Myogenic reactivity is reduced in small renal arteries isolated from relaxin-treated rats

JACQUELINE NOVAK,1 ROLANDO J. J. RAMIREZ,1 ROBIN E. GANDLEY,1 O. DAVID SHERWOOD,2 AND KIRK P. CONRAD1,3
1 Departments of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine and Magee-Womens Research Institute, Pittsburgh 15213; 2 Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213; and 3 Department of Molecular and Integrative Physiology and College of Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Received 25 October 2001; accepted in final form 12 April 2002

Myogenic reactivity is reduced in small renal arteries isolated from relaxin-treated rats. Am J Physiol Regulatory Integrative Comp Physiol 283: R349–R355, 2002; 10.1152/ajpregu.00635.2001.—Administration of the ovarian hormone relaxin to nonpregnant rats vasodilates the renal circulation comparable to pregnancy. This vasodilation is mediated by endothelin (ET), the ETB receptor, and nitric oxide. Furthermore, endogenous relaxin mediates the renal vasodilation and hyperfiltration that occur during gestation. The goal of this study was to investigate whether myogenic reactivity of small renal and mesenteric arteries is reduced in relaxin-treated rats. Thus relaxin administration to nonpregnant rats mimics pregnancy, insofar as myogenic reactivity of small renal and mesenteric arteries is reduced in both conditions.

Chronic treatment with relaxin decreases blood pressure and vasoconstrictor responses in spontaneously hypertensive rats (14, 20). In addition, relaxin has been reported to increase coronary blood flow and decrease platelet aggregation via nitric oxide (NO) and guanosine 3′,5′-cyclic monophosphate (1). Chronic infusion of relaxin in conscious rats produces renal vasodilatation and hyperfiltration comparable to that observed during pregnancy (2, 6, 7). This renal vasodilatory effect of relaxin occurs in intact and ovariectomized female rats (7) and in male rats (6). Furthermore, elimination of circulating relaxin or its activity during pregnancy in rats prevents the gestational increases in renal function (18). In the gravid rat, the gestational renal vasodilation and hyperfiltration are mediated by endothelin (ET), which activates ETB receptors on the vascular endothelium, thereby releasing NO (4, 5). The ET/NO pathway also underlies the renal vasodilation and hyperfiltration produced by relaxin administration to nonpregnant female rats (6, 7).

Another maternal adaptation to pregnancy is attenuation of the constrictor responses of small renal arteries to increases in intraluminal pressure in vitro; this reduction in myogenic reactivity is also mediated by the ETB receptor and NO (8). Therefore, the primary goal of the present investigation was to test whether chronic administration of relaxin to virgin rats elicits...
reduced myogenic reactivity of small renal arteries comparable to the gravid condition, and so, to determine the role of the endothelial ETB receptor and NO in mediating the reduced myogenic reactivity. A secondary objective was to test whether chronic administration of relaxin also reduces the myogenic reactivity of small arteries from another vascular bed, the mesentery. We chose this vascular bed because our group has experience with small mesenteric arteries and both relaxin treatment (14) and pregnancy (11, 16, 17) have been shown to alter vascular responses in these arteries.

METHODS

Animals and tissues. Long-Evans rats were purchased from Harlan Sprague Dawley (Frederick, MD) at 12–14 wk of age. All animals were housed in the animal facility of Magee-Womens Research Institute. The experimental protocols were conducted in accordance with the guidelines of our Institutional Animal Care and Use Committee. Animals were fed PROLAB RMH2000 diet (PMI, Brentwood, MO) containing 0.48% Na+, and water was provided ad libitum while animals were maintained on a 12:12-h light-dark cycle. For chronic infusion of relaxin, an osmotic minipump was inserted subcutaneously in the back of the animal under isoflurane anesthesia. After shaving and cleaning the skin with alcohol and betadine, a small incision was made and the minipump containing recombinant human relaxin (Connetics, Palo Alto, CA), highly purified porcine relaxin (22), or vehicle was inserted. The vehicle for the recombinant human relaxin was 20 mM sodium acetate at pH 5, and the vehicle for the porcine relaxin was Ringer solution. The incision was closed with surgical staples. Either the model 2001 or 2ML1 7-day osmotic minipump (Durect Corp, Cupertino, CA) was used to deliver relaxin at a dose of 4 µg/h, which yields concentrations of circulating relaxin similar to those measured on gestational days 12–14 in pregnant rats (20–40 ng/ml) (7). In pregnant rats, circulating relaxin is first detectable on gestational day 8 (21), and ~5 days later (midgestation in the rat) when levels have reached 20–40 ng/ml, pregnancy-induced renal vasodilation is maximal (2). In the present study, rats were euthanized with pentobarbital sodium (60 mg/kg body wt ip) and exsanguinated after 5 days of relaxin infusion. One kidney was removed and placed in an ice-cold HEPES-buffered physiological saline solution (PSS), a modified Kreb’s buffer composed of (in mmol/l) 142 sodium chloride, 4.7 potassium chloride, 1.17 magnesium sulfate, 2.5 calcium chloride, 1.18 potassium phosphate, 10 HEPES, and 5.5 glucose, and was maintained at pH 7.4 and 37°C. Renal artery dissection was performed similar to that described by Gandley and colleagues (8). The kidney was bisected longitudinally and the renal medulla was removed. The renal arteries were then exposed by dissecting the tissue overlying them. Typically, the main renal artery divided into three branches on each half of the kidney. These further branched into two small interlobar arteries that gave rise to the arcuate arteries. Segments of the interlobar arteries were isolated for the experiments. Small mesenteric arteries were also investigated. For these studies, a section of mesentery 5–10 cm distal to the pylorus was removed and placed in ice-cold PSS as described above. Mesenteric artery dissection was then conducted according to Meyer et al. (16).

Myogenic reactivity. To evaluate the myogenic reactivity of small arteries isolated from rats treated with relaxin or vehicle, segments of renal interlobar or second-order mesenteric arterial branches were used (unpressurized inner diameter, 100–200 µm). For each protocol, one arterial segment from each rat was then transferred to the isobaric arteriograph (Living Systems, Burlington, VT) and mounted on two glass micropipettes suspended in the chamber containing 3 ml of PSS maintained at pH 7.4 and 37°C. After residual blood was washed from the lumen of the vessel, the distal cannula was occluded to prevent flow. The proximal cannula was attached to a pressure transducer, a pressure servocontroller, and a peristaltic pump. The servocontroller maintained a selected intraluminal pressure, which could be rapidly changed in a stepwise manner. The arteriograph was placed on the stage of an inverted microscope with a video camera to provide an image of the vessel. Arterial diameter was obtained by an electronic dimension analyzing system (Living Systems). After being mounted between the two glass micropipettes, the arteries were allowed to equilibrate at 60 mmHg for 1 h. The artery was then slightly constricted by 20% of its initial diameter at 60 mmHg with phenylephrine (PE). The mean concentration of PE used for each group to constrict the vessel by 20% is listed in Materials. This small amount of constriction is used to induce equivalent tone in the arteries and has been shown to optimize myogenic reactivity (13).

For each set of experiments, only one of the following two different myogenic protocols was conducted. Myogenic protocol 1: After PE constriction, the intraluminal pressure was decreased to 20 mmHg and allowed to stabilize for 10 min. Then, the pressure was increased in a stepwise manner from 20 to 120 mmHg in 20-mmHg increments at ~4- to 6-min intervals (see Figs. 1A, 2, A and B, and 5). Data were expressed as percent change in diameter from the initial diameter at 20 mmHg. Myogenic protocol 2: An alternate myogenic protocol was used for some of the experiments (see Figs. 1B and 3, A and B). This protocol requires less time to complete and also allows a direct comparison with previous work from our lab (8). After PE constriction, diameter at 60 mmHg was recorded and then the intraluminal pressure was rapidly increased in a stepwise manner from 60 to 80 mmHg. The pressure was maintained at 80 mmHg until the artery stabilized at a new diameter (~4–6 min). This new diameter was recorded and then the pressure was returned to 60 mmHg. After another stabilization period, the entire process was repeated three or four times. The data are expressed as percent change in diameter at 80 mmHg compared with the diameter at 60 mmHg. The responses from each vessel were averaged. The second protocol yielded assessments of myogenic reactivity comparable to the first method since both showed reduced myogenic reactivity in relaxin-treated rats (see RESULTS).

Myogenic reactivity was also tested on renal arteries after removal of the endothelium by passing several air bubbles (total volume ~0.5 ml) through the lumen of the vessel while mounted in the arteriograph (10, 12, 15). At the end of the experiment, the absence of endothelium was confirmed by a <10% relaxation to 1 × 10−4 M methacholine after a constriction to 50% of initial diameter with PE. Only 1 of 14 arteries studied failed to meet this criterion, and therefore, the data from this vessel were excluded from the analysis. Myogenic reactivity was investigated following pretreatment of the small renal vessels with the NO synthesis inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) at 0.25 mmol/l for 15 min. In another set of arteries, additional experiments were conducted following incubation of the artery with the ETB receptor antagonist RES-701–1 at 10 µmol/l for 15 min (Kayo Hakko Kogyo, Japan, see Refs. 4, 6, and citations therein).
**Agonist concentration-response curves in small renal arteries.** For responses to the vasodilator sodium nitroprusside (SNP), the renal arteries were first constricted to 50% of their original diameter with PE, and then cumulative doses were tested using concentrations ranging from $1 \times 10^{-8}$ to $1 \times 10^{-6}$ M. The diameter was recorded at each agonist concentration after stabilization.

**Percent tone.** The percent basal tone (tone at 60 mmHg) was determined for each artery. At the end of the experiment, the relaxed (passive) diameter was measured after incubating the arteries in calcium-free HEPES PSS with papaverine ($1 \times 10^{-4}$ M) and EGTA ($1 \times 10^{-4}$ M). Percent tone was then calculated by the equation: $\%$ tone = $\left(\frac{D_r - D_{PSS}}{D_r}\right) \times 100$, where $D_r$ is the relaxed diameter in the calcium-free buffer and $D_{PSS}$ is the diameter in HEPES PSS.

**Statistical analysis.** Data are expressed as means ± SE. The $n$ refers to the number of rats that was investigated. One artery from each animal was used per protocol. All data were analyzed by two-factor repeated-measures ANOVA except for data from Figs. 1B and 3, A and B, in which the group means were compared by unpaired $t$-test. A $P$ value <0.05 was considered to be statistically significant.

**RESULTS**

Relaxin treatment did not significantly alter the concentration of PE necessary to constrict the small renal arteries by 20% ($P = 0.2$). The mean concentration of PE used in the vehicle-treated group was $2.5 \pm 0.6 \times 10^{-7}$ M PE ($n = 10$ rats). In the arteries from relaxin-treated animals, the concentration of PE was $3.5 \pm 0.5 \times 10^{-7}$ M PE ($n = 13$ rats). The PE concentration tended to be higher in the relaxin-treated group. However, the percent basal tone (tone at 60 mmHg) of the arteries from this group of rats also tended to be lower compared with those from the vehicle-treated cohort, $3.2 \pm 1.1$ vs. $6.2 \pm 1.6\%$ ($P = 0.2$), which most likely accounts for slightly higher PE concentrations required in the vessels from the relaxin-treated rats. Thus relaxin did not significantly change the sensitivity of the vessels to PE, at least at the low concentrations used here.

Relaxin treatment decreased the myogenic reactivity of small renal arteries (Fig. 1, A and B). In the renal arteries from the vehicle-treated rats, the diameter changed very little over the entire range of pressure. By contrast, the renal arteries from the relaxin-treated animals increased their diameter so that the percent change in diameter was significantly greater than the vehicle-treated rats at each pressure step. The curves are statistically different by repeated-measures ANOVA ($P = 0.026$). Figure 1B portrays the average myogenic response from three or four pressure steps of 60 to 80 mmHg. Representative tracings are also shown in Fig. 1B, inset. These results are expressed as percent change in diameter at 80 mmHg compared with the diameter at 60 mmHg. The mean responses are greater in the relaxin-treated animals compared with the vehicle-treated controls and are statistically different by unpaired $t$-test ($P = 0.0002$). Both assessments of myogenic reactivity yielded similar results and indicate that relaxin attenuates this blood vessel behavior in small renal arteries.

**Fig. 1.** Myogenic reactivity of small renal arteries. A: rats were treated with vehicle or relaxin for 5 days before isolation of 1 artery from each animal for study of myogenic reactivity (see METHODS). Responses are displayed as a percent change in diameter (Dm) from initial diameter at 20 mmHg ± SE. B: see legend to Fig. 1A. This is a single 20-mmHg pressure step from 60 to 80 mmHg, and data are expressed as a percent change in diameter at 80 mmHg from the initial diameter at 60 mmHg. The pressure step is performed 3 to 4 times per vessel (1 artery/animal). The individual responses for each vessel are averaged and then combined with the average responses of the other vessels (see METHODS). *$P = 0.0002$. Inset: tracings from a representative relaxin and vehicle experiment.

During NO synthase blockade, the myogenic reactivity of arteries from relaxin-treated rats was significantly increased ($P = 0.004$; Fig. 2A). In fact, in the presence of L-NAME, the myogenic responses were not significantly different from the responses of the arteries from vehicle-treated rats also in the presence of L-NAME (compare Fig. 2A, □ and Fig. 2B, ○, $P = 0.3$). L-NAME treatment did not significantly change the myogenic responses of arteries from the vehicle-treated rats (Fig. 2B).

Denudation of the endothelium increased the myogenic reactivity of the small renal arteries from relaxin-treated animals (Fig. 3A). After removal of the endothelium, the average myogenic response of the arteries from the relaxin-treated rats was $-1.3 \pm 1.3\%$ compared with $6.8 \pm 1.0\%$ change in diameter in the arteries from relaxin-treated rats with intact endothelium.
lium (see Fig. 1B). In fact, after the removal of endothelium, the responses of arteries from relaxin-treated rats were not significantly different from the responses of the arteries from the vehicle-treated controls (P = 0.6). This figure only includes small renal arteries that failed to relax to the endothelium-dependent vasodilator methacholine, thus confirming successful removal of the endothelium (13 of 14 arteries).

Likewise, blockade of the ETB receptor increased the myogenic reactivity of renal vessels from relaxin-treated animals to levels observed in small renal arteries from vehicle-treated animals (P = 0.4; Fig. 3B). Furthermore, there were no significant differences between myogenic responses of the deendothelialized and ETB antagonist-treated arteries from the vehicle- or relaxin-treated groups in Fig. 3, A and B (P = 0.9 open bars and P = 0.14 filled bars).

Cumulative concentration-response curves for the endothelium-independent vasodilator SNP were similar for the arteries from the relaxin- and vehicle-treated rats (Fig. 4). This indicates that vascular smooth muscle sensitivity to NO was not changed by relaxin treatment.

The myogenic responses of small mesenteric arteries isolated from relaxin-treated animals were significantly less than those responses from vehicle-treated controls (Fig. 5). Thus these results are comparable to the data from the small renal arteries. The amount of PE needed to constrict the mesenteric arteries by 20% of their initial diameter tended to be higher in the relaxin-treated rats compared with vehicle-treated rats (12.8 ± 0.5 × 10⁻⁷ vs. 5.7 ± 0.5 × 10⁻⁷ M, P = 0.1). Once again, percent tone tended to be lower in the arteries from the relaxin-treated group (0.7 ± 0.7 vs.
2.3 ± 0.8%, P = 0.2), which most likely accounts for slightly higher PE requirements.

DISCUSSION

Myogenic reactivity is defined as the response (either constriction or dilation) of an artery to a rapid change in intraluminal pressure. Here we show that arteries from relaxin-treated rats constrict less than those from vehicle-treated rats in response to an increase in intraluminal pressure. This is similar to the reduced myogenic responses of renal arteries isolated from midgestation pregnant rats (8). In the latter, the ET<sub>B</sub> receptor and NO mediate the decreased myogenic reactivity, because RES-701–1, a specific ET<sub>B</sub> receptor antagonist, and L-NAME, a NO synthase inhibitor, reversed the reduced myogenic reactivity (8). In the present study, the reduced myogenic reactivity of small renal arteries from relaxin-treated nonpregnant rats is also attributable to the ET<sub>B</sub> receptor and NO. Furthermore, in both pregnant and relaxin-treated nonpregnant rats, removal of the vascular endothelium increases the myogenic reactivity so that the responses are similar to those of the arteries from virgin and vehicle-treated rats, respectively (present study and Ref. 8). We previously showed that circulating relaxin mediates the reduced myogenic reactivity of isolated small renal arteries in gravid rats, because when relaxin-neutralizing antibodies are administered or pregnant rats are ovariectomized, the myogenic reactivity is restored to virgin levels (18).

Removal of endothelium by air perfusion had no effect on the arteries from virgin or vehicle-treated rats (8, current study). This finding conflicts with a previous report by Liu and colleagues (12) who showed that air perfusion decreased myogenic reactivity of canine small renal arteries. The volume of air and perfusion pressure used in each study may account for the divergent results. In the current study, several air bubbles with a total volume of 0.5 ml were infused at ~10 mmHg, whereas Liu and colleagues (12) infused 3–5 ml at a pressure of ~100 mmHg. Possibly, the large volume of air and the high perfusion pressure damaged the underlying vascular smooth muscle.

The role of NO in the reduced myogenic reactivity of small renal arteries from midterm pregnant and relaxin-treated virgin rats could be due to increased synthesis/bioavailability of NO, enhanced sensitivity of vascular smooth muscle to NO, or both. SNP was used to address the possible contribution of increased vascular smooth muscle sensitivity to NO. Concentration-response curves to SNP for small renal arteries from relaxin- and vehicle-treated rats are not significantly different, indicating that sensitivity to NO is not altered by relaxin treatment. Likewise, renal arteries from pregnant rats are not more sensitive to NO (8). Therefore, the reduced myogenic reactivity in arteries from pregnant and relaxin-treated rats is probably the result of increased production or bioavailability of NO. Most likely the production is increased via the endothelial ET<sub>B</sub> receptor subtype.

To address whether the reduced myogenic reactivity of small arteries is specific to the kidney or a more widespread phenomenon, we evaluated the myogenic responses of small mesenteric arteries isolated from relaxin- and vehicle-treated rats. Comparable to the renal arteries, myogenic reactivity is also reduced in mesenteric arteries. This finding suggests that the relaxin-induced reduction in myogenic reactivity may be a generalized phenomenon and not merely a function of the renal vasculature. In fact, earlier work by Massicotte and colleagues (14) indicated that relaxin treatment reduced vasoconstrictor responses in the...
perfused mesenteric arterial bed of the spontaneously hypertensive rats. In addition, our results are comparable to pregnancy in that mesenteric arteries from pregnant rats also exhibit reduced myogenic reactivity (so far only investigated near term, 11, 16, 17). Future studies are needed to address whether the reduced myogenicity in the mesenteric arteries is also mediated by the ETB/NO pathway.

Our finding of reduced myogenic reactivity in the face of insignificant changes in PE sensitivity is not altogether surprising because they are two distinctly different blood vessel behaviors. Myogenic reactivity is triggered by a pressure stimulus, whereas PE is directly mediated by α-adrenergic receptors. The ET/NO pathway that mediates the reduced myogenic reactivity of small renal arteries from midterm pregnant and relaxin-treated nonpregnant rats is most likely initiated by the pressure stimulus, which releases ET and its precursor, big ET, from the endothelium (19). Possibly, pulsatile arterial pressure serves to stimulate ET release in vivo.

The parallels between relaxin administration to nonpregnant rats and pregnancy are striking. The changes in renal hemodynamics (2, 7) and myogenic reactivity of small renal arteries (8, present study) induced by relaxin treatment are similar to pregnancy. The mechanism for both of these phenomena involves ET, the endothelial ETB receptor, and NO (4–6, 8, present study). The renal vasoconstrictor effect of ANG II is also attenuated in both pregnant and nonpregnant rats treated with relaxin (3, 5, 7). In addition, during pregnancy, both plasma osmolality and sodium concentrations decrease, and these changes are mimicked by relaxin administration to virgin rats (7, 23). Finally, the gestational increase in renal hemodynamics, the decrease in myogenic reactivity, and the reduction in plasma sodium and osmolality are all blocked by relaxin neutralizing antibodies or ovarietomy, thus indicating that relaxin mediates these maternal adaptations to pregnancy (18).

**Perspectives**

An important caveat is that relative to the afferent arteriole, the interlobar artery makes only a minor contribution to vascular resistance and autoregulation in the kidney. Nevertheless, we showed that the renal vasodilation and hyperfiltration elicited both by pregnancy (2) and relaxin treatment of nonpregnant rats (7) are fully recapitulated by the dynamic and complex behavior of myogenic reactivity in the interlobar artery. That is, myogenic reactivity is reduced in these small renal arteries from pregnant and relaxin-treated rats (8, present study). Furthermore, this reduction is reversed by inhibitors of NO synthase and of the ETB receptor when they are incubated with the blood vessel in vitro (8, present study), analogous to the inhibition of renal vasodilation and hyperfiltration by these inhibitors when infused in vivo (4–7). Thus the reduced myogenic reactivity of small renal arteries is a convenient functional bioassay for the renal hemodynamic changes of pregnancy, thereby providing a critical link between our ongoing investigations of the molecular mechanisms in the small renal arteries and the physiological phenomenon of renal vasodilation and hyperfiltration.

We thank V. McClain for expert clerical support. We also thank E. Unemori from Connetics (Palo Alto, CA) for generously providing the recombinant human relaxin.

This work was supported by National Institutes of Health Grant RO1-HD30325.

**REFERENCES**


