.3 ± 2.5% decrease in diameter
in response to an increase in pressure of 20 mmHg. In

Table 1. Myogenic reactivity of small renal arteries
from virgin and midgestation rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Virgin</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Treated</td>
</tr>
<tr>
<td>NMA</td>
<td>3.8 ± 2.1</td>
<td>3.3 ± 2.2</td>
</tr>
<tr>
<td>Time control</td>
<td>2.9 ± 1.1</td>
<td>0.3 ± 1.6</td>
</tr>
<tr>
<td>D-NAME</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>BQ-123</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE in %; no. of rats studied are depicted in the
parentheses. N7-monomethyl-l-arginine (l-NMA) and nitro-d-argi-
nine methyl ester (d-NNAME) were used at a final concentrations of 0.1
and 0.25 mmol/l, respectively. BQ-123 (a selective ETA receptor
subtype antagonist) was used at 10 μmol/l. The vehicle for RES-
701-1 (0.02% Na2CO3-5% dextrose) was used for the time control
experiments. *P < 0.05 initial pregnant vs. initial virgin response;
†P < 0.01 treated vs. initial for arteries from pregnant rats. Data
shown is for a 20-mmHg increase in pressure, comparable results
were observed with a 40-mmHg pressure increase.
the presence of exogenous NO, a 20-mmHg increase in pressure resulted in a 7.3 ± 1.5% increase in diameter.

The major role for both endothelin and nitric oxide in the reduced myogenic reactivity of small renal arteries from midterm pregnant rats was underscored by the observation that endothelial denudation also restored the myogenic reactivity (Fig. 4B). Moreover, treatment of the denuded vessels with RES-701–1 did not significantly change the myogenic reactivity in vessels from either group of rats (data not shown). None of these aforementioned interventions significantly affected the robust myogenic reactivity of vessels from the virgin animals.

To evaluate whether the restoration of reduced myogenic reactivity in the arteries from pregnant rats by blockade of the ET$_B$ receptor subtype was mediated through reduced NO, we tested the combined treatment of RES-701–1 and L-NAME (Fig. 4C). An initial myogenic response of 6.3 ± 0.6% was increased to 1.0 ± 1.7% with 10 μmol/l RES-701–1 alone ($P < 0.03$, $n = 4$ rats). After new media was added with 0.25 mmol/l L-NAME and 10 μmol/l RES-701–1, the same myogenic response was observed, 1.0 ± 2.0% ($P < 0.02$ vs. initial).

To exclude the possibility of augmented NO signaling in the vessels from the gravid rats, we evaluated dose-response curves for the NO donor SNP for the small renal arteries from seven virgin and midgestation rats (Fig. 5). The vessels were preconstricted with phenylephrine to 50% of their initial diameter. Then SNP was cumulatively added. There was no significant difference in sensitivity of the renal arteries from virgin and pregnant rats (EC$_{50}$ 2.2 ± 1.1 vs. 1.2 ± 0.6 × 10$^{-8}$ mol/l $P = 0.6$) to SNP, indicating comparable responsiveness of the vascular smooth muscle to exogenous NO.

**DISCUSSION**

Renal arteries from midgestation rats were found to have reduced myogenic reactivity compared with arteries from virgin control animals. The response of renal arteries from virgin rats to rapid pressure increases is consistent with a pressure range where myogenic reactivity is initially observed in arteries of comparable size (8, 29). This finding of reduced myogenic reactivity of small renal arteries from midterm pregnancy is compatible with the profound renal vasodilation that occurs at this gestational stage in the rat (4).
Inhibition of NOS using either l-NAME or l-NMA restored the reduced myogenic reactivity in the small renal arteries from midterm pregnant rats to levels observed in the arteries from virgin animals. This observation again correlates with our work in chronically instrumented pregnant rats of the same gestational age, insofar as inhibition of NOS also restored GFR and ERPF to virgin control levels (6). The present study further identified the ET\textsubscript{B} receptor subtype as a contributing factor to the reduced myogenic reactivity during pregnancy. Treatment of arteries with the ET\textsubscript{B} receptor subtype antagonist RES-701–1 also restored the reduced myogenic reactivity to levels found in renal arteries from virgin rats. Importantly, this effect was duplicated using the nonselective ET\textsubscript{AB} receptor antagonist SB-209670, suggesting that the effect of RES-701–1 was not merely a nonspecific action of the drug. In contrast, the ET\textsubscript{A} receptor subtype antagonist BQ-123 had no effect on the myogenic reactivity in arteries from either pregnant or virgin rats. Our previous study reported a decrease in cGMP content in isolated renal arteries of comparable size treated with RES-701–1 or SB-209670 (5). Taken together, these findings implicate a major role for the endothelial ET\textsubscript{B} receptor subtype in mediating the reduced myogenic reactivity of small renal arteries from midterm pregnant rats, presumably by stimulation of endothelial nitric oxide.

The vasodilatory substance mediating the reduced myogenic reactivity of small renal arteries from midgestation rats, presumably NO, was shown to be of endothelial origin. In endothelial denuded arteries, the myogenic reactivity was comparably robust in vessels from midgestation pregnant and virgin rats. Several reports indicated perturbation in myogenic reactivity depending on the method of endothelial removal. We used the air bubble technique in the present study (14, 20, 23). In our experience using either the air bubble or an antibody technique to denude arteries (data not shown), myogenic reactivity is maintained in arteries from virgin rats, whereas it may be compromised by mechanical removal (9, 18).

The myogenic response after a combination of RES-701–1 and l-NAME treatment was not different from either treatment alone (see Figs. 3A and 4C). This suggested that a common vasodilatory pathway was inhibited. It is unlikely that the failure to show either additivity or synergism was due to a “ceiling effect,” because we observed that the myogenic response is fully capable of establishing a new vessel diameter that is actually smaller than baseline despite the 20-mmHg increase in intraluminal pressure (data not shown).

Small differences in arterial diameter have a direct and profound impact on blood flow, as the vascular resistance is proportional to the fourth power of the radius. If the modest difference in arterial diameter observed in this study reflects the behavior of the whole renal vasculature, then the ET\textsubscript{B} receptor activation during pregnancy will impact renal blood flow. The myogenic reactivity described in this study is a model for a portion of the myogenic mechanisms previously described in whole kidney preparations (12, 15, 24). This response may not be representative of the myogenic mechanism previously described in the preglomerular arterioles (2, 3, 15, 16). Rapidly changing transmural pressure causes alterations in arterial wall tension. In the kidney, increases in pressure that change wall tension result in a myogenic response by increasing Ca\textsuperscript{2+} influx, primarily through voltage-dependent calcium channels. Studies in other vascular beds indicate that this may not be the only stretch-responsive mechanism for calcium entry, implicating other calcium channels or changes in intracellular calcium sensitivity (19, 30, 32).

In vivo studies examining tubuloglomerular feedback and renal blood flow have found fully functional renal autoregulation during pregnancy, despite the renal vasodilation present at midgestation (1, 26, 33). It is presently unclear how these observations relate to the findings reported herein. The contribution, if any, of small renal arteries on the in vivo myogenic mechanism attributed primarily to afferent arterioles is as yet unknown. Numerous factors not a part of our model system may also contribute to the observations reported in vivo, including the presence of flow, circulating vasoactive substances, and the myogenic capacity of smaller arterioles within the renal vasculature.

In contrast to late pregnancy (9), no significant changes were detected in the physical properties of the arterial wall at midgestation. Late in pregnancy, the stress-strain characteristics of the renal arteries indicated that they were more distensible, which may impact myogenic reactivity. Thus the change in myogenic reactivity during pregnancy occurs before changes in the physical properties of the renal arteries.

Reactivity of renal arteries to the adrenergic agonist phenylephrine indicated no difference in contractile capacity at midgestation. Additionally, the response of
renal arteries to a low dose of phenylephrine in the
myogenic studies was comparable between the groups.
The response of the renal arteries to cumulative doses of SNP also suggested no difference in relaxation capacity in response to NO. The latter indicates that the response of the renal vascular smooth muscle to nitric oxide is not altered at midgestation.

In conclusion, the endothelium mediates reduced myogenic reactivity in small renal arteries from midterm pregnant rats, likely through NO produced in response to the action of endothelin on the endothelial ETB receptor subtype. The present results are entirely consistent with the findings in conscious pregnant rats using NOS inhibitors and ETB receptor subtype antagonists in which gestational renal vasodilation and hyperfiltration were abrogated (5, 6). Further investigation is needed to determine which step(s) along the ET-NO vasodilatory pathway is altered in the renal vasculature of pregnant rats.

We thank Miklos Gellai for the generous gift of SB-209670, as well as for helpful discussion and valuable advice. We are also grateful to Drs. Nakanishi and Ogawa for generous contribution of RES-701–1. Finally, we thank Laurie Kerchner, Sue Kauffman, and Theresa Miles for technical and clerical support.

This work was supported by the National Institutes of Health Grants RCDA-K04-HD-01908, R01-HD-30325, and P32-ES-05717.

REFERENCES


