Impact of gender and endothelin on renal vasodilation and hyperfiltration induced by relaxin in conscious rats

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Received 17 April 2000; accepted in final form 22 May 2000

Danielson, Lee A., Laurie J. Kercher, and Kirk P. Conrad. Impact of gender and endothelin on renal vasodilation and hyperfiltration induced by relaxin in conscious rats. Am J Physiol Regulatory Integrative Comp Physiol 279: R1298–R1304, 2000.—Chronic administration of the hormone relaxin elicits renal vasodilation that is dependent on nitric oxide (NO) in both conscious intact and ovariectomized female rats. Our first objective was to test whether the hormone, when administered to approximate serum concentrations found in midterm pregnant rats, induces renal vasodilation in males. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) increased significantly, on average, by 33 and 49% over baseline, respectively, after 5 days of recombinant human relaxin (rhRLX) administration to 12 conscious male rats by subcutaneous osmotic minipump. There were also significant decreases in hematocrit, plasma osmolality, and sodium concentration. Another objective was to determine whether endogenous endothelin (ET; via the endothelial ETB receptor) mediates the NO-dependent renal vasodilation produced by relaxin. rhRLX or vehicle was administered to conscious female rats (n = 9 and 8 rats, respectively). On the fifth day, baseline GFR and ERPF were both increased, on average, by 20–30% in the rats administered rhRLX (P < 0.05 vs. vehicle). Next, the specific ETB-receptor antagonist RES-701-1 was infused intravenously over 4 h in both groups of rats. In response to RES-701-1, there was a significant decline in both GFR and ERPF in the rats receiving rhRLX such that renal function converged in the two groups of animals. We conclude 1) relaxin induces marked changes in the renal circulation and in osmoregulation regardless of gender and 2) relaxin-induced renal vasodilation and hyperfiltration are mediated by endothelin through the endothelial ETB receptor subtype and NO.

glomerular filtration; renal blood flow; renal circulation; osmoregulation; nitric oxide; endothelin B receptor; RES-701-1; male and female rats

ONE OF THE EARLIEST AND MOST remarkable of physiological changes to occur during normal pregnancy is the vasodilation of nonreproductive organs. In this regard, the renal circulation plays a major role. A significant rise in both effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) is evident during the first few weeks of human pregnancy, with maximal increases of 40–80% above preconception levels observed by the end of the first or beginning of the second trimester (reviewed in Ref. 6). In chronically instrumented conscious rats, a significant rise in renal function is evident by gestational day 6 with a peak of 20–40% above preconception levels measured during gestational days 12–16 (3, 6). (Rat gestation lasts 22 days.)

The mechanisms underlying the profound renal vasodilatory response of pregnancy have remained elusive. Although vasodilatory prostaglandins are unlikely to play a major role except during certain stressful and unphysiological circumstances (4, 8), nitric oxide (NO) has been discovered to be an important mediator in the conscious rat model (6, 7, 20). Furthermore, endothelin (ET) via the endothelial ETB receptor, mediates the NO-dependent renal vasodilation and hyperfiltration of pregnancy in this species (5, 6).

Ultimately, these local renal mechanisms are likely to be under systemic hormonal control. In this regard, we deduced that the hormone relaxin (RLX) is a likely candidate (9). RLX is a low-molecular-weight protein of ~6,000 Da belonging to the insulin-growth factor family that circulates during the luteal phase of the menstrual cycle and throughout gestation in women. RLX is also a pregnancy hormone in rats. Endogenous rat relaxin is not detectable in the serum of nonpregnant rats nor in pregnancy until gestational day 8 (19). In both species, circulating levels derive from the corpus luteum (reviewed in Ref. 19). The hormone likely mediates the marked reduction in plasma osmolality that transpires during gestation (9, 25, 26). Analogous to the pregnant condition, chronic administration of the hormone to nonpregnant rats produces renal vasodilation and hyperfiltration that is dependent on NO (9).

In contrast to many of the biological actions of RLX on the reproductive tract (19), the renal vasodilation and hyperfiltration persist despite ovariectomy, suggesting the absence of either an intermediary or per-
missive role for estrogen and progesterone (9). Accordingly, the first objective of the present investigation was to extend this observation by testing whether RLX can produce comparable changes in the renal circulation of male rats. If so, then the hormone is intrinsically a renal vasodilator irrespective of gender.

Another objective was to further test the parallel between relaxin- and pregnancy-induced renal vasodilation and hyperfiltration. As already mentioned, we previously showed that endothelin and the endothelial ET<sub>B</sub> receptor lie upstream of the NO-dependent renal circulatory changes in pregnancy. Therefore, in the present work we tested whether the endothelin system plays a similar role in the NO-dependent renal vasodilation and hyperfiltration elicited by relaxin.

METHODS

Animal preparation. Long-Evans female and male rats of 10–14 wk of age were used. Those animals studied at the University of New Mexico were purchased from Harlan Sprague Dawley (Indianapolis, IN) and were fed a PROLAB RMH 2500 diet containing 0.40% sodium (PME Feeds, St. Louis, MO). The rats investigated at the Magee-Womens Research Institute were purchased from Harlan Sprague Dawley (Frederick, MD) and were fed PROLAB RMH 2000 diet containing 0.48% sodium (PME Feeds). The rats were maintained on a 12:12-h light-dark cycle in fully accredited Animal Resource Facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care. All experiments were approved by the Institutional Animal Care and Use Committee of the University of New Mexico School of Medicine or of the Magee-Womens Research Institute.

Before surgical preparation, the rats were habituated to Plexiglas experimental cages (Braintree Scientific, Braintree MA) over 5 days as previously described (3, 4, 7, 8). The surgical procedures have also been previously described in detail (3, 4, 7, 8). After surgery, the rats were returned to their home cages and provided 5% dextrose in water during the first 2 days of surgical recovery for additional hydration and nourishment. Seven to ten days of recovery were permitted, during which time the rats were trained once more to the experimental cage.

Experimental protocol: male rats. After being placed in the experimental cage, a 100-μl blood sample was drawn from the arterial catheter into a heparinized tube for measurement of baseline plasma osmolality, sodium concentration, and hematocrit before the administration of any fluids. This catheter was subsequently connected to a Statham pressure transducer (Gould P23 ID) and a Gould Universal amplifier to measure mean arterial pressure (MAP), which was displayed on a Gould 5800 Series Signal Conditioner Cage and TA11 chart recorder. Next, a bolus of inulin [IN; 0.2 ml of a 20% stock solution/100 g body wt (BW)] and para-aminohippurate (PAH; 0.1 ml of a 2% working solution/100 g BW) was given over 1 min into the venous catheter, followed by a constant infusion of the two reagents at a rate of 0.5 and 0.1 mg·min<sup>−1·100 g BW</sup><sup>−1</sup>, respectively. The flow rate was 19 μl/min, delivered by a model 200 Syringe Pump (KD Scientific, Boston MA). Finally, the obturator in the bladder catheter was removed, and the latter was extended with a short piece of polyethylene tubing to facilitate the collection of urine.

After an equilibration period of 60 min, three 30-min urine collections with midpoint blood samples were obtained, to determine the renal clearances of IN and PAH, which provide measures of GFR and ERPF, respectively. The technique of urine collection has proven to be reliable (4, 7, 8). Indeed, after reaching steady state in this study, the excretion rates of IN and PAH were 91 ± 3 and 100 ± 4% of their respective infusions. After baseline MAP and renal function were measured, an osmotic minipump (model 2ML1, Alza, Palo Alto, CA) containing either recombinant human RLX (rhRLX; n = 12 rats) or vehicle (n = 7) was implanted subcutaneously on the back using light ether anesthesia. The infusion rate of rhRLX was 4 μg/h, a rate that we previously showed to produce serum levels comparable to midgestation in the female rat when ERPF and GFR are maximal during pregnancy in this species (Ref. 3 and 9, and see RESULTS). Five days later, MAP, GFR, ERPF, and other parameters were again assessed as described above. At this time, the excretion rates of IN and PAH were 91 ± 3 and 98 ± 2% of their respective infusion rates. At the end of the experiment, 1.0 ml of blood was collected for determination of serum rhRLX.

Experimental protocol: female rats. After surgical recovery, an osmotic minipump (model 2ML1, Alza, Palo Alto, CA) containing either rhRLX (n = 9 rats) or vehicle (n = 8) was implanted subcutaneously on the back using light ether anesthesia. The infusion rate of rhRLX was 4 μg/h. Experiments were conducted on day 5 of rhRLX or vehicle administration. MAP and renal function were measured as described above.

After an equilibration period of 60 min, three 30-min urine collections with midpoint blood samples were obtained, to determine baseline renal clearances of IN and PAH. The technique of urine collection was again reliable. After reaching steady state, the excretion rates of IN and PAH at baseline were 99 ± 3 and 100 ± 2% of their respective infusions for the rats administered rhRLX and 100 ± 2 and 99 ± 2% for those given vehicle. After the determination of baseline MAP and renal function, an infusion of RES-701-1, a selective ET<sub>B</sub>-receptor antagonist (5, 22, 24), was started at a rate of 10 μg/min (flow rate 12 μl/min) through the venous catheter. Next, six 40-min renal clearances were obtained during the infusion of the RES-701-1. The average recovery rates for both IN and PAH in the urine were comparable to those described above, i.e., > 95% of the infusion rates. At the end of the experiment, 1.0 ml of blood was collected for determination of serum rhRLX.

In an additional three rats each administered rhRLX or vehicle, identical experimental procedures as described above were applied, except that the vehicle for RES-701-1, as described below, was infused instead of RES-701-1.

Analytical techniques. IN concentration in plasma and urine was measured by the anthrone method, and PAH was determined by the method of Bratton and Marshall as modified by Smith (see citations in Ref. 3). Plasma sodium was measured by a Kodak Ektachem Instrument (Rochester, NY). Plasma osmolality was determined by freezing-point depression (Advanced Osmometer, model 3MO, Advanced Instruments, Needham Heights, MA). All urine and plasma samples from MWRI were coded and sent to the University of New Mexico for analysis of IN, PAH, sodium, and osmolality (by L. A. Danielson). The rhRLX in serum was measured by Beverly Grove of Connetics (Palo Alto, CA) using a quantitative sandwich immunoassay (9, 23), again in a blinded fashion.

Preparation of drugs. PAH and IN were prepared as previously reported (3, 4, 7, 8). The rhRLX was generously provided by Dr. Elaine Unemori of Connetics at a concentration of 1.5 mg/ml in 20 mM sodium acetate (pH 5.0). The rhRLX was diluted with additional 20 mM sodium acetate for instillation in the osmotic minipumps, or the 20 mM sodium acetate buffer was administered alone as vehicle. The flow
rate of the osmotic minipumps was ~10 \mu l/h. The ET-receptor antagonist RES-701-1, a selective ET B receptor subtype antagonist purified from the broth of *Streptomyces* (22), was prepared at 37°C in a dilute 0.02% sodium carbonate solution containing 5% dextrose (5).

**Statistical analysis.** There were a total of 12 male rats administered rhRLX and 7 administered vehicle. Five of the rats receiving rhRLX and three given vehicle were studied at the University of New Mexico School of Medicine (by L. A. Danielson). The remaining rats in the two groups were investigated at the University of Pittsburgh School of Medicine, Magee-Womens Research Institute (by L. J. Kercher and K. P. Conrad). The data obtained from the two laboratories were comparable, and therefore, combined. Data are expressed as means \pm SE. MAP and renal function measured during the three renal clearance periods were averaged for each experiment. The results obtained at baseline and after 5 days of rhRLX or vehicle infusion were compared by paired \( t \)-tests (Fig. 1). In Table 1, we applied unpaired \( t \)-tests. A \( P \) value of <0.05 was taken to be significant.

A total of 11 female rats were administered rhRLX and 10 administered vehicle. Five in each group were studied at the University of New Mexico School of Medicine (by L. A. Danielson). The remaining rats in the two groups were investigated at the University of Pittsburgh School of Medicine, Magee-Womens Research Institute (by L. J. Kercher and K. P. Conrad). The data obtained by the two laboratories were comparable, and therefore, combined. Data are expressed as means \pm SE. MAP and renal function measured during the six renal clearance periods during the infusion of RES-701-1 or its vehicle were also averaged. Two-factor, repeated measures analysis of variance was employed to analyze the data presented in Fig. 2. If significant main effects or interactions were observed, then group means were compared by the method of Contrasts (SuperANOVA, Abacus Concepts, Berkeley CA). In Table 2, we used unpaired \( t \)-tests. A \( P \) value of <0.05 was again taken to be significant.

**RESULTS**

**Male rats.** The results for MAP and renal function are portrayed in Fig. 1. The chronic administration of rhRLX did not significantly affect MAP. In contrast, the hormone significantly increased both GFR and ERPF, while reducing effective renal vascular resistance (ERVR). Effective filtration fraction was reduced from 0.32 \pm 0.01 at baseline to 0.29 \pm 0.01 during rhRLX administration (\( P < 0.05 \)). The vehicle time-control studies showed stability of both MAP and renal function.

Table 1 depicts the data for hematocrit, plasma osmolality, and sodium concentration. These variables were relatively constant in the vehicle time-control experiments. However, in those rats receiving rhRLX for 5 days, there was a significant decline in all three parameters. RLX was not detectable in any of the rats administered vehicle \((n = 7)\). In those administered rhRLX, the mean concentration was 12.3 \pm 0.7 ng/ml \((n = 12)\).

**Female rats.** Figure 2 portrays the results using the specific ET B-receptor antagonist RES-701-1. At baseline, both GFR and ERPF were significantly increased, and ERVR was reciprocally reduced by 20–30% in the
rats administered rhRLX for 5 days compared with vehicle infusion. Effective filtration fraction was $0.32 \pm 0.01$ and $0.29 \pm 0.01$ in the vehicle- and RLX-treated rats, respectively ($P < 0.05$). MAP was not significantly affected.

Administration of RES-701-1 had no significant effect on MAP and renal function in the vehicle-treated rats, although ERVR tended to be increased. In contrast, RES-701-1 reduced both GFR and ERPF and increased MAP and ERVR in the rats treated with rhRLX (all $P < 0.05$ vs. baseline). During the infusion of the ETB-receptor antagonist, GFR, ERPF, and ERVR converged in the two groups of rats by the end of the second renal clearance period (data not shown).

Table 2 depicts the data from the rhRLX- or vehicle-treated rats administered the vehicle for RES-701-1 instead of the antagonist (time-control). At baseline, GFR and ERPF were increased, and ERVR was reduced in the rats administered rhRLX for 5 days compared with baseline values observed in the rats receiving vehicle instead of rhRLX (all $P < 0.05$). These differences were maintained during the administration of the vehicle for RES-701-1, showing stability of renal function over the 240-min infusion period. On day 5 of rhRLX administration, the mean serum concentration was $16.6 \pm 1.5$ ng/ml. Serum rhRLX was not detected in any of the rats administered vehicle instead of rhRLX except (inexplicably) for one animal with a value of 0.84 ng/ml.

The male rats responded more robustly to the rhRLX than did the females despite receiving the same dosage and reaching somewhat lower serum levels of the hormone. Both GFR and ERPF were higher in the male rats during rhRLX administration ($P < 0.05$ vs. females).

**DISCUSSION**

The major findings are 1) chronic administration of rhRLX to conscious male rats elicits renal vasodilation and hyperfiltration and reduces plasma osmolality and sodium concentration, as well as hematocrit; 2) the renal vasodilation and hyperfiltration produced by 5-day administration of rhRLX to conscious female rats were blocked by RES-701-1, a specific antagonist of the ETB receptor; and 3) MAP was increased by RES-701-1 only in rats administered rhRLX and not in control animals receiving vehicle instead of rhRLX.

### Table 2. Time-control studies

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>GFR, µl/min</th>
<th>ERPF, µl/min</th>
<th>ERVR, mmHg·ml⁻¹·min</th>
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<tr>
<td></td>
<td>B</td>
<td>V</td>
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<tr>
<td>Vehicle</td>
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<tr>
<td>($n = 3$ rats)</td>
<td>113 ± 4</td>
<td>113 ± 6</td>
<td>2,189 ± 108</td>
<td>2,308 ± 26</td>
</tr>
<tr>
<td>rhRLX</td>
<td>112 ± 7</td>
<td>115 ± 8</td>
<td>3,082 ± 31a</td>
<td>3,112 ± 138a</td>
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</table>

Values are means ± SE. MAP, mean arterial pressure; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; ERVR, effective renal vascular resistance; B, baseline; V, vehicle for RES-701-1. *$P < 0.05$ rhRLX vs. vehicle (for rhRLX).
The first major finding is intriguing because RLX is traditionally considered to be a female hormone. RLX is not believed to circulate in male rats (19), nor was it detectable by immunoassay in our vehicle-infused animals (see RESULTS). Perhaps under physiological conditions, RLX production and action within the central nervous system (CNS) may influence osmoregulation and renal function in male (and female) rats, or possibly, the hormone is synthesized by the kidney and acts locally to modulate the renal circulation. Indeed, Gunnersen and co-workers (15) reported RLX mRNA in the brain of both male and female rats, and in the kidneys of females by RT-PCR (kidneys from male rats were apparently not tested).

Weisenger and co-workers (25) reported that ovariectomized rats treated with synthetic hRLX for 7 days demonstrated a significant reduction in baseline plasma osmolality with unchanging plasma arginine vasopressin (AVP), indicating a shift in the osmoregulatory threshold for AVP release (25). In our own work, we found a profound reduction in plasma osmolality by 12 or 14 mosmol/kgH2O during chronic administration of either rhRLX or purified porcine RLX, respectively, to intact female rats. We also observed a significant decline in hematocrit, possibly reflecting plasma volume expansion (9). By using mice rendered deficient in RLX by gene targeting, late-pregnant mice demonstrated a significantly higher plasma osmolality than the wild-type animals (26). Taken together, these data strongly suggest that the osmoregulatory changes that occur during normal pregnancy are mediated by RLX. The present results further indicate that exogenous RLX can evoke osmoregulatory changes regardless of gender. Circulating RLX may act directly on the circumventricular organs in the CNS or indirectly by reducing total peripheral vascular resistance, thus effecting release of AVP and alteration of the threshold for thirst. Local production of RLX and its action on receptors in the CNS may also modulate osmoregulation (11, 21).

We previously reported that chronic administration of rhRLX or of purified porcine RLX to conscious virgin female rats induced renal vasodilation and hyperfiltration comparable in magnitude to that observed during midgestation in this species (9). Indeed, the RLX infusion rate in that study (and the present) was targeted to yield the serum concentration of ~20 ng/ml that is found in midgestation, a gestational stage when renal vasodilation and hyperfiltration are maximal (3). Surprisingly, unlike previous studies evaluating the action of either endogenous or exogenous RLX on the female reproductive tract (19), the renal effects of the hormone persisted despite ovariectomy, thereby excluding an intermediary or permissive role for the sex steroids (9). The latter observation prompted the present investigation in males, which shows that RLX can induce marked renal vasodilation and hyperfiltration irrespective of gender (and despite somewhat lower serum levels of RLX than found in midterm pregnant rats). In fact, the hormone was apparently more efficacious in male rats, producing higher levels of GFR and ERPF than in the females. However, our experiments in the males and females were not conducted concurrently; a side-by-side comparison is really needed to corroborate this apparent gender difference.

The efficacy of RLX in males found in the present study is consistent with previous reports showing coronary blood flow and other cardiac effects of RLX in hearts isolated from male rats (2, 16). However, a major difference lies in the time required for the onset of RLX action: by extrapolation from our previous work in female rats, a relatively prolonged exposure is needed for alteration of renal and osmoregulatory function (9), whereas the effects on the heart are rapid (2, 16).

Gellai and colleagues (12, 14) reported a major physiological role for endogenous ET and the endothelial ETB receptor subtype in maintaining the low vascular resistance of the renal circulation in conscious male rats. They also proposed that the NO pathway mediated the vasoconstrictor influence of endogenous ET in the kidney (12, 13). Recently, we showed that both the specific ETB-receptor antagonist RES-701-1 and the mixed ETA/ETB-receptor antagonist SB-209670 significantly increased MAP and ERVR and reduced ERPF in conscious (nonpregnant) female rats (6). In the present work, we used an infusion rate of RES-701-1 three times lower than that in our previous study (10 vs. 30 μg/min), mainly due to the short supply of RES-701-1 and unavailability of additional compound. In the control rats administered vehicle (instead of rhRLX), we observed no significant change in MAP or renal function, although ERVR tended to increase in response to RES-701-1. Although this effect of RES-701-1 is diminished compared with that found in our previous study (6), it is most likely due to the lower (virtually subthreshold) dosage employed in the present investigation.

In contrast, the 10 μg/min infusion rate of RES-701-1 elicited significantly more renal vasoconstriction in those rats administered RLX. Administration of rhRLX for 5 days significantly increased both GFR and ERPF and reduced ERVR as previously reported (9). When RES-701-1 was infused into the rats receiving rhRLX, GFR and ERPF were reduced, and ERVR was elevated to levels observed in the control animals administered the vehicle for rhRLX instead of hormone. These results are analogous to those previously reported for conscious, midterm pregnant, and nonpregnant control rats, in which RES-701-1 led to a convergence of GFR, ERPF, and ERVR in the two groups of animals due to more robust renal vasoconstriction in the gravid rats (5). In that study, concurrent administration of RES-701-1 and the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) to midterm pregnant rats produced no further decline in renal function than either agent given alone, suggesting the inhibition of a common vasodilatory pathway (5). Indeed, earlier work showed that NO mediated the renal vasodilation and hyperfiltration in conscious midterm pregnant rats (7). The renal vasodilatory response to RLX was also shown to be mediated by NO, and the hormone caused an attenuation of the renal vasoconstrictor response to angiotensin II (9), again mimicking...
the pregnant condition (7). The present study extends the parallel between RLX- and pregnancy-induced renal vasodilation and hyperfiltration, insofar as both are abrogated by ETa receptor blockade. Thus the physiological role of ET and the endothelial ETa Receptor subtype in maintaining low renal vascular tone via the NO pathway in the nonpregnant condition is accentuated not only in midterm pregnant rats but also in nonpregnant rats administered rhRLX.

We found that there was a convergence of renal function in the RLX- and vehicle-treated rats after the second 40-min renal clearance during infusion of RES-701-1 (data not shown). In contrast, the response to RES-701-1 was more gradual in the pregnancy study (701-1 (data not shown). In contrast, the response to second 40-min renal clearance during infusion of RES-

function in the RLX- and vehicle-treated rats after the

in nonpregnant rats administered rhRLX.

centuated not only in midterm pregnant rats but also

the NO pathway in the nonpregnant condition is ac-

tively by 30% (1, 10).

Interestingly, we observed exaggerated hypertensive responses to both L-NAME (9) and RES-701-1 (present study) in conscious female rats during chronic infusion of rhRLX. These findings are in contrast to those obtained for nonpregnant control and midterm pregnant rats, in which the hypertensive responses to these inhibitors were not different between the two groups of animals (5, 7). One possible explanation for this discrepancy is that baroreflexes may have obscured potential differences in the pressor effects between nonpregnant and gravid rats. Indeed, using ganglionic-blocked rats, Nathan and colleagues (18) reported a greater pressor response to L-NAME in gravid rats compared with nonpregnant controls.

In future studies, we plan to directly test the hypothesis that the renal vasodilatory effect of RLX is locally mediated at the level of small renal arteries. If so, RLX binding sites then need to be investigated in the kidney, particularly on small renal arteries. Unfortunately, the RLX receptor has not been cloned. Nevertheless, previous work using biotinylated RLX suggested binding sites associated with blood vessels at least in reproductive organs of women (17). If the newly described renal vasodilatory property of RLX in rats also pertains to humans, then it is tempting to contemplate a potential therapeutic role for RLX in renal diseases that afflict men as well as women, particularly in light of the well-known matrix-degrading attributes of the hormone (19, 23).

We thank Dr. Elaine Unemori of Connetics, Palo Alto, CA, for generously providing the recombinant human relaxin and Beverly Grove of Connetics for measuring the serum levels of the hormone. Drs. Tatsuhiro Ogawa and Satoshi Nakanishi of Kyowa Hakko Kogyo, Tokyo Research Laboratories, generously provided the RES-701-1. We also gratefully acknowledge the expert clerical assistance of Sue Kauffman.

This work was supported by National Institute of Child Health and Human Development Research Career Development Award K04 HD-01098 and Grant RO1 HD-30325.

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