Attenuation of Gonadotropin-Releasing Hormone Reflex to Coitus by α_1-adrenergic Receptor Blockade in the Rabbit (44287)

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Abstract. **The coitally induced gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) surge in the rabbit is preceded by an enhanced secretion of hypothalamic norepinephrine (NE). To investigate if adrenergic receptors are essential for the GnRH/LH surge, we administered a specific α_1 receptor blocker, prazosin, into either the arcuate nucleus-median eminence (AME) or the third cerebroventricle (3rd V) of tethered, freely moving intact female rabbits *via* push-pull perfusion (PPP). Dual cannulae for PPP and drug infusion were placed stereotaxically either into the AME or AME and 3rd V of each rabbit after insertion of a permanent femoral venous catheter for serial blood sampling. During an experiment, continuous PPP samples and 10-min intermittent blood samples were collected for 5–6 hr. Females received either prazosin or control medium (artificial cerebrospinal fluid into an AME or saline into a 3rd V cannula) for 4 hr, beginning 1 hr before coitus. Intraventricular infusion of prazosin significantly ($P < 0.05$) suppressed both the postcoital GnRH and the LH surges. Administration of prazosin into the AME also attenuated the magnitude of the postcoital GnRH surge ($P < 0.05$) whereas postcoital LH values were not decreased below that of the control group ($P > 0.05$). The results suggest that α_1 receptors are physiologically active in the initiation of the postcoital GnRH release. These findings, along with our earlier report of enhanced postcoital NE secretion, reinforce the hypothesis that NE plays an essential role in the preovulatory GnRH/LH surge in rabbits.**

[P.S.E.B.M. 1998, Vol 218]

Previous findings have suggested that hypothalamic norepinephrine (NE) has an important role in ovulation in the rabbit, a reflex ovulator. Intraventricular administration of NE induces secretion of the luteinizing hormone (LH) and ovulation (1, 2) whereas dibenamine, an adrenergic blocking agent, inhibits ovulation (3). Recent observations also suggest that noradrenergic activities (i.e., NE secretion (4, 5), synthesis, and reuptake (6)) increase during the coitally induced preovulatory LH surge. The following evidence suggests that NE activates the LH surge by stimulating gonadotropin releasing hormone (GnRH) release: (1) neural terminals that contain catecholamine synapse in proximity with GnRH neurons in the hypothalamus (7); (2) NE stimulates GnRH/LH release in intact and ovariectomized/estrogen-treated rabbits (8); (3) an increase in hypothalamic NE release accompanies an increase in GnRH release during the postcoital LH surge (4, 5); and (4) neither pituitary sensitivity to GnRH (9) nor LH release is affected when the pituitary is exposed to NE *in vitro* (10) or *in situ* (11). However, no direct evidence has shown an effect of an antagonist of NE on GnRH release during the LH surge. Moreover, the specific adrenergic receptors that are involved in this process have not been elucidated.

Previous *in vivo* studies demonstrate that α_1-adrenergic receptors are abundant in the rat hypothalamus (12), and they participate in regulation of LH secretion (13). In the rabbit, hypothalamic neuropeptide Y and GnRH are con-

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This research was supported by the National Institutes of Health Grants RR-00163, HD-30316 and HD-18185.

Received September 8, 1997. [P.S.E.B.M. 1998, Vol 218]
Accepted January 27, 1998.

0037-9727/98/2183-0204$10.50/0

comitantly stimulated by the ovulating agent cupric acetate (14). Moreover, an α_1 but not an α_2, adrenergic antagonist blocks neuropeptide Y-induced GnRH release (15); however, the relevance of these findings to coitally induced GnRH release is not clear. In the present study, we report a clear diminution of the coitally induced GnRH/LH surge by intracranial infusion of an α_1-adrenergic receptor blocker, prazosin, in intact female rabbits.

Materials and Methods

Animals. Adult female New Zealand White (NZW) rabbits (3.5–4.5 kg) were obtained from Western Oregon Laboratories (Corvallis, OR) and housed in single cages in a temperature (22° ± 2°C)- and light (lights on, 0700–1900)-controlled room. The rabbits were fed 150 g of rabbit chow (Ralston-Purina, St. Louis, MO) daily, and water was available *ad libitum*. The protocols for experiments performed were approved by the institution's Animal Care and Use Committee.

Surgery. Each rabbit received a femoral vein catheter. Surgical anesthesia was initiated with an iv injection of ketamine (40 mg/kg BW), acepromazine (0.5 mg/kg) and xylazine (5 mg/kg), and was maintained by inhalation of 2.5% halothane (Upjohn Co., Kalamazoo, MI) and 30% nitrous oxide-70% oxygen. An indwelling catheter (PV-6, Bolab, Lake Havasu City, AZ) was inserted into the vena cava *via* the femoral vein to a level below the renal bifurcation. The catheter extension was routed subcutaneously to the top of the calvarium and exteriorized *via* a tunnel to the push-pull cannulae (PPC) and headpiece. The catheter was filled with a concentrated heparin solution (1000 IU/ml; Upjohn Co.) and sealed.

Immediately following the successful implantation of the indwelling catheter, a pair of PPC were stereotaxically implanted bilaterally either into the arcuate nucleus–median eminence (AME) of each rabbit, or the pair of cannulae were implanted so that one was in the AME and the other in the third ventricle (3rd V). The construction of the PPC assembly (stylet-outer cannula headpiece) and the procedures for PPC placement have been described in detail elsewhere (4, 5, 8). Briefly, the dura and sagittal sinus were exposed by removal of a 3-mm × 5-mm skull fragment at bregma. A longitudinal incision was made in the dura, and the sinus was retracted laterally. A sterile PPC assembly was then lowered slowly into the AME at a position 0.2 mm anterior to bregma, 0.3 mm lateral to midline, and 17.5 mm ventral to the dural surface. A PPC aimed at the 3rd V was 2.5 mm posterior to the AME cannula at midline and 15 mm ventral to the dural surface. The PPC assembly was secured to the skull with anchor screws and dental cement (Co-oralite Dental Mfg. Co., Rancho Cordova, CA), and the fascia and skin were sutured around the base of the PPC assembly. Flo-Cillin was administered prior to returning the animal to its cage for recovery.

Push-Pull Perfusion Procedure. To provide an undisturbed environment for the rabbits, a multichannel fluid-swivel-tethering system was used for remote sampling (4, 5) of both push-pull perfusate (PPP) and venous blood from an adjacent room. Behaviors were also monitored *via* closed circuit television. Mating occurred in a cage with two chambers (each 50 × 50 × 50 cm) separated by a transparent removable plexiglass divider. The divider was removed during the 10-min interval of male and female interaction; it was replaced after a single copulation. Each chamber had food and water available *ad libitum*. Each female was acclimated to the chamber and connected to the swivel/tether two or more times before an experiment. To enhance mating rates, intact females were primed with a sc injection of 3 μg estradiol benzoate (EB, Sigma Chemical Co., St. Louis, MO) in sesame oil the night before a mating trial. The flow of sterile perfusion medium, Krebs-Ringer phosphate buffer (KRP), was balanced between the push and pull pumps to a flow rate of 5 μl/min as previously described (8). Perfusate fractions (50 μl total volume) were collected continuously into plastic tubes on ice every 10 min for a total of 5–6 hr. Perfusate samples were acidified immediately during collection to pH 2.0–3.0 with 10 μl 1 *N* acetic acid and stored at −20°C until assayed for GnRH. Each rabbit was subjected to PPP twice. The two trials were separated by at least 21 days with alternate control or prazosin infusion in a random order.

Prazosin (Mylan Pharmaceuticals Inc., Morgantown, WV) was administered either into the third cerebroventricle *via* an icv cannula with saline as carrier or locally into the AME *via* a PPP cannula with prazosin dissolved in KRP during PPP. Saline was selected as a carrier in the icv trials because the solubility of prazosin in saline was approximately twice the amount of that in KRP. A higher concentration of prazosin was desirable for icv infusion to reduce the fluid volume introduced into the third cerebroventricle. Saline alone infused icv did not alter the pattern of GnRH/LH secretion (preliminary trials, data not shown).

Sequential blood samples (0.6 ml) were obtained *via* the catheter at the end of each 10-min perfusate collection. Samples were placed into ice-cold heparinized (50 IU/tube) glass tubes and centrifuged at 4°C for 30 min (1500*g*). The plasma fractions were collected and stored at −20°C until assayed for LH.

Experimental Design. *Experiment 1: Effect of 3rd V infusion of prazosin on GnRH and LH release.* AME-PPP was performed for 5 hr. At the initiation of hr-2 of PPP—one hr prior to mating—females received either saline ($n = 5$) or prazosin (2 mg/ml saline, [$n = 5$]) infused into the 3rd V at a rate of 3 μl/min until the end of the experiment. At the end of hr-2, the tethered, freely moving female was provided access to a vasectomized male for 10 min or less (i.e., as soon as copulation occurred).

Experiment 2: Effect of AME infusion of prazosin on GnRH and LH release. Blood and PPP samples were collected for 6 hr. At the end of hr-2 and 1 hr before mating, females either continued receiving KRP only ($n = 5$) or

prazosin (Sigma Chemical Co., St. Louis, MO) at a dosage of 1 mg/ml KRP (n = 5) for 4 hr. At the end of hr-3 of PPP, a vasectomized male was allowed 10 min or less with the female for coital interaction. PPP and blood sampling continued for an additional 3 hr. Coitus occurred in all control and prazosin-treated females in these studies.

Assays. The radioimmunoassay (RIA) procedure for GnRH was the same as that reported previously (16, 17). Synthetic GnRH (Sigma, St. Louis, MO) was used both for the reference standard and for radioiodination. GnRH antiserum (EL-14) was used at a final dilution of 1:504,000 with binding of 20%–30% and a sensitivity of 0.13–0.21 pg/tube at 90% displacement in the standard curve. Coefficients of variation (CV) between and within assays were 13% and 8%. Plasma LH was measured by a homologous rabbit RIA (8). The sensitivity of the LH assay ranged from 0.23–0.62 ng/ml. Inter- and intra-assay coefficients of variation for the LH assay were 10% and 8%.

Statistics. Changes in the release of GnRH and LH within groups were analyzed using analysis of variance (ANOVA) for repeated measures with a *post hoc* Fisher's multiple range test. Differences between hormone patterns of different groups were determined with two-way ANOVA. All samples with nondetectable values were assigned values equal to the particular assay sensitivity with appropriate adjustments for the loss in degrees of freedom. Differences in postcoital hourly mean values between control and prazosin-treated animals were analyzed by ANOVA followed by *post hoc* Duncan's multiple range comparisons. Group data are reported as mean ± 1 SEM.

Results

The individual patterns of AME-GnRH and plasma LH concentrations before and after coitus in two females, each of whom received both icv saline and prazosin during separate experimental trials, are presented in Figure 1. Coitus induced a distinct release of GnRH and LH in both females #906 and #875 after saline whereas these postcoital neuroendocrine changes were blocked (#875) or attenuated (#906) after prazosin administration.

Figure 2 depicts simultaneous AME concentrations of GnRH and plasma levels of LH in all females that were infused with icv saline (n = 4) or prazosin (n = 5) before and after coitus (data were lost in one icv-saline animal due to an unsuccessful PPP). In the saline-infused controls, a postcoital GnRH and LH surge was observed in each of four females. These mean GnRH/LH values remained elevated during a postcoital 3-hr interval (Fig. 2A). In sharp contrast, the elevated postcoital GnRH/LH changes were suppressed by icv prazosin infusion (Fig. 2B).

Precoital concentrations of AME-GnRH and plasma LH were unaltered ($P > 0.05$) by either icv infusion of saline (GnRH: preinfusion [pif] levels averaged 0.1 ± 0.04 vs. infusion [if] levels of 0.04 ± 0.02 pg/ml; LH [pif] levels averaged 0.56 ± 0.2 vs [if] values of 0.61 ± 0.35 ng/ml, respectively) or prazosin (GnRH; pif levels averaged 0.04 ± 0.02 vs. if values of 0.03 ± 0.01 pg/ml; LH pif values averaged 0.43 ± 0.1 vs. if values of 0.46 ± 0.1 ng/ml, respectively).

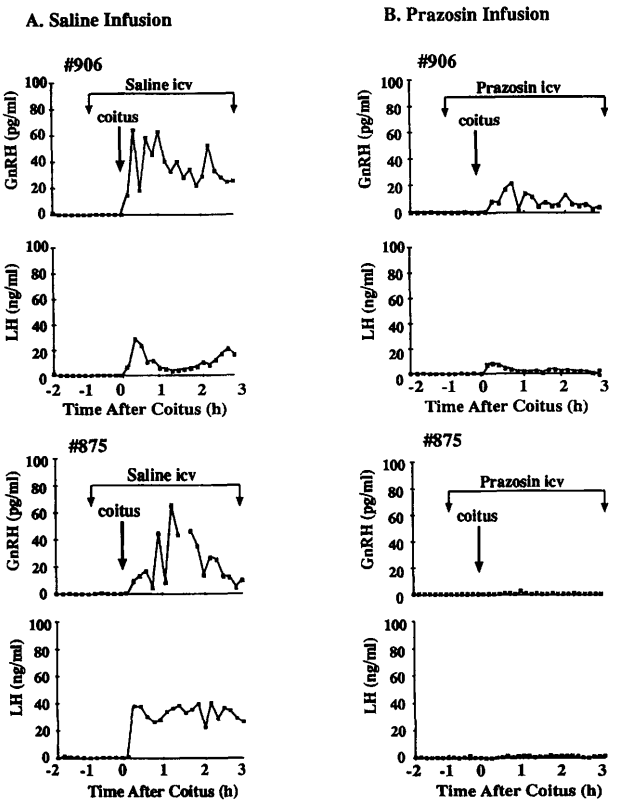

Figure 1. Individual patterns of AME GnRH and plasma LH in two females (#906 and #875) with alternate saline or prazosin infusion into the third ventricle. Arrows indicate the time of mating and the beginning and ending of third ventricle (icv) infusion.

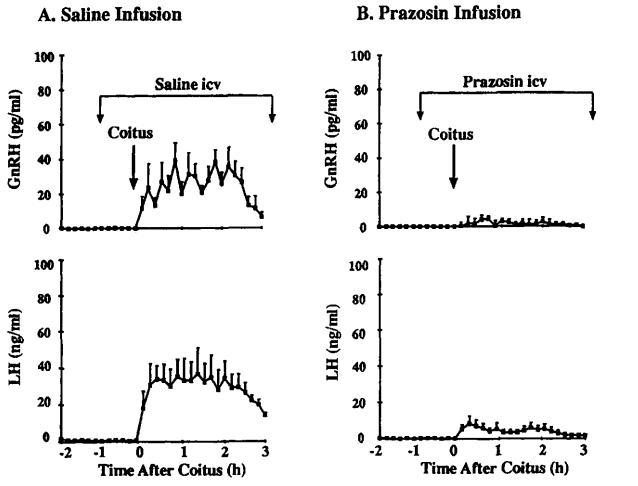

Figure 2. Mean + standard error (SE) levels of AME GnRH and plasma LH before and after coitus in female rabbits during third ventricle infusion of either saline (panel A; n = 4) or prazosin (panel B; n = 5). Arrows indicate the time of mating and the initiation and cessation of icv infusion.

Localization of the adrenergic receptor blockade action to the AME region was attempted by direct perfusion of prazosin. Figure 3 illustrates the profiles of AME-GnRH

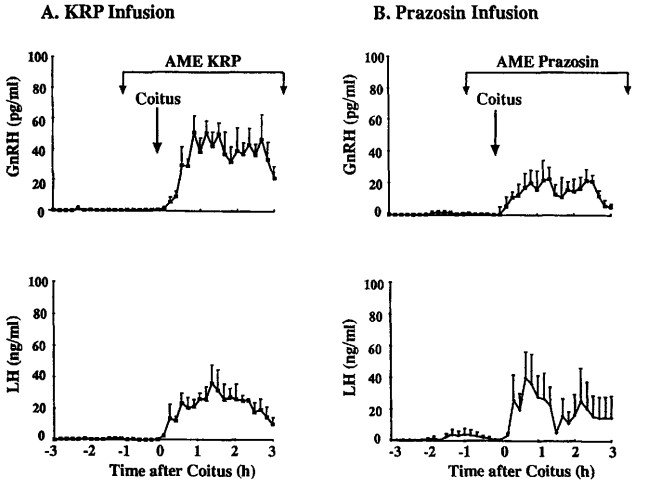

Figure 3. Mean + standard error (SE) levels of AME GnRH and plasma LH before and after coitus in AME KRP-(panel A; $n = 4$) or prazosin-infused (panel B; $n = 5$) female rabbits. Arrows indicate the time of mating and the beginning and ending of AME infusion.

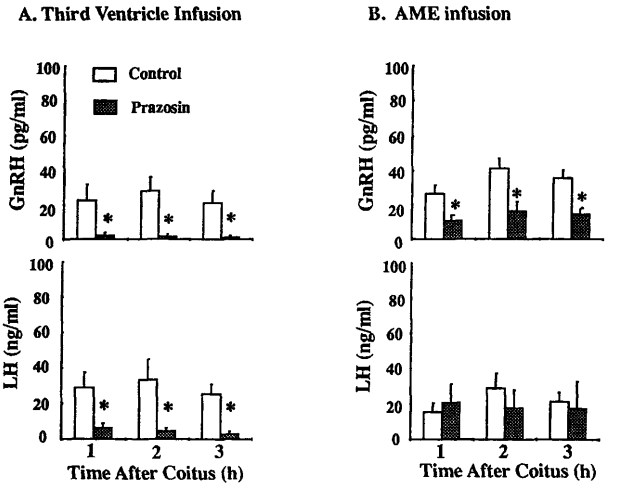

Figure 4. Hourly mean levels (with 1 SE) of AME GnRH and plasma LH after coitus following either third ventricle (panel A) or AME (panel B) infusion of control medium (open bar) or prazosin (solid bar). Mean levels of postcoital GnRH in prazosin-treated rabbits were lower than those in control animals regardless of the site of administration whereas mean LH levels were suppressed by icv, but not AME, prazosin. $^*P < 0.05$ compared to respective controls.

and plasma LH patterns in these control ($n = 4$) and prazosin-infused ($n = 5$) rabbits before and after coitus (data were lost in one control animal due to an unsuccessful PPP). In four control females, a pronounced surge in both GnRH and LH occurred after coitus (Fig. 3A). AME-GnRH values reached a peak (50.1 ± 7.6 pg/ml) near 1 hr and remained elevated above premating values for 3 hr. Postcoital plasma LH increased approximately 56-fold above precoital levels (27.1 ± 5.5 vs. 0.5 ± 0.1 ng/ml, respectively). In the five females that received AME-prazosin before coitus, the LH response after coitus varied greatly (individual data not shown) although postcoital concentrations of both GnRH and LH were elevated above precoital values ($P < 0.05$, Fig. 3B).

To contrast postcoital GnRH and LH profiles between rabbits that were given prazosin into the 3rd V or into the AME, the hourly mean levels of both GnRH and LH were determined within each group and compared by analysis of variance (Fig. 4). After prazosin administration into the ventricle, both AME-GnRH and plasma LH were significantly ($P < 0.05$) suppressed below levels in control animals at each postcoital hour (hr-1, hr-2, and hr-3; Fig. 4A). Following prazosin infusion directly into the AME, plasma LH levels were not altered significantly ($P > 0.05$), albeit the GnRH concentrations were reduced below control levels at each of the three postcoital hours studied ($P < 0.05$, Fig. 4B).

Discussion

Previous studies suggest that brain noradrenergic activities are activated during the preovulatory GnRH/LH surge in several mammalian species. For example, it is known that terminals of catecholamine neurons synapse near GnRH-containing cells in the hypothalamus (7) and that NE stimulates GnRH/LH release (8). In female rabbits coitus induces an immediate and marked release of hypothalamic NE and GnRH that prompts release of pituitary LH (5). Also, enhanced gene expression of tyrosine hydroxylase, the rate limiting enzyme for NE synthesis, and NE transporter protein, the key protein for presynaptic NE reuptake, in brainstem noradrenergic neurons occurs during the preovulatory LH surge (6). In this study, intracranial infusion of prazosin, a potent α_1-adrenergic antagonist, effectively attenuated preovulatory GnRH/LH secretion. The postcoital GnRH surge was nearly fully blocked by intraventricular administration of prazosin whereas local prazosin infusion into the AME region was less effective, but it significantly attenuated the postcoital GnRH secretion (Fig. 3B and Fig. 4B). This difference in prazosin blockade of GnRH between the 3rd V and AME infusion probably was related to either the concentration of prazosin that we could administer or the site of NE action in the hypothalamus. The maximal solubility of prazosin in the Krebs Ringer Phosphate (KRP) buffer that was used for medium in the GnRH PPP system was 1 mg/ml of KRP, whereas in saline, the solvent that we infused intraventricularly, 2 mg of prazosin was dissolved per ml. While we adjusted the rate of infusion (3 μl/min intraventricularly vs. 5 μl/min in the AME), we have no knowledge of the total dosage delivered into the two different sites. Moreover, infusion of prazosin directly into the AME, an area that is located *outside* the blood-brain barrier, reflects a localized effect of α_1 receptor blockade; chemicals infused into the third cerebroventricle are likely diffused to several NE-innervated hypothalamic areas that are *inside* the blood-brain barrier. Regardless of the "site-dosage" issue, the results provide the first direct evidence that an α_1-receptor antagonist can block the postcoital GnRH/LH surge in rabbits. The observations suggest that α_1-adrenergic receptors play a key role in the activation of GnRH release.

The involvement of α_1-adrenergic receptors in postcoital GnRH release is in agreement with previous findings

showing that prazosin, but not yohimbine, an α_2-adrenergic antagonist, blocked NPY-induced GnRH release in ovariectomized (OVX) estradiol-treated rabbits (15), and that phentolamine, a potent α-receptor blocker (both α_1 and α_2), inhibits pulsatile GnRH release in OVX rabbits (18). It is also consistent with reports in other species; phentolamine or prazosin suppresses GnRH release in OVX monkeys (19, 20). In the rat, prazosin blocks the circadian pattern of LH release observed in OVX estrogen-treated rats and the proestrus LH surge in intact rats (21, 22). We could speculate that NPY acts upstream to NE, since NE is the logical ligand for α_1 receptor activation of GnRH. However, we do not know the mechanism by which NE and NPY interact, particularly in response to coitus. We also don't know why prazosin did not completely block the mating-induced GnRH/LH surge. Perhaps NE and NPY are not the only chemicals that are involved in this process. The finding that a 50% reduction in postcoital GnRH can occur without a significant suppression of the LH surge (Fig. 3B and Fig. 4B) argues for the strength of the model in the study of the hypothalamohypophyseal ovarian axis (23). Although the GnRH surge is required to induce the postcoital LH surge, the total endogenous GnRH surge may not be needed in this process. Indeed, Karsch and colleagues reported that, in the sheep, although the endogenous GnRH surge is clearly necessary to induce the LH surge, only 26% of the amplitude of the endogenous GnRH surge is enough to elicit an LH surge similar to the endogenous LH surge (24).

The administration of prazosin in this study did not significantly alter basal GnRH and LH release. GnRH and LH levels in intact female rabbits are low, and any suppression of endogenous NE activity by blocking α_1-adrenergic receptors is not detectable statistically.

Interestingly, 1 hr of intraventricular infusion of prazosin before coitus did not significantly affect the mating behavior in female rabbits. Substantial evidence suggests that NE is an important transmitter in modulating sex steroid regulation of behavioral activity, and α_1-adrenergic receptors have been implicated in the enhancement of lordosis behavior in female rats and guinea pigs (25, 26). In the present study, intraventricular treatment with prazosin suppressed the postcoital GnRH and LH surge without an effect on mating, suggesting that hypothalamic α_1-adrenergic pathways play a minor role, if any, in regulating sexual behavior in this species. Earlier studies demonstrated that although mating behavior and gonadotropic secretion are tightly associated in the rabbit, the two functions may be regulated differently. Lesions located in the mammillary region produce permanent anestrous without affecting basal and coitus-evoked gonadotropic release (27). In contrast, lesions placed in the medial basal hypothalamus block coitus-induced ovulation in rabbits without affecting estrous behavior (28). If α_1-receptors are important in copulatory behavior in rabbits, one might have expected differences in coital activity between AME and 3rd V infusions of prazosin.

In summary, the results of this study indicate that intracranial administration of the α_1-adrenergic antagonist, prazosin, suppresses postcoital GnRH and LH release in the rabbit. These observations suggest that the involvement of hypothalamic NE in the preovulatory GnRH/LH release is mediated by α_1-adrenergic receptors. The information gained extends our earlier findings of increased NE activity during the process and reinforces the hypothesis that NE plays an important role in postcoital release of the GnRH surge in rabbits.

The authors thank Cyrus J. Lee for his excellent technical assistance and Jennifer Wall for her secretarial expertise. We are grateful to Drs. William Ellinwood, Harold Papkoff, and Albert Parlow, and to the National Hormone and Pituitary Program of the NIH for providing reagents for RIAs.

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