Relaxin Increases Cardiac Output and Reduces Systemic Arterial Load in Hypertensive Rats

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Abstract—Chronic administration of recombinant human relaxin (rhRLX) to conscious, normotensive rats (male and female) increases cardiac output (CO) and global arterial compliance (ACg) and reduces systemic vascular resistance (SVR) with no change in mean arterial pressure (MAP). Effects (magnitude and temporal pattern) of relaxin on systemic hemodynamics and arterial properties in hypertensive animal models are not known. Accordingly, the major goal of the present study was to determine the cardiovascular effects of rhRLX in hypertensive rats using 2 models: Long–Evans rats chronically administered angiotensin II (AII) and spontaneously hypertensive rats (SHR). CO and systemic arterial load, as quantified by SVR and ACg, were obtained using methods reported previously by us. In rats with AII-induced hypertension, acute rhRLX administration (up to 6 hours) significantly increased CO and ACg (24.9±3.9 and 34.3±12.6% above baseline, respectively) and significantly decreased SVR (17.2±3.5%) without changing MAP. In contrast, acute rhRLX administration to SHR and normotensive rats for up to 6 hours failed to produce any significant changes in CO, ACg, SVR, or MAP. However, chronic rhRLX administration (1 to 7 days) to SHR yielded significant changes (24.0±8.1 and 22.3±6.6% increases in CO and ACg, respectively, and a 13.3±5.3% decrease in SVR, with no change in MAP). In conclusion, rhRLX increases CO and reduces arterial load in hypertensive rats without reducing MAP. However, the time course of response to rhRLX treatment is dependent on the model of hypertension such that rats characterized by AII-mediated hypertension responded more rapidly to rhRLX administration than SHR.

Key Words: angiotensin II ▪ hypertension, arterial ▪ cardiac output ▪ hormones ▫ rats, spontaneously hypertensive ▫ vascular resistance ▪ vasodilation ▪ arterial compliance

Relaxin is an ovarian hormone secreted by the corpus luteum during gestation in rodents and humans. Its role in the reproductive system has been well documented.1 More recent studies have revealed that the hormone acts outside the reproductive system and plays a role in regulating renal and cardiovascular function.2 We have previously shown that chronic administration of recombinant human relaxin (rhRLX) to nonpregnant female and male normotensive rats elicits a vasodilatory response resulting in increased cardiac output (CO) as well as reduced steady and pulsatile components of the arterial load.3,4 Furthermore, the magnitude of change in arterial load in response to relaxin treatment is dependent on the baseline value of arterial load.4 That is, rats that exhibited higher systemic vascular resistance (SVR) or lower global arterial compliance (ACg) at baseline responded more robustly to relaxin administration. Based on this observation, we reasoned that relaxin administration may be particularly effective in chronic hypertension in which arterial load is typically elevated. In fact, St-Louis et al and Massicotte et al3,4 showed that relaxin administration to female spontaneously hypertensive rats (SHR) caused a significant decrease in mean arterial pressure (MAP) as early as 6 hours after the onset of relaxin infusion that persisted throughout the 5 days of relaxin infusion. However, these studies did not examine the effects of relaxin on other hemodynamic variables and arterial properties. Other studies have also reported that relaxin antagonizes the actions of angiotensin II (AII) in rats.5,8 Therefore, the primary goal of this study was to investigate the impact of rhRLX on systemic hemodynamics and arterial properties in 2 models of hypertension: rats made hypertensive by chronic administration of AII and SHR.

Relaxin-induced changes in systemic hemodynamics and arterial properties were observed in normotensive rats at the earliest time point studied (3 days), and these changes persisted throughout the entire 7 days of rhRLX infusion.3,4 In other studies, we showed that rhRLX vasodilates the renal circulation as early as 1 to 2 hours after the onset of intravenous infusion of the hormone.9 Therefore, the second goal of the present study was to determine whether the changes in systemic hemodynamics and arterial properties produced by relaxin would be observed during short term (up...
to 6 hours) intravenous infusion of the hormone, especially in the hypertensive rats, wherein arterial load is elevated at baseline.

**Methods**

Methodological details regarding surgical procedures, instrumentation, experimental protocols, data acquisition and analysis are presented in the online supplement, available at [http://www.hypertensionaha.org](http://www.hypertensionaha.org). Briefly, hemodynamic data (aortic pressure waveform and CO) were acquired in conscious rats to characterize systemic arterial load in terms of SVR and AC, as described previously by us. Female Long–Evans rats chronically administered AII and male SHR were used as 2 models of systemic hypertension. To determine the short-term effects of rhRLX, each group of rats were administered an intravenous bolus (2.0 μg/0.3 mL bolus of rhRLX followed by an infusion rate of 4 μg/hour). CO, SV, HR, and MAP during rhRLX administration are presented as percentages of baseline. *P<0.05 vs baseline (post hoc Fisher’s least significant difference).

**Acute Administration of rhRLX to AII-Treated Rats (n=7)**

As detailed in the Methods, each rat was administered rhRLX intravenously for a 6-hour period on days 2 and 5 after the initiation of the AII treatment. Because there was no statistically significant difference between days 2 and 5 for any of the systemic hemodynamics and arterial properties (by 2-factor repeated-measure ANOVA), the results were averaged. Control values of heart rate (HR), stroke volume (SV), CO, and MAP before AII administration were 399±7 bpm, 0.34±0.02 mL, 135±6 mL/min, and 111±2 mm Hg, respectively. After treatment with AII (defined as the baseline condition for rhRLX treatment), HR, SV, CO, and MAP were 367±14 bpm, 0.31±0.02 mL, 114±4 mL/min, and 138±6 mm Hg, respectively. Figure 1 illustrates the temporal patterns of changes in these hemodynamic variables (expressed as percentages of baseline) in response to short-term intravenous infusion of rhRLX. Short-term rhRLX treatment resulted in a significant increase from baseline in CO within 2 hours after the initiation of the infusion. This increase in CO was attributable to increases in SV and HR (Figure 1).

Control value of SVR before AII administration was 49.9±2.1 mm Hg/mL. After treatment with AII (ie, the baseline condition for rhRLX treatment), SVR increased to 74.6±3.9 mm Hg/mL (P<0.001). Figure 2 illustrates the temporal effects of acute intravenous infusion of rhRLX on systemic arterial properties. There was a significant decrease from baseline in SVR within 2 hours after the onset of rhRLX infusion, and AC, as indicated by both measures (AC and SV/PP), was significantly increased within 4 hours.

As in our previous work, we calculated a composite mean change from baseline for each variable by averaging values at all successive time points during the infusion of rhRLX that were characterized by a significant change from baseline but were not significantly different from each other. This yielded increases in CO and AC of 24.9±3.9 and 34.3±12.6% from baseline, respectively, and a decrease in SVR of 17.2±3.5% (all P<0.05 versus baseline). The serum concentration of rhRLX measured in blood obtained after 6 hours of infusion was 26.0±0.6 ng/mL.

Three rats were subjected to the same protocol, except they were administered the vehicle for rhRLX rather than rhRLX. There were no significant changes from baseline in systemic hemodynamics or arterial properties during the 6-hour vehicle infusion (data not shown).
Acute Administration of rhRLX to Male SHR (n=7)
Baseline values of HR, SV, CO, and MAP were 385±13 bpm, 0.33±0.02 mL, 129±7 mL/min, and 175±7 mm Hg, respectively. As detailed in the Methods, systemic hemodynamics and arterial properties were assessed continuously during the 6-hour infusion of rhRLX. There were no statistically significant changes in the systemic hemodynamics or arterial properties among the data for various time points during the 6-hour infusion. Therefore, Figure 3 illustrates the combined mean change from baseline of the systemic hemodynamic variables measured over the 6-hour period. Compared with baseline, there was a slight but statistically significant increase in HR (Figure 3A), which was offset by a small but not statistically significant decrease in SV (Figure 3B), such that CO remained unchanged (Figure 3C). Similarly, MAP was unchanged during the short-term rhRLX infusion (Figure 3D).

Baseline values of SVR, AC, and SV/PP were 83.7±5.6 mm Hg · s/mL, 3.8±0.2 μL/mm Hg, and 5.3±0.4 μL/mm Hg, respectively. Figure 4 illustrates the combined mean change of the systemic arterial properties obtained during the 6-hour infusion of rhRLX. Short-term infusion of rhRLX did not yield any statistically significant changes.

Chronic Administration of rhRLX to Male SHR (n=7)
There were no statistically significant differences in systemic hemodynamics and arterial properties assessed at days 1, 4, and 6 of rhRLX administration by subcutaneous osmotic minipump. Therefore, combined mean changes of these variables obtained over the 6-day chronic infusion of rhRLX are illustrated in Figures 3 and 4. Chronic rhRLX treatment resulted in a significant increase in HR compared with baseline (Figure 3A). Similarly, SV was significantly increased from baseline (Figure 3B). The increase in HR and SV resulted in a significant increase in CO (Figure 3C). MAP was not significantly altered during the chronic rhRLX infusion (Figure 3D). SVR was significantly reduced from baseline during the chronic rhRLX infusion (Figure 4A), and both measures of AC were significantly increased from baseline (Figure 4B and 4C). The serum concentration of rhRLX measured in blood obtained after 7 days of infusion was 29.8±0.76 ng/mL.
RhRLX treatment (post hoc Fisher’s least significant difference).

Major findings of the present study are as follows: (1) rhRLX reduces arterial load (a decrease in SVR and an increase in ACg) and increases CO in hypertensive rats, without any effect on MAP; and (2) the temporal pattern of these changes is model specific. Whereas rats with AII-induced hypertension respond to rhRLX administration rapidly (within 2 hours), SHR, like normotensive rats, respond more slowly (at least 1 day). To our knowledge, this investigation of relaxin on systemic hemodynamics and arterial properties in hypertensive animal models is unprecedented.

In previous work, we reported that chronic administration of rhRLX to normotensive rats, elicits a systemic vasodilatory response that is observed as early as 3 days after the onset of rhRLX infusion.3,4 In other work, we reported that rhRLX elicits a vasodilatory response in the renal circulation that is observed during acute (within 1 to 2 hours) and chronic (2 to 5 days) administration of the hormone.7,9 In fact, the hormone mediates the renal circulatory (and osmoregulatory) changes of pregnancy in this species.10 In the current study, we did not observe a systemic vasodilatory response when the hormone was administered acutely to normotensive rats for 1 to 6 hours. This finding suggests that certain organ beds such as the kidneys are able to respond more quickly to the vasodilatory actions of relaxin. (Because baseline renal blood flow is only ≈20% of CO, the expected increase in CO caused by a 20% to 40% increase in renal blood flow by rhRLX is not readily detectable.). Consistent with this concept is the observation that after preconstriction with phenylephrine, small renal but not mesenteric or coronary arteries isolated from rats demonstrate an immediate relaxation response to rhRLX (J. Novak and K. P. Conrad, unpublished data, 2004). Furthermore, Fisher et al reported that isolated human gluteal arteries from rats demonstrate an immediate relaxation response to relaxin.11 Therefore, it is possible that when administered acutely, relaxin preferentially vasodilates certain organ beds and is ineffective in vasodilating others, at least on a short-term basis. Of note, acute intravenous infusion of rhRLX did result in a slight but significant increase in MAP similar to previously reported observations.12

Interestingly, rhRLX did elicit a rapid systemic vasodilatory response when acutely administered to rats with AII-mediated hypertension. In fact, the present work in hypertensive rats was spurred by our previous observation showing that normotensive rats with higher magnitudes of arterial load at baseline responded more robustly to chronic relaxin treatment.4 Rats with AII-mediated hypertension responded with an even greater increase in CO and decrease in arterial load in a much shorter period of time than normotensive rats (vide supra). Pregnancy, and more specifically relaxin, has been shown previously to antagonize the vascular actions of AII. Conscious pregnant rats were less responsive to the renal and systemic vasoconstrictory effects of AII, both at midgestation13 and late gestation.14,15 Chronic administration of relaxin to nonpregnant rats also attenuated AII-induced renal vasoconstriction.7 Finally, Samuel et al reported recently that relaxin reduced cardiac collagen synthesis and accumulation in myofibroblasts that had been stimulated with AII.8

In contrast to AII-treated rats, SHR did not respond to acute infusion of rhRLX. This finding was contrary to our expectations because recent reports demonstrated that AII contributes to hypertension in SHR through the angiotensin type 1 receptor16,17 and because relaxin has been shown to be a functional AII antagonist.7,8 The lack of response was not attributable to inadequate serum concentrations of rhRLX. In fact, the circulating levels in all the studies in hypertensive animals were ≈2× higher than normotensive animals. We do not have a ready explanation for why SHR did not respond acutely to rhRLX; however, they did respond to chronic rhRLX administration. Based on our previous work,3 the reduction in SVR and increase in ACg attributable to chronic rhRLX administration results from a reduction in vascular smooth muscle tone and remodeling of arterial structure. Because relaxin possesses angiogenic attributes,2,18 angiogenesis may also contribute to the changes in systemic hemodynamics and arterial properties.4

In our work examining the effects of exogenous relaxin administration on systemic hemodynamics and arterial prop-

Figure 4. Temporal changes in arterial properties in response to acute and chronic rhRLX administration in male SHR (n=7). For acute administration, rats were given an intravenous 2 μg/0.3 mL bolus of rhRLX followed by an infusion rate of 4 μg/hour. For chronic administration, rhRLX was delivered by subcutaneous osmotic minipump at 4 μg/hour. SRV (A), and 2 measures of ACg ACarea (B) and SV/PP (C) during rhRLX administration are presented as percentages of baseline. Bar graphs represent the combined mean change throughout the 6 hours of acute or days 1, 4, and 6 of chronic rhRLX administration. *P<0.05 vs baseline; †P<0.05 vs short-term rhRLX treatment (post hoc Fisher’s least significant difference).

Acute Administration of rhRLX to Female Long–Evans Normotensive Rats (n=7)
The only significant change was a small (<10%) increase in MAP (see online supplement).

Discussion

Major findings of the present study are as follows: (1) rhRLX reduces arterial load (a decrease in SVR and an increase in ACg) and increases CO in hypertensive rats, without any effect on MAP; and (2) the temporal pattern of these changes is model specific. Whereas rats with AII-induced hypertension respond to rhRLX administration rapidly (within 2 hours), SHR, like normotensive rats, respond more slowly (at least 1 day). To our knowledge, this investigation of relaxin on systemic hemodynamics and arterial properties in hypertensive animal models is unprecedented.
properties, including the current study, we observed that the decline in SVR elicited by rhRLX is unaccompanied by a significant change in MAP. In fact, this situation resembles pregnancy in humans and other species in which a profound reduction in SVR is associated with only a modest decline in MAP.19–21 The decline in SVR is offset by a comparable rise in CO, thereby maintaining MAP. Four mechanisms may contribute to this reciprocal increase in CO during relaxin administration. First, relaxin is a positive chronotropic agent in vitro and in vivo (at least in rats), an observation reported by us and several others.3,4,22–24 Second, relaxin produces a positive inotropic effect in isolated ventricular tissue from rat and guinea pig heart.23–25 Third, the fall in SVR and increase in arterial compliance reduce venular afterload, thereby abetting the increase in CO. Finally, because MAP does not fall, our studies suggest that relaxin is a pure arterial vasodilator, thus permitting venoconstriction or reduction in passive venous compliance that maintains the ventricular end-diastolic volume or preload despite the decline in SVR. To our knowledge, there are no published studies on relaxin and the venous circulation. However, Edouard et al conducted a longitudinal study examining venous behavior throughout normal pregnancy in women, and they observed increased venous tone in the lower limb beginning in the first trimester that was strongly correlated with left ventricular diastolic diameter.26 In summary, based on these previous findings and the present work, we speculate that relaxin may exert different actions in the venous and arterial circulations. Specifically, relaxin elicits a vasodilatory response in the arterial circulation while augmenting venous tone either directly or indirectly (eg, via potentiation of humoral venoconstriction or baroreflex-mediated sympathetic tone). Consequently, the fall in SVR induced by relaxin is paralleled by an increase in CO, and it is by this mechanism that MAP is maintained during relaxin administration.

Of note, other studies have reported that contrary to what we observed in this investigation, MAP is significantly reduced in SHR in response to acute and chronic infusion of relaxin.5,6 These studies differed from the current investigation in that the animals used were female SHR. Therefore, to determine whether female SHR show a different response to relaxin infusion than males, we obtained arterial pressure measurements before and during rhRLX infusion in 7 female SHR. (Note: Because of their small size (<210 g), we only instrumented the animals with the mouse pressure catheter. In our experience, ligation of the femoral artery in small rats leads to hind limb ischemia.) Five days after implantation of the mouse pressure catheter in the right carotid artery, the rats were briefly anesthetized with isoflurane and implanted with an osmotic minipump that delivered rhRLX at 4 μg/hour for 6 days. The serum level of rhRLX after 6 days of infusion was 28.6±2.16 ng/mL. Baseline values of HR and MAP were 405±2 bpm and 171±2 mm Hg, respectively. The temporal responses of these hemodynamic variables to rhRLX infusion are illustrated in Figure 5. When compared with baseline, HR was significantly increased during rhRLX infusion comparable to the male SHR. There was a small but statistically significant decrease in MAP 6 hours after infusion of rhRLX. However, MAP was not significantly different from baseline at any of the subsequent time points studied. We attribute the small decrease in MAP at 6 hours to the hypotensive effects of isoflurane anesthesia. The hypotensive response to isoflurane anesthesia has been well documented in various strains of rats, and SHR have been shown to be more sensitive in this regard.27,28 We do not have an immediate explanation for why we were unable to reproduce the results of St-Louis et al and Massiochotte et al,5,6 differences in surgical procedures and experimental approaches may contribute to this discrepancy.

Perspectives
rhRLX increases CO and AC\(_r\), and reduces SVR without any changes in MAP in normotensive and hypertensive rat models. Thus, relaxin mimics the vasodilatory influences of normal pregnancy, during which MAP declines only slightly relative to the marked reduction in SVR.19–21 Based on this constellation of cardiovascular actions, we speculate that rhRLX may be particularly useful in preeclampsia, in which CO and AC\(_r\) are reduced and SVR and MAP are increased, resulting in marked and selective organ hypoperfusion. In this setting, rhRLX administration may improve maternal organ perfusion without unduly lowering MAP, thereby avoiding compromise of uterine blood flow. Interestingly, the vasodilatory action of relaxin was observed in the hypertensive animal models despite endothelial dysfunction.

Acknowledgments
This project was supported by National Institutes of Health grant RO1 HL67937 and McGinnis chair endowment funds. We are grateful to Elaine Unemori, PhD, of Connetics Corporation, Palo Alto, Calif, and BAS Medical, Palo Alto, Calif, for providing the rhRLX and the antibodies for the rhRLX ELISA.

References


27. Seyde WC, Durieux ME, Longnecker DE. The hemodynamic response to isoflurane is altered in genetically hypertensive (SHR), as compared to normotensive (WKY) rats. Anesthesiology. 1987;66:798–804.


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Hypertension. 2005;46:745-750; originally published online September 19, 2005;
doi: 10.1161/01.HYP.0000184230.52059.33

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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