Blunted Responses to Vasoconstrictors in Mesenteric Vasculature but not in Portal Vein of Spontaneously Hypertensive Rats Treated with Relaxin (42857)

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Abstract. **Relaxin (RLX), an ovarian polypeptide hormone that is particularly associated with gestation in viviparous species, has recently been shown to decrease blood pressure in virgin spontaneously hypertensive rats (SHR) upon chronic infusion. In this investigation, vascular reactivity to angiotensin II, arginine-vasopressin, and norepinephrine was studied in the perfused mesenteric artery and isolated portal vein of control and RLX-treated virgin spontaneously hypertensive rats. The latter received an intravenous infusion of 75 ng/hr purified rat RLX for 2 days, whereas the controls were given an equal infusion of saline. All of the animals were then killed and their tissues processed for *in vitro* study. In the perfused mesenteric artery, the concentration-response curves for arginine-vasopressin and norepinephrine were shifted to the right by a factor of about 2 ($P < 0.05$ and $P < 0.005$, respectively) after RLX treatment. In the isolated portal vein, the response to angiotensin II was not affected; the effect of norepinephrine was slightly displaced to the right (increase in EC_{50}) and the maximum response remained unchanged. These results demonstrate that RLX treatment for 42 hr blunted the vascular response to vasoconstrictor agents in the mesenteric vasculature and are consistent with similar observations reported previously in the same tissue of 20-day-old pregnant rats. It is concluded that RLX may be involved in the blunted response to vasoconstrictor agents during gestation in the rat.** [P.S.E.B.M. 1989, Vol 190]

Pregnancy in humans and rats is generally accompanied by a decrease in blood pressure (1, 2). Although the timing of this reduction is different in humans and rats, it is generally assumed that the hypotensive effect of pregnancy is the consequence of lowered vascular responsiveness to pressor agents (3–5). Recently, we described a chronic vasodepressor action of relaxin (RLX) (2), an ovarian polypeptide hormone, which is present in large amounts in the rat during the second half of pregnancy (6). In fact, this decrease in blood pressure in humans (1) and rats (2) corresponds to the levels of RLX in plasma (6, 7). In non-pregnant spontaneously hypertensive rats (SHR), systolic blood pressure is markedly diminished 24 hr after the beginning of intravenous chronic infusion of purified rat RLX (2).

To investigate the possible involvement of RLX in the regulation of blood pressure during gestation in the rat, we undertook experiments to determine if the hormone can decrease the responsiveness to vasoconstrictor agents in a similar way as does the eve of parturition in SHR (4, 8). We measured the effects of angiotensin II (AII), [8-arginine]vasopressin (AVP), and norepinephrine (NE) in two isolated vascular organs, the perfused mesenteric artery and isolated portal vein of RLX-treated SHR and their saline-infused counterparts. Recent results from this laboratory (8) have demonstrated that the reactivity of these tissues to vasoconstrictors is decreased at the end of gestation in the rat. Our present data indicate that RLX could be involved in these blunted responses to pressor agents noted during gestation in this species.

Materials and Methods

Female SHR (13–14 weeks old) were obtained from Taconic Farms (Germantown, NY). A previously de-

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scribed method was used to measure blood pressure in these conscious animals (4). In brief, the left carotid artery and jugular vein were cannulated, under light ether anesthesia, with PE-50 and PE-20 polyethylene tubing, respectively (Clay-Adams). The catheters were passed under the skin, brought out at the scruff of the neck, and secured with silk threads. The carotid catheter was connected via a Gould-Statham pressure transducer (P23ID) to a Grass polygraph #7. After surgery, the rats were allowed to recover from the anesthesia (approximately 30 min) in metal-wire cages, where they stayed for the rest of the experiment and were maintained on a regular rat diet with water *ad libitum*. Purified rat RLX (75 ng/hr), diluted in saline, was infused (50 μl/hr) with a compact infusion pump (model 975; Harvard) via the jugular vein catheter, while control SHR received only a saline infusion. The experiments were performed after 42 hr of infusion. At this point, the rats were decapitated, and their mesenteric vascular bed and portal vein were processed for *in vitro* study.

Perfusion of the rat mesenteric vessels was performed as described previously (8–10). In brief, the superior mesenteric artery was quickly cannulated with a PE-90 polyethylene catheter via an incision in the abdominal aorta; the vascular bed was dissected at the intestinal border and mounted in an organ bath maintained at 37°C. The mesenteric bed was then perfused, by means of a peristaltic pump (Minipulse II; Gilson), with Krebs solution at a constant flow rate of 2.0 ml/min. The perfusion pressure was monitored with a pressure transducer (P23ID; Gould-Statham) and recorded on a Grass polygraph #7. AVP and NE were given as infusions via a sidearm of the perfusate inflow. The solution was preheated to 37°C and bubbled with a mixture of 95% O_2:5% CO_2.

Basal perfusion pressure, between 10 and 15 mm Hg at the beginning of the experiment, remained stable throughout and was not different between the control and experimental groups. The mesenteric bed was perfused for 45 min before being challenged with a standard dose of 100 ng of AVP (as a 0.1-ml bolus injection) to measure the reactivity of the preparation. The concentration-response curves for AVP and NE were then obtained by consecutive additions of alternating and increasing concentrations of the drugs, with sufficient time between each concentration to allow full recovery from the previous response. Each concentration of AVP and NE was infused until the response achieved a stable plateau.

The isolated portal vein was processed according to the procedure of Couture *et al.* (11), with some modifications (8). A polyethylene catheter (PE-90; Clay-Adams) was introduced at the junction of the splenic and mesenteric veins and pushed into the portal vein, which was then dissected out of the animal along with the catheter. The tissue was placed in oxygenated Krebs at room temperature and cleaned from fat and surrounding connective tissues. The vein, cut longitudinally to obtain a strip 1.5-cm long, was suspended in a glass-jacketed tissue bath by means of silk threads (6-0 size; Ethicon, Sommerville, NJ) between a hook at the bottom of the bath and an isometric force transducer (model 1030; Transmed) connected to a Grass polygraph #7. The tissue was stretched to a passive tension of 0.3 g, which resulted in the maximum active force for the portal vein of both SHR and WKY as reported (8, 12). Tissues were allowed to equilibrate for 45 min while frequently changing the Krebs solution bathing the preparation. Concentration-response curves for AII and NE were measured by adding alternatively increasing concentrations of both agents. The tissue was washed frequently before the addition of the next agonist concentration to allow full recovery of the preparation and to avoid tachyphylaxis.

The Krebs solution used in these experiments contained (in mmol/liter): NaCl, 118; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $NaHCO_3$, 25; $CaCl_2$, 2.5; KCl, 4.7; and dextrose, 5.5. The pH was between 7.42 and 7.45 at 37°C; the solution was aerated with a gas mixture of 95% O_2:5% CO_2.

Each experimental concentration-response curve was fitted by computer-assisted nonlinear regression analysis, using the ALLFIT program (13) to determine the concentration producing 50% of the maximal response (EC_{50}) and maximum asymptote of the response. The statistical data were evaluated by Student's *t* test for unpaired samples. Since EC_{50} is logarithmically distributed, each experimental value was converted to the negative logarithm (pD_2) and means ± SE were calculated to perform statistical analysis. The changes in mean blood pressure (Fig. 1) were compared by Student's *t* test for paired samples.

Relaxin used in the present studies was purified in our laboratory from pregnant (19–21 days) rat ovaries according to the method of Sherwood (14), modified by Walsh and Niall (15). Potency was measured by our standard bioassay on potassium chloride precontracted uterine segments (2, 16). In this assay, purified rat relaxin is equipotent to purified porcine relaxin obtained from the NIH. Other agents used in these studies are arginine^8-vasopressin (AVP) and aspartic^1-isoleucine^5-angiotensin II (AII) obtained from Peninsula (Belmont, CA) and L-arterenol hydrochloride (norepinephrine, NE) obtained from Sigma (St. Louis, MO).

Results

The blood pressure response to chronic infusion of purified rat RLX is shown in Figure 1. Mean blood pressure, at the beginning of the experiment, was 146 ± 3 mm Hg in the RLX-treated group ($n = 9$) and 140 ± 5 mm Hg in saline-infused animals ($n = 10$). After starting the infusion of relaxin, mean blood pressure did not change significantly before 6 hr, but a decrease

of 19 mm Hg was recorded after 9 hr and this significant decrease persisted until 30 hr. In saline-infused SHR, blood pressure did not change significantly over the time of measurements, except a short lasting increase of 9 mm Hg at 6 hr. Changes in mean blood pressure are not reported after 30 hr of infusion because the catheter in the carotid artery of some animals was blocked and we could not obtain reliable measurements of blood pressure. In the remaining animals, the pressure continued to be decreased, in RLX-infused SHR (≈20 mm Hg) and stayed unchanged in saline-infused rats. Nevertheless, the infusion of RLX or saline was continued for up to 42 hr.

At this time point, the rats were killed and their *in vitro* responses to the vasoconstrictor agents were measured in the perfused mesenteric vascular bed and isolated portal vein. Figure 2 depicts these results in the mesenteric bed as the percentage of maximum response. In the RLX-treated group, the responses to both AVP and NE were shifted to higher concentrations of the vasoconstrictor agents. In the portal vein, the response to NE, but not to AII, was displaced to the right by RLX infusion (Fig. 3). This indicates lower sensitivity to AVP and NE in the mesenteric vasculature and to NE in the isolated portal vein in relaxin-treated animals by comparison to the saline-infused controls. Tables I and II also show the maximum response and

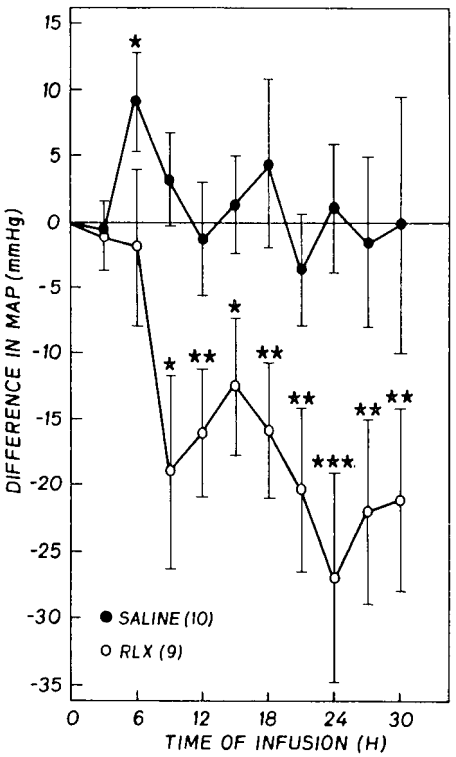

Figure 1. Change in mean arterial pressure after the infusion of saline (●) and relaxin (○) in virgin SHR. Abscissa: time of infusion in hours; ordinate: change in mean arterial pressure in mm Hg. $^{*}P < 0.05$, $^{**}P < 0.025$, $^{***}P < 0.01$ vs preinfusion period.

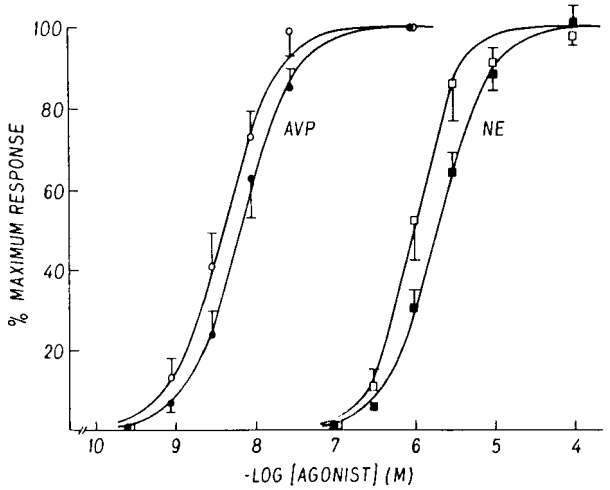

Figure 2. Concentration-response curves of AVP (○, ●) and NE (□, ■) in the perfused mesenteric vascular bed of saline-infused (○, □) and relaxin-infused (●, ■) SHR. The curves are the best fit to the experimental points which are shown as mean ± SE. Abscissa: molar concentration of agonist; ordinate: response in percentage of maximum response.

sensitivity to the vasoconstrictors in the mesenteric vascular bed and portal vein, respectively. The maximum responses to AVP and NE were not significantly changed in the mesenteric vasculature of RLX-infused rats but sensitivity to both vasoconstrictors was significantly decreased. No statistical differences were observed, however, in either the maximum response or sensitivity to AII and NE in the isolated portal vein.

Discussion

The end of gestation in the rat is generally accompanied by a decrease in blood pressure (2), a decline in sensitivity to vasoconstrictor agents, both *in vivo* (4) and *in vitro* (8), and an increased plasma level of immunoreactive RLX (6). The present study was undertaken to try to link these observations in unmated female SHR by infusing purified rat RLX over a period of 42 hr and by measuring blood pressure and the vascular response to vasoconstrictor agents *in vitro*. The results obtained confirm our previous report (2) that RLX infusion (75 ng/hr) produces a substantial decrease in the blood pressure of unmated female SHR. Furthermore, this infusion of the peptide hormone in unmated female rats elicited a drop in the vascular response to AVP and NE in the mesenteric vasculature, but not to AII and NE in the portal vein. These results indicate that RLX may be one of the pregnancy influences responsible for the decrease in blood pressure and in the vascular sensitivity of the blood vessels to vasoconstrictor agents at the end of gestation in the rat.

RLX is a peptide hormone from the corpus luteum of pregnancy, and the amount of this hormone present in the blood is proportional to the number of conceptuses and consequently of corpora lutea (17, 18). It has

Table I. Maximum Effect and EC_{50} of AVP and NE in the Perfused Mesenteric Artery of Control and Relaxin-Treated SHR

	N^a	Maximum effect (mm Hg)		$pD_2{}^b$ (EC_{50} in M)	
Vasopressin					
Control	8	207 ± 26	NS[c]	8.40 ± 0.11 (3.97×10^{-9})	$P < 0.05$
RLX-infused	10	223 ± 17		8.12 ± 0.09 (7.68×10^{-9})	
Norepinephrine					
Control	7	167 ± 21	NS	5.98 ± 0.11 (1.04×10^{-6})	$P < 0.05$
RLX-infused	10	205 ± 13		5.70 ± 0.07 (2.05×10^{-6})	

[a] Number of perfusion experiments.
[b] Mean ± SE of −log EC_{50} of individually fitted curves.
[c] NS, not significant.

Table II. Maximum Effect and EC_{50} of AII and NE in the Isolated Portal Vein of Control and Relaxin-Treated SHR

	N^a	Maximum effect (g)		$pD_2{}^b$ (EC_{50} in M)	
Angiotensin II					
Control	8	0.52 ± 0.09	NS[c]	8.20 ± 0.04 (6.3×10^{-9})	NS
RLX-infused	9	0.43 ± 0.06		8.19 ± 0.06 (6.5×10^{-9})	
Norepinephrine					
Control	8	0.56 ± 0.09	NS	6.45 ± 0.09 (3.6×10^{-7})	NS
RLX-infused	9	0.51 ± 0.08		6.31 ± 0.09 (4.9×10^{-7})	

[a] Number of vessels tested.
[b] Mean ± SE of −log EC_{50} of individually fitted curves.
[c] NS, not significant.

been shown recently that, in pregnant spontaneously hypertensive rats, the magnitude of hemodynamic changes, including the decrease in blood pressure, was proportional to the number of fetuses (19). In this latter study, the number of fetuses was adjusted by surgery on Day 8 of gestation. This procedure was shown (18) to decrease proportionally the activity of the corpora lutea, including the ovarian and plasma contents of relaxin.

Using the same dosage of purified rat RLX as in the present study, we observed a similar drop in blood pressure in our previous investigation 24 hr after starting the RLX infusion (with osmotic minipumps) (2). It was also noted that this blood pressure decreasing effect of rat RLX lasted for at least 4 more days. This study also duplicates the results of a previous experiment in our laboratory (8) in which we measured, at 20–21 days of pregnancy in SHR, the vascular response to vasoconstrictors in the same tissues as reported here. Indeed, in comparing both sets of results, it can be observed that they are nearly identical. In both the perfused mesenteric artery and portal vein, the maximum response to the vasoconstrictors was not affected by gestation (8) or by RLX treatment (this study). In the mesenteric vascular bed, sensitivity to AVP (measured as increases in EC_{50}) was decreased by a factor of 1.6 ($P < 0.05$) in pregnant rats and 1.9 ($P < 0.05$) in RLX-treated rats. For NE, these reductions in sensitivity were respectively 1.4 ($P < 0.005$) and 2.0 ($P < 0.05$). In the portal vein, the response to AII was not significantly decreased in either situation (1.3 and 1.03, respectively), whereas for NE the decline in sensitivity was significant during pregnancy (1.4, $P < 0.05$) but not in RLX-infused rats (1.4, nonsignificant). The similarity of the results pre-

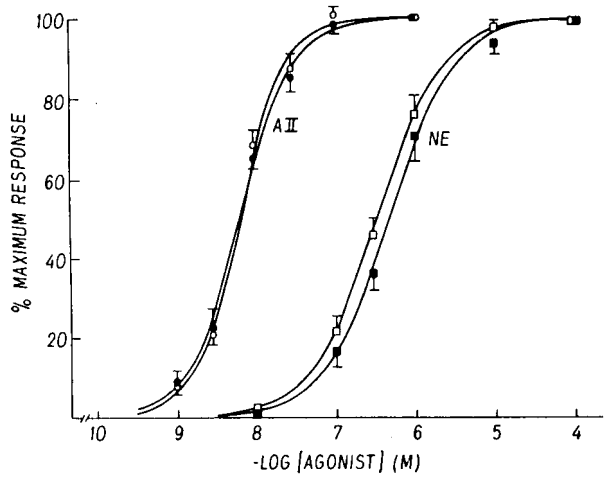

Figure 3. Concentration-response curves of AII (○, ●) and NE (□, ■) in the isolated portal vein of saline-infused (○, □) or relaxin-infused (●, ■) SHR. The curves are the best fit to the experimental points which are shown as mean ± SE. Abscissa: molar concentration of agonist; ordinate: response in percentage of maximum response.

sented in both studies suggests a common mechanism of this decrease of responsiveness to vasoconstrictor agents.

The possible vascular actions of RLX are an issue which has been re-surfacing in the last few years. The first evidence of vasodilation of vessels by RLX was reported by Casten *et al.* (20, 21), whose work was derived from the observation that Raynaud's phenomenon completely disappears during early pregnancy. They suggested, after several clinical studies in both men and women, that an ovarian hormone RLX may have beneficial effects in healing Raynaud's lesions via its actions on the capillary system. Later, Hisaw *et al.* (22) provided evidence that RLX is the hormone responsible for endothelial cytomorphosis during pregnancy in the monkey, a phenomenon characterized by the proliferation of endothelial cells in blood vessels of the uterine myometrium below the implantation site. More recently, Bigazzi *et al.* (23) extended their first observation (24) that RLX when applied topically to the microcirculation of the mesocecum of the rat dilates the venules and antagonizes the contractile effects of norepinephrine and promethazine in this circulatory bed.

These findings, along with the present data on the decrease in blood pressure by rat RLX (2), represent a series of circumstantial evidence suggestive of a role for RLX in circulatory regulation during pregnancy. Whether or not RLX affects total peripheral resistance remains to be clarified since the site and type of action of RLX in the blood vessels do not appear evident at this point in time and will therefore be the objective of future studies.

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