Time course and dose response of relaxin-mediated renal vasodilation, hyperfiltration, and changes in plasma osmolality in conscious rats

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Danielson, Lee A., and Kirk P. Conrad. Time course and dose response of relaxin-mediated renal vasodilation, hyperfiltration, and changes in plasma osmolality in conscious rats. J Appl Physiol 95: 1509–1514, 2003. First published June 20, 2003; 10.1152/japplphysiol.00545.2003.—The pregnancy hormone relaxin elicits renal vasodilation, hyperfiltration, and osmoregulatory changes when chronically administered to conscious, nonpregnant rats. The objective in this study was to determine the dose response and time course of hormone action, as well as the time required for recovery on stopping its administration. The threshold dose of recombinant human relaxin (rhRLX) for renal vasodilation and reduction in plasma osmolality was 0.15 μg/h when given by subcutaneous osmotic minipump for 2 days (an infusion rate that achieved circulating levels of ~6 ng/ml). The peak response was observed during the 0.4 μg/h infusion rate (serum rhRLX of ~11 ng/ml), which was comparable to our previous work using a 4.0 μg/h (serum rhRLX of ~20 ng/ml). In contrast, a dose of 40 μg/h was ineffective (serum rhRLX of ~80 ng/ml). When 4.0 μg/h rhRLX was administered by osmotic minipump for shorter periods (<24 h), renal circulatory and osmoregulatory changes were observed by <6 h. After removal of the osmotic minipump, these changes persisted for at least 12 h, but they were fully restored by 24 h. Even briefier administration of 4.0 μg/h rhRLX by intravenous infusion showed an onset of action in the kidney by 1–2 h. In contrast, the 40 μg/h dose of rhRLX elicited minimal effects, and comparable to our earlier report, 4.0 μg/h purified porcine relaxin was also relatively ineffective during short-term intravenous administration. In conclusion, the effect of relaxin on the renal circulation and osmoregulation is biphasic, insofar as high doses are relatively inactive, and the onset of action is more rapid than previously believed. These findings may be important to consider when evaluating relaxin in the treatment of renal disease.

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In short, the objective of the present investigation was threefold: to determine the dose response and time course of relaxin-induced renal vasodilation, hyperfiltration, and osmoregulatory changes, as well as to determine the time required for the recovery of these variables after cessation of relaxin administration.

METHODS

Animal preparation. Female Long Evans rats of 10–14 wk of age were purchased from Harlan Sprague Dawley (Indianapolis, IN). The rats were fed PROLAB RMG 2500 diet containing 0.4% sodium (PME Feeds, St. Louis, MO). The rats were maintained on a 12:12-h light-dark cycle at the University of New Mexico Animal Resource Facility, a fully accredited program approved by the Association for Assessment and Accreditation of Laboratory Animal Care. All rat protocols were approved by the Institutional Animal Care and Use Committee.

Before surgical procedures, rats were habituated to a Plexiglas experimental cage (Braintree Scientific, Braintree, MA) from 1 to 5 h/day for a minimum of 5 days. The rats were then retrained 7–10 days after surgery and before the onset of the experimental protocol.

The surgical preparation has been previously described in detail (2). Briefly, under halothane anesthesia and with the use of the aseptic technique, catheters were implanted in the abdominal aorta and inferior vena cava via the femoral artery and vein, respectively, and externalized subcutaneously between the scapulae. These catheters were filled with a 1:1 mixture of 50% dextrose and heparin (1,000 U/ml) to keep them patent. A catheter made of Silastic-covered stainless-steel tubing was secured in the bladder with a purse-string suture and exteriorized through the ventral abdominal wall. The stainless-steel cannula was then stoppered with a Silastic-covered pin (18-gauge blunt needle), thus allowing the animals to urinate through the urethra while in their home cages. After surgery, the rats were returned to their home cages, provided with a 5% dextrose solution for hydration and additional nourishment for 48 h, and allowed 7–10 days of recovery before experimentation. All catheters were assembled in house and gas sterilized before surgery.

Measurement of renal function. After 7–10 days of recovery from surgery, renal function and mean arterial blood pressure (MAP) were measured in age-matched, chronically instrumented conscious rats. The rats were placed in the experimental cage, and after the obturator was removed from the bladder catheter, the bladder was rinsed with a small amount of sterile water and the cannula was extended with a short polyethylene tube that led to a collection container. Approximately 100 μl of blood were collected from the femoral arterial catheter for measurement of plasma osmolality (Posmol), sodium, and hematocrit. The arterial catheter was then attached to a Statham pressure transducer (Gould P23 ID) and a Gilson Universal amplifier and chart recorder (model ICT-2H Duograph, Gilson Medical Electronics, Middleton, WI). MAP was continuously recorded. The arterial catheter was also used for collection of blood samples at the midpoint of each timed urine collection. The femoral venous catheter was attached to a Sage infusion pump (model ICT-2H Duograph, Gilson Medical Electronics, Needham Heights, MA). The levels of rhRLX in serum were assayed by standard techniques, as reported previously (2).

Analytical techniques. Plasma and urine In and pAH were assayed by standard techniques, as reported previously (2). Posmol was measured by a freezing-point depression instrumentosmometer (model 3MO, Advanced Instruments, Needham Heights, MA). The levels of rhRLX in serum were measured by a quantitative ELISA immunoassay (11, 17). Brieﬂy, a 96-well microtiter plate (Falcon polystyrene U bottom, Becton Dickinson, Franklin Lakes, NJ) was coated overnight with affinintiy-puriﬁed anti-rhRLX rabbit polyclonal antibody. Standards and rat sera were diluted in PBS containing Tween-20, BSA, and goat IgG (Sigma Chemical, St. Louis, MO), and 100 μl were added to each well in duplicate or triplicate, respectively. After overnight incubation at 4°C, the wells were washed, and 100 μl of afﬁnity-puriﬁed, peroxidase-conjugated anti-rhRLX rabbit polyclonal antibody were added to each well, followed by incubation at room temperature while shaking for 3 h. After appropriate washing, tetramethylbenzidine solution
was added, and color development was stopped with phosphoric acid. Plates were read on a microtiter plate spectrophotometer (Labsystems MultiSkani RC, Fisher Scientific) at a wavelength of 450 nm with a reference wavelength of 630 nm. After correction for the blank, sample concentrations were determined by entering data into a four-parameter logistic curve-fitting program. Concentrations were corrected for the dilution factor and reported. Validation of the assay included spiking rat plasma at three levels: 50, 200, and 500 pg/ml, which yielded recoveries of 98, 95, and 102%, respectively. Low-, medium-, and high-level controls (n = 10 each) in the range of the standard curve (11.72–750 pg/ml) were then used to validate the intra-assay coefficient of variation. These values were 5.1, 1.9, and 3.5%, respectively. The interassay variation was 6.9, 12.1, and 13.1%, respectively, when the low, medium, and high controls were evaluated over 10 plates. The detection limit of the assay was 5 pg/ml. This assay does not detect either rat or pRLX.

Preparation of drugs. pAH (Merck & Co., West Point, PA) and IN (Sigma Chemical) were freshly prepared on the morning of the experiment by using Ringer solution as diluent. In, rect, Cupertino, CA) was used to deliver the rhRLX or vehicle (20 mM sodium acetate, pH 5.0). The rhRLX (from Elaine Unemori, Connectics, Palo Alto, CA) was supplied as a 5.0 mg/ml solution in 20 mM sodium acetate, pH 5.0. For intravenous infusion, both rhRLX and pRLX (the latter provided by O. David Sherwood as a lyophilized preparation) were diluted in Ringer solution.

Statistical analysis. Data are presented as means ± SE. Baseline renal clearances and clearances during the various doses of rhRLX infusion were averaged. The data was analyzed by using two-factor repeated-measures multivariate ANOVA. If significant main effects or interactions were observed, then group means were compared by using Tukey’s test (18). \( P < 0.05 \) was considered statistically significant.

RESULTS

Several doses of rhRLX were administered by Alzet minipump 1003D: 0.04, 0.15, 0.4, and 40 \( \mu \)g/h for 48 h. These infusions produced serum concentrations of 1.2 ± 1.0, 6.2 ± 1.5, 10.7 ± 2.3, and 80.2 ± 10.5 ng/ml, respectively. Both the 0.15 and 0.4 \( \mu \)g/h infusion rates increased GFR and ERPF, and decreased effective renal vascular resistance, with significance being reached at 0.4 \( \mu \)g/h (Fig. 1, A–C). MAP was not significantly affected by relaxin administration (data not shown). The increases in GFR and ERPF produced by the 0.4 \( \mu \)g/h infusion rate were comparable to those for rhRLX at 4 \( \mu \)g/h, as previously reported (5, 6, 11). We previously demonstrated that the serum rhRLX concentration attained with the 4 \( \mu \)g/h infusion averaged ~20 ng/ml, a level found on gestational day 11, when pregnancy-induced renal vasodilation is virtually maximal in this species (2, 5, 6, 11, 16). Of interest, the influence of rhRLX dosage on renal function was biphasic, because after 48 h of rhRLX administration at 40 \( \mu \)g/h, GFR, ERPF, and effective renal vascular resistance were comparable to baseline (Fig. 1, A–C). The Posmol was significantly decreased during the 0.15 and 0.4 \( \mu \)g/h but not during the 40 \( \mu \)g/h infusion rates compared with baseline (Fig. 1D).

GFR and ERPF were significantly increased as early as 6 h after the infusion of rhRLX by subcutaneous osmotic minipump at 4 \( \mu \)g/h was begun (Fig. 2, A and B). By 12 h, GFR and ERPF were at their peak and
comparable to our earlier studies in which we measured renal function after 2 and 5 days of rhRLX administration (5, 6, 11). Serum rhRLX concentrations at 6, 12, and 24 h were 10.7 ± 1.1, 14.0 ± 2.4, and 16.8 ± 2.7 ng/ml, respectively. Posmol was decreased at all time points compared with baseline (Fig. 2C). After the 24-h time point, the osmotic minipumps were removed. Renal function and Posmol returned to levels observed in vehicle-infused rats by 24 h (Fig. 3).

To investigate even shorter durations of infusion, rhRLX or pRLX was administered through the femoral venous catheter. rhRLX produced a significant increase in GFR and ERPF by as early as 1–2 h after the intravenous infusion was begun compared with vehicle administration (Fig. 4, A and B). Here, serum rhRLX concentration averaged 6.8 ± 1.0 ng/ml after 6 h of infusion. pRLX was considerably less effective than rhRLX when administered at the same infusion rate (4 μg/h).

Finally, renal function was measured at baseline and during intravenous infusion of vehicle or 40 μg/h rhRLX. This high dose of rhRLX modestly increased GFR relative to vehicle and baseline at 1, 3, and 4 h, but then returned to baseline values by 5 and 6 h (Fig. 5A). ERPF was significantly increased relative to vehicle infusion only at the 2-h time point (Fig. 5B). Serum rhRLX averaged 47.9 ± 12.1 ng/ml after 6 h of infusion.

**DISCUSSION**

We previously reported that rhRLX produced significant renal vasodilation and hyperfiltration after 2 and 5 days of chronic infusion at 4.0 μg/h (5, 6, 11). This infusion rate was designed to approximate serum levels comparable to those observed in midterm pregnant rats when gestational renal vasodilation is maximal (2, 16). In the present study, we evaluated a dose-response relationship between relaxin and renal function after 2
days of infusion. We observed the threshold dose to be \( \sim 0.15 \) \( \mu g/\)h, which produced an average serum concentration of \( \sim 6 \) ng/ml, whereas the peak response was observed during an infusion rate of \( 0.4 \) \( \mu g/\)h (serum rhRLX of \( \sim 11 \) ng/ml). The latter was comparable to the renal circulatory effects of the \( 4.0 \) \( \mu g/\)h infusion rate previously reported (5, 6, 11). Higher infusion rates of \( 40 \) \( \mu g/\)h, which achieved a mean serum concentration of \( \sim 80 \) ng/ml did not increase renal function. This serum level is comparable to that observed during late pregnancy in the rat, when renal function is returning to prepregnant values (2, 16). One possible explanation (among many) for the biphasic effect of relaxin is that high serum concentrations preferentially vasodilate other organ beds such as the uterus (13). Thus the kidneys may be effectively deprived of blood flow during late gestation in the rat.

We previously reported that renal function was unaffected during a 4-h period after a 2.0-\( \mu g \) intravenous bolus and 4 \( \mu g/\)h infusion of pRLX (6). This result, however, conflicted with our more recent work showing that incubation of small renal arteries with rhRLX in vitro for 3 h reduced myogenic reactivity (Novak and Conrad, unpublished observations). Therefore, we revisited the issue of time course. When rhRLX was administered by subcutaneous osmotic minipump at 4.0 \( \mu g/\)h, the onset of action in the renal circulation was \( \leq 6 \) h. Of additional interest, when the osmotic minipumps were subsequently removed, renal vasodilatation and hyperfiltration persisted for at least 12 h with complete restoration of renal function observed by 24 h.

After a 2.0-\( \mu g \) intravenous bolus and 4 \( \mu g/\)h infusion of rhRLX, significant renal vasodilatation and hyperfiltration were observed by as early as 1 to 2 h, which then persisted throughout the 6-h period of study. However, at the same dose, pRLX was relatively inactive between 1 and 6 h of infusion, comparable to our laboratory’s earlier report (6). Nevertheless, pRLX is equally effective as rhRLX on the renal circulation when administered for 2 or 5 days by osmotic minipump (6). Thus pRLX is considerably less potent than rhRLX when administered acutely but is comparable when administered chronically. Presently, we do not have a ready explanation for these discrepant results between the two relaxin preparations during acute administration.

Although the infusion of rhRLX for 6 h at 40 \( \mu g/\)h significantly increased GFR at the 1-, 3-, and 4-h time

Fig. 4. Effect of intravenous infusion of porcine relaxin (pRLX; 4 \( \mu g/\)h), rhRLX (4 \( \mu g/\)h), and vehicle (Ringer solution) on glomerular filtration rate (A) and effective renal plasma flow (B). Each point represents mean \( \pm \) SE; \( n = 6 \) rats each for the vehicle and pRLX groups; \( n = 13 \) rats for the rhRLX group. *\( P < 0.05 \) pRLX or rhRLX vs. vehicle.

Fig. 5. Effect of intravenous infusion of rhRLX (40 \( \mu g/\)h) or vehicle (Ringer solution) on glomerular filtration rate (A) and effective renal plasma flow (B). Each point represents mean \( \pm \) SE; \( n = 5 \) rats per treatment. *\( P < 0.05 \) vs. vehicle.
contribution of porcine and rhRLX, respectively. The expert clerical compared with infusion rates of 0.4 or 4.0
g/h are consistent with those obtained after 2 days of infusion, i.e., a minimal effect on renal function (if any)
with rates of 0.4 or 4.0 g/h. In addition to mediating the renal circulatory adap-
tations to pregnancy (14), relaxin also initiates the osmoregulatory adjustments (14). After 2 days of
rhRLX administration, the threshold dose for a decline in Posmol was 0.15 μg/h, which was identical to the
threshold dose for the renal circulatory changes. Further reduction was observed during the 0.4 μg/h infusion
that produced levels of Posmol comparable to the 4 μg/h infusion rate, as previously reported (5, 6, 11).
Of note, the 40 μg/h dose failed to significantly reduce Posmol. This result was unexpected, because Posmol
remains decreased during late gestation in rats in the face of high serum concentrations of relaxin that are
comparable to those reached with the 40 μg/h infusion (2, 12). One potential explanation for this apparent
inconsistency is that the moderate levels of serum relaxin observed during early to midgestation in the
rat initiate the decline in Posmol (14), whereas the maintenance of hypoosmolality with advancing gesta-
tion is independent of the hormone. During short-term administration of rhRLX by subcutaneous osmotic
minipump at 4.0 μg/h, the Posmol was significantly decreased after as little as 6 h of infusion. On cessation
of infusion, Posmol remained significantly decreased at 12 h but was restored by 24 h.

In view of both the renal vasodilatory (6) and matrix-degrading (17) attributes of relaxin, it has been sug-
gested that the hormone may be efficacious in the treatment of various renal diseases (1). So far, the
hormone has been tested in several rat models of renal disease: the bromoethylamine model of renal intersti-
tial fibrosis (9), renal mass reduction by infarction or surgical excision of both poles (8), and cyclosporin
nephrotoxicity (10). In all cases, amelioration of renal injury was observed. The findings of the present study
related to the biphasic dose response of relaxin and relatively rapid onset of action may facilitate further
evaluation of the hormone in the treatment of both chronic and acute renal diseases.

In summary, the present results provide further insight into the action of relaxin on the renal circula-
tion and osmoregulation in conscious rats. Surprising-
ly, the onset of action is considerably more rapid than previously believed (6), i.e., within hours, and the
dose response is biphasic, with high doses being rela-
tively inactive. Overall, both the dose response and time course effects of rhRLX on renal function and
Posmol were comparable.

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DISCLOSURES

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