

# The Role of Neuropeptide Y in the Progesterone-Induced Luteinizing Hormone-Releasing Hormone Surge *in Vivo* in Ovariectomized Female Rhesus Monkeys\*

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## ABSTRACT

Progesterone induces a LHRH surge in estrogen-primed ovariectomized rhesus monkeys, with a concomitant increase in the pulse frequency of neuropeptide Y (NPY) release. However, the role for NPY in the positive feedback action of progesterone on LHRH release in primates is unknown. The present study examines the effect of an antisense oligodeoxynucleotide for NPY messenger RNA (AS NPY) on the progesterone-induced LHRH surge *in vivo* using push-pull perfusion. The AS NPY was directly infused into the stalk-median eminence (S-ME), whereas perfusates were collected for assessment of LHRH release. For a control, a scrambled oligodeoxynucleotide was

infused. The results indicate that 1) the scrambled oligodeoxynucleotide did not interfere with the progesterone-induced LHRH surge, 2) whereas AS NPY blocked the progesterone-induced increase in LHRH release, and 3) no LHRH surges were induced by oil as a control for progesterone, but the AS NPY also reduced LHRH release in oil controls. These data suggest that 1) AS NPY infusion into the S-ME results in reduction in LHRH release; and 2) NPY release in the S-ME is important for the positive feedback effects of progesterone on LHRH release in estrogen-primed ovariectomized monkeys. (*Endocrinology* 141: 1772–1779, 2000)

**I**N PRIMATES, as in other species, an increase in serum estradiol precedes the preovulatory LH surge (1). However, the first detectable increase in progesterone occurs after the initial increase in midcycle LH release, and a significant increase in progesterone has been observed several hours before the LH peak (2, 3), indicating that in primates estrogen induces preovulatory LH release, whereas progesterone facilitates or augments it. In fact, we have shown that progesterone injection after a small dose of estrogen in ovariectomized (OVX) monkeys results in a LH surge with a short latency (6–9 h) and a short duration (12 h) (4–9) and that this surge is a direct consequence of an increase in LHRH release induced by progesterone (9).

It has been shown in rats and rabbits that neuropeptide Y (NPY) in the hypothalamus plays an important role in the modulation of pulsatile LHRH release and the preovulatory LH surge (10, 11). In primates as well, we have reported that infusion of NPY in the stalk-median eminence (S-ME) stimulates LHRH release (12), whereas infusion of an NPY antibody or antisense oligodeoxynucleotide for NPY messenger RNA (mRNA; AS NPY) into the S-ME suppresses LHRH pulses (13, 14), and NPY pulses precede LHRH pulses in OVX monkeys as well as during the progesterone-induced LH surge (9, 13). Moreover, the pulse frequency of NPY pulses increases along with an increase in the LHRH pulse

frequency when progesterone induces a LHRH surge in estrogen-treated OVX monkeys (9), suggesting that NPY neurons may mediate positive feedback effects of progesterone in primates. Nonetheless, it is unclear whether NPY neurons play an obligatory or a permissive role in the control of steroid-induced LHRH release in primates. As little information on the mechanism of the preovulatory LHRH surge in primates is available, and there are distinct species differences in neuroendocrine characteristics between primates and rodents (6, 15), it is important to study the triggering mechanism of the steroid-induced LHRH surge in nonhuman primates. Therefore, the present study investigates the effects of an antisense oligodeoxynucleotide for NPY mRNA (AS NPY) on progesterone-induced LHRH release in estrogen-primed OVX female rhesus monkeys. This study expands upon our previous work demonstrating that AS NPY infusion suppresses both NPY and LHRH pulses in OVX female monkeys (14).

## Materials and Methods

### Animals

Twenty-nine female rhesus monkeys (*Macaca mulatta*), ranging from 6.6–18.3 yr of age and weighing 5.3–9.7 kg, were used in this study. All monkeys were ovariectomized 4 months to 2.5 yr before the experiment. The animals were housed under conditions described previously (7, 16) and provided a standard diet of Purina monkey chow (Ralston Purina Co., St. Louis, MO) supplemented with fresh fruit. Water was available *ad libitum*. The protocol for this study was reviewed and approved by the animal care and use committee, University of Wisconsin, and all experiments were performed under the guidelines established by the NIH and USDA.

### Steroid treatments and blood sampling

The steroid treatment protocol used was similar to that reported previously (6, 7). A 4-cm SILASTIC brand capsule (Dow Corning Corp.,

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Midland, MI) containing 17 $\beta$ -estradiol (E<sub>2</sub>) was implanted sc 14 days before sc injection of 30  $\mu$ g estradiol benzoate (EB). Twenty-four hours after EB treatment, progesterone (2.5 mg in 0.25 ml corn oil) or oil (0.25 ml corn oil or sesame oil), as a control for progesterone, was injected sc. Serum samples (1.0 ml) were collected through indwelling saphenous catheters at time points relative to progesterone (or oil) injection (-3, 0, 3, 6, 9, and 12 h) for evaluation of LH levels.

### Push-pull perfusion

At least 8 weeks before push-pull perfusion experiments began, a cranial pedestal was implanted under halothane anesthesia (16, 17). The method for push-pull perfusion of the S-ME in conscious monkeys was similar to that previously described (16). Briefly, a concentric cannula with double lumens was stereotaxically inserted into the S-ME of anesthetized monkeys 3 days before the start of the perfusion experiments. The placement of the tip of the cannula was confined to an area 1.0 mm posterior and ventral to the tip of the infundibular recess and laterally within 1.0 mm of the midline. On the third day after cannula insertion, modified Krebs-Ringer phosphate buffer solution (artificial cerebrospinal fluid) with bacitracin (4 U/ml; Sigma, St. Louis, MO) was infused into the S-ME through the push cannula at a rate of 23  $\mu$ l/min using a peristaltic pump (Minipulse 3, Gilson, Inc., Middleton, WI). Perfusate was collected (on ice) from the pull cannula at 10-min intervals using an identically calibrated pump. Each perfusate sample was aliquoted into two vials (150  $\mu$ l for LHRH and 75  $\mu$ l for other neuromodulators) before storage at -70 C.

### Experimental design

**Exp 1.** To determine the effects of progesterone on LHRH release and LH release, push-pull perfusion at 10-min intervals and blood samplings at 3-h intervals were conducted simultaneously. Twelve female OVX monkeys that had been implanted with E<sub>2</sub> capsules 2 weeks before the experiment and injected with 30  $\mu$ g EB 24 h before the experiment were used. After 4 h of control perfusion, 2.5 mg progesterone in corn oil or corn oil alone were injected sc, and perfusate collection was continued until 12 h after progesterone or oil injection. The time of progesterone or oil injection was designated time zero. All 12 monkeys received progesterone injection (n = 12), whereas 11 of the 12 monkeys received oil injection (n = 11) in random order in successive experiments. LHRH in perfusates, but not NPY, was measured in this experiment.

**Exp 2.** To determine whether suppression of NPY release alters the progesterone-induced LHRH surge in conscious OVX rhesus monkeys, we infused an antisense oligodeoxynucleotide for NPY mRNA (AS NPY) into the S-ME using a push cannula, whereas perfusate samples were collected through a pull cannula, as described previously (14). The AS NPY (5'-CCA GTC GCT TGT TAC CTA GC-3') is a 20-base oligonucleotide corresponding to the N-terminus of human NPY immediately downstream from the initiation codon (GenBank accession no. M.15789), as described previously (14). An oligonucleotide (5'-CTC CGC TTC AGT ACG CTA GT-3') containing the same bases in scrambled sequence (SC NPY) was used as a control. Both AS NPY and SC NPY were synthesized at the Biotechnology Center, University of Wisconsin (Madison, WI). We examined the specificity of the AS NPY and SC NPY using the GenBank Primate Database (FASTA Program, Genetics Computer Group, Madison, WI) as described previously (14). The AS NPY and SC NPY were then desalted and resuspended in artificial cerebrospinal fluid under sterile conditions. LHRH levels in perfusates were measured by RIA. NPY levels were not measured in this experiment.

**Exp 3.** To determine whether the effects of AS NPY on LHRH release in the progesterone-treated group differ from those in the oil-treated group, AS NPY was infused into the S-ME of animals that received oil injection. We did not examine the effects of SC NPY on LHRH release in animals treated with oil, because SC NPY did not cause any significant effect on LHRH release in a previous study (14). LHRH and NPY levels in perfusates were measured by RIAs.

Exp 2 and 3 were conducted concurrently on 17 OVX monkeys, in which E<sub>2</sub> capsules were implanted 2 weeks before and EB was injected 24 h before the initiation of the experiment. After 2 h of control perfusion, AS NPY or SC NPY at 10  $\mu$ M was continuously infused for 10 h, followed

by an additional 4 h of control perfusion. Progesterone in corn oil or corn oil alone was injected sc 2 h after the initiation of infusion of AS NPY or SC NPY, and the time point at which progesterone or oil injection was designated time zero. Eleven of the 17 monkeys were used for progesterone with AS NPY (n = 11), 12 of the 17 monkeys were used for progesterone with SC NPY (n = 12), and 5 of the 17 monkeys were used for oil with AS NPY (n = 5). Eleven of the 17 monkeys were assigned to 2 different treatments, and none of animals was tested twice with the same treatment.

### Hormone assays

LHRH concentrations in the perfusate fractions were measured by RIA, using anti-LHRH serum (R1245), as described previously (17). A 150- $\mu$ l aliquot of perfusate was used for the LHRH assay. The sensitivity of the LHRH assay was 0.05 pg/tube at 95% binding. The intra- and interassay coefficients of variation were 7.9% and 10.1%, respectively.

NPY concentrations in the perfusate fractions were measured by RIA, using anti-NPY serum, provided by Dr. K. Chihara, Kobe University (Kobe, Japan) (18). This assay was modified from a previously reported assay (19). A 75- $\mu$ l aliquot of perfusate was used for NPY assay. The sensitivity of the NPY assay was 0.7 pg/tube at 95% binding, and the intra- and interassay coefficients of variation were 10.8% and 13.3%, respectively.

LH concentrations in serum samples were measured in duplicate (100  $\mu$ l) by RIA as described previously (7), using anti-LH serum (R13, pool D), provided by Dr. G. Niswender. The reference standard used was WDP-x81-1720, provided by the NICHD. The sensitivity of the assay was 2.0 ng/tube at 90% binding. The intra- and interassay coefficients of variation were 4.2% and 5.9%, respectively.

### Pulse analysis

LHRH pulses in Exp I were analyzed using the Pulsar algorithm (20). Pulses of LHRH were depicted using parameters identical to those previously reported, *i.e.* cut-off criteria for G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, and G<sub>5</sub> were 3.8, 2.6, 1.9, 1.6, and 1.2, respectively (7, 17).

### Statistical analysis

**Exp 1.** To determine changes in mean LHRH release, pulse amplitude, and interpulse intervals during the progesterone-induced LH surge, the results from the six 2-h periods after progesterone or oil injection were compared with those from the two 2-h periods before injection using two-way ANOVA with repeated measures in which the variables were treatments and time. *Post-hoc* analysis of changes occurring during specific time periods after progesterone treatment was conducted using the Student-Newman-Keuls multiple range test. Effects of progesterone on LH release were similarly examined by two-way ANOVA followed by *post-hoc* analysis using the Student-Newman-Keuls multiple range test.

**Exp 2 and 3.** To determine whether the progesterone-induced LHRH surge was blocked by AS NPY, 2 h means of LHRH release after the AS or SC NPY treatments were compared with 2 h means of LHRH before the treatments using two-way ANOVA, followed by the Student-Newman-Keuls *post-hoc* analysis. Similarly, to examine whether AS NPY effects with the progesterone treatment differ from those with the oil treatment, the 2 h means of LHRH release after AS NPY in the progesterone and oil treatment groups were compared with the 2 h means before AS NPY using two-way ANOVA followed by the same *post-hoc* analysis. The effects of AS NPY on NPY release in the oil-treated group were similarly examined using one-way ANOVA.

The statistical analysis was conducted using raw values, and significance was attained at  $P < 0.05$ . For graphic presentation, mean LHRH and mean NPY levels were expressed with normalized data, *i.e.* mean LHRH release and mean NPY release during a 2-h period before the injection of progesterone or oil (Exp 1) or the initiation of AS NPY or SC NPY infusion (Exp 2 and 3) were designated 100% in each animal, and the remaining data were calculated accordingly.

**Results**

*Effects of progesterone on LH release and pulsatile LHRH release*

Progesterone injection 24 h after EB injection resulted in a LH surge with an increase in LHRH release (Fig. 1). The pulse frequency and/or pulse amplitude of LHRH pulses started to increase approximately 3 h after progesterone. A higher frequency of LHRH release continued, whereas a large pulse amplitude was not sustained in most cases (Fig. 1). In contrast, oil injection did not alter LH release or LHRH release (Fig. 1).

Group data indicated that the LH increase induced by progesterone was significantly ( $P < 0.001$ ) larger than that in the oil control (Fig. 2D). *Post-hoc* analysis indicated that mean LH levels after progesterone were significantly higher than those before progesterone and the corresponding mean LH levels in the oil control ( $P < 0.01$  to 0.05).

Overall, mean LHRH release and pulse amplitude in the progesterone-treated group were significantly higher than those in the oil control group ( $P < 0.05$  for both; Fig. 2, A and B). *Post-hoc* analysis indicated that mean LHRH levels and mean pulse amplitude during the 4–12 h after progesterone were significantly larger than those before progesterone and

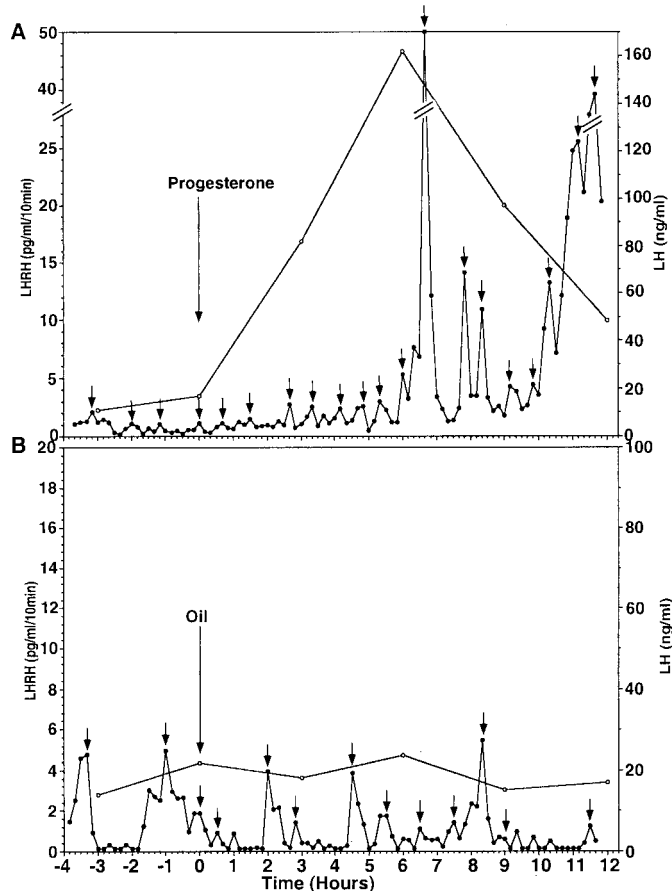


FIG. 1. An example of the effects of progesterone (A) or oil (B) on pulsatile LHRH release in the S-ME (solid circles) and LH release (open circles) in OVX monkeys. Progesterone (2.5 mg) or oil was injected sc at time zero in animals treated with EB (30  $\mu$ g) 24 h earlier. Arrows indicate LHRH pulses, detected using Pulsar.

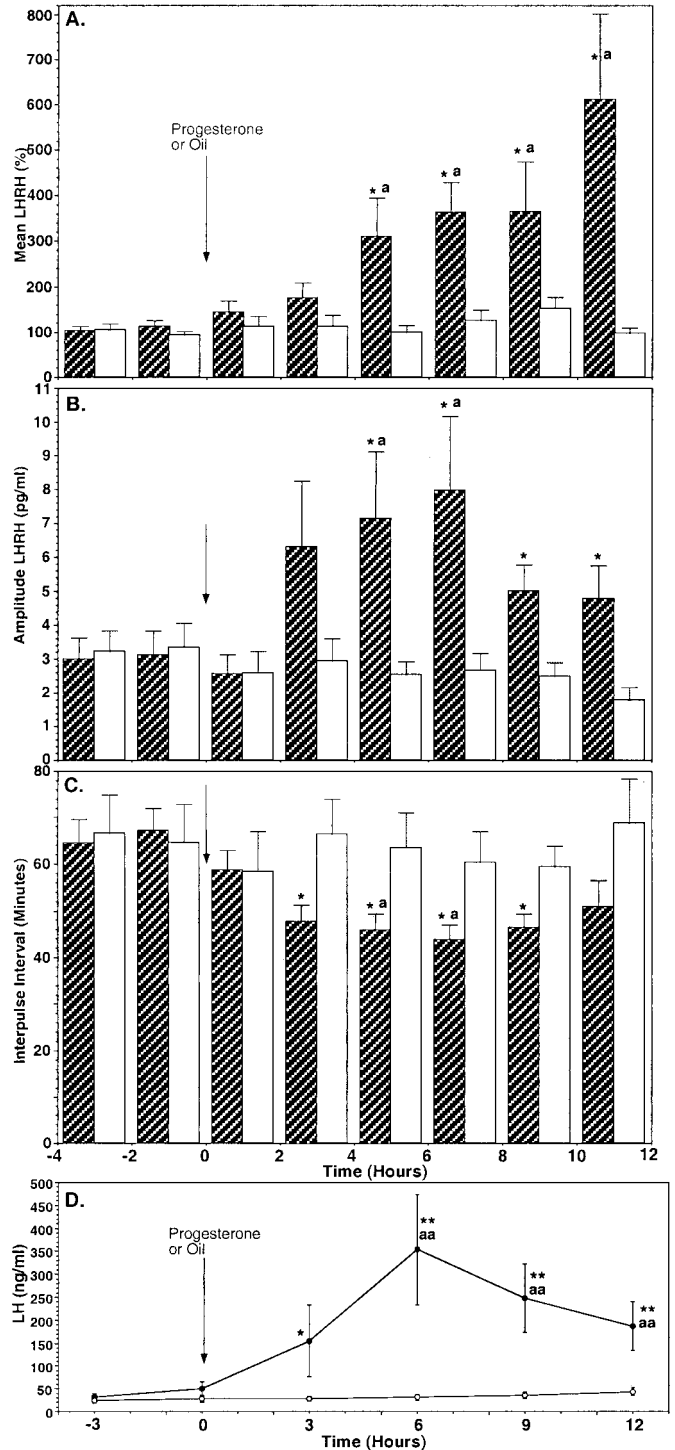


FIG. 2. Effects of progesterone (hatched bars;  $n = 12$ ) or oil (open bars;  $n = 11$ ) on mean LHRH release (A), pulse amplitude (B), and interpulse interval (C). Effects of progesterone (solid circles;  $n = 12$ ) or oil (open circles;  $n = 11$ ) on LH release in the same animals are shown in D. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (vs. data before progesterone). a,  $P < 0.05$ ; aa,  $P < 0.01$  (vs. corresponding data in oil control).

the corresponding LHRH means in the control group ( $P < 0.05$ ). Similarly, the overall mean of the interpulse interval after progesterone was significantly shorter than that in the

oil control ( $P < 0.05$ ; Fig. 2C), and mean interpulse intervals during the 2–10 h after progesterone were significantly shorter than that before progesterone or the corresponding means in the oil control.

*Effects of AS NPY on progesterone-induced LHRH release*

Progesterone induced a robust LHRH increase in animals infused with SC NPY (Fig. 3, A and B; overall significance,  $P < 0.05$ ). Although the first LHRH increase was observed between 2–4 h in 7 of 12 animals in this group, *post-hoc* analysis indicated that the LHRH increase did not reach statistical significance until 4–6 h after progesterone injection, and the LHRH peak occurred at 6–8 h ( $P < 0.01$ ). The progesterone-induced LHRH increase lasted for at least 12 h after progesterone injection ( $P < 0.05$ ), when the experiment was terminated.

In contrast, infusion of AS NPY clearly suppressed the progesterone-induced LHRH release (Fig. 4, A and B), compared with the results from the SC NPY group. Overall significance between AS NPY and SC NPY was  $P < 0.01$ . Mean LHRH levels after progesterone injection in the AS NPY group did not significantly fluctuate over time ( $P > 0.1$ ). *Post-hoc* analysis indicated that mean LHRH levels at 4–12 h after progesterone injection in the AS NPY group were significantly lower than corresponding LHRH means in the SC NPY group ( $P < 0.05$ ).

*Comparison of the effects of AS NPY on LHRH release in the progesterone- and oil-treated animals*

AS NPY suppressed LHRH release in the oil-treated group (Fig. 5;  $P < 0.01$ ). Further, the AS NPY-induced LHRH suppression tended to be more prominent in the oil-treated group than that in the progesterone-treated group, although statistical significance between the two treatment groups was not attained ( $P = 0.08$ ). *Post-hoc* analysis indicated that mean LHRH levels after AS NPY in the oil-treated group were significantly lower than those before AS NPY, except for the first 2 h ( $P < 0.05$  to 0.001), but mean LHRH levels at 2–12 h after oil injection in the AS NPY group were not significantly different from the corresponding LHRH means after progesterone injection in the AS NPY group.

*Effects of AS NPY on NPY release in estrogen-primed, oil-treated animals*

AS NPY infusion into the S-ME clearly suppressed NPY release starting within the first 2 h ( $P < 0.05$ ; Fig. 6). *Post-hoc* analysis indicated that mean NPY levels at all time points after the initiation of AS NPY infusion were lower than those before infusion ( $P < 0.05$ –0.001).

**Discussion**

The first experiment confirms our previous observation (9) that progesterone induces a LHRH increase in estrogen-

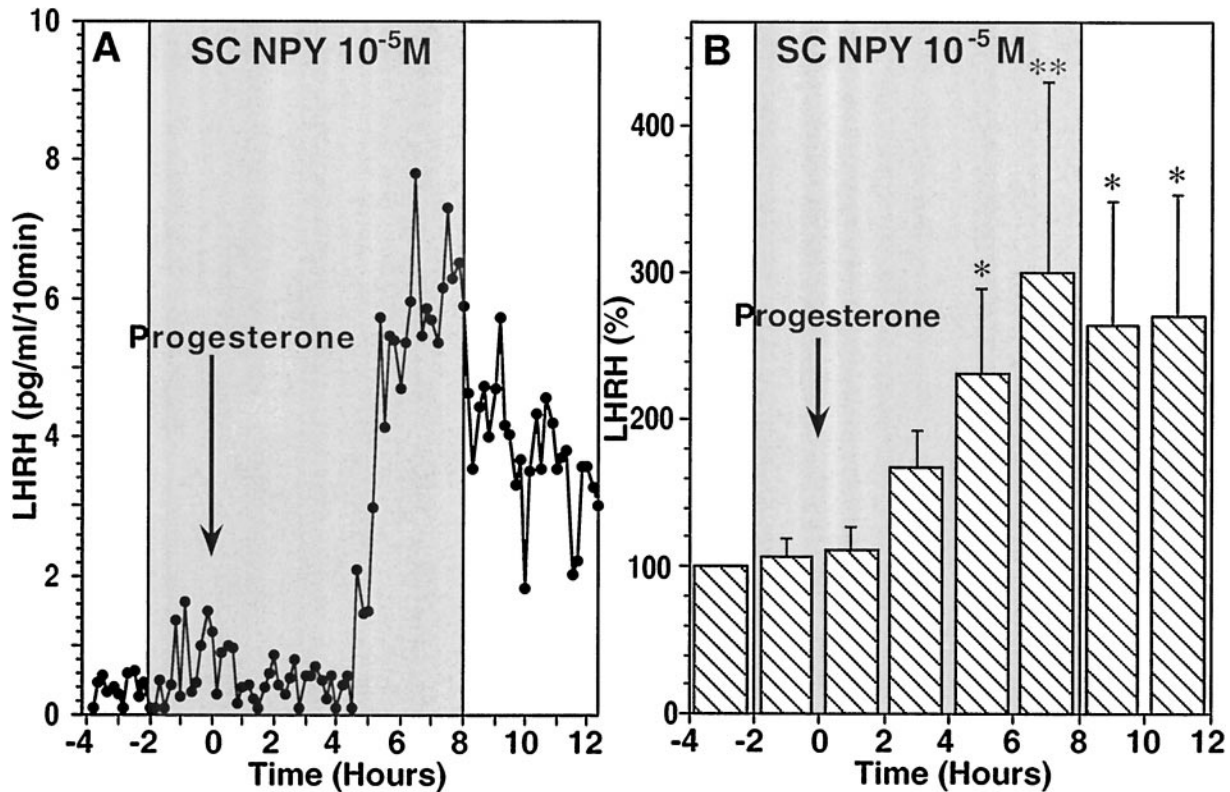


FIG. 3. Effects of a scrambled oligodeoxynucleotide for NPY mRNA (SC NPY) on the progesterone-induced LHRH surge. An individual example (A) and the group mean (B) are shown. Progesterone was injected at time zero, and 2 h before progesterone injection SC NPY infusion was initiated through a push cannula, whereas perfusates were collected continuously through a pull cannula. Note that SC NPY did not alter the progesterone-induced LHRH surge. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  [ vs. data before SC (within group)].

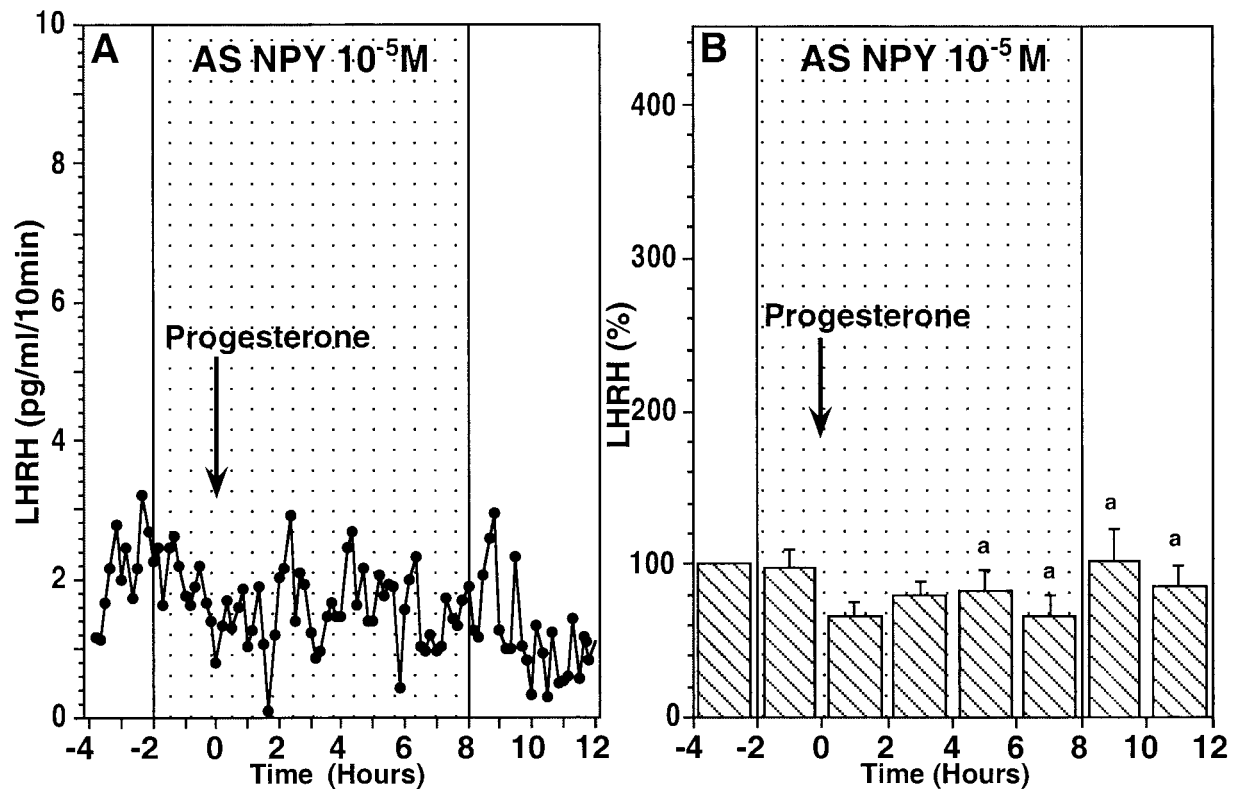


FIG. 4. Effects of an antisense oligodeoxynucleotide for NPY mRNA (AS NPY) on the progesterone-induced LHRH surge. An individual example (A) and the group mean (B) are shown. Mean LHRH levels before and after progesterone injection (time zero) are shown. Progesterone was injected at time zero, and 2 h before progesterone injection AS NPY infusion was initiated through a push cannula, whereas perfusates were collected continuously through a pull cannula. Note that AS NPY suppressed the progesterone-induced LHRH surge. a,  $P < 0.05$  vs. corresponding data from SC (between groups).

primed OVX monkeys. A LH increase after progesterone was accompanied by a decrease in the interpulse interval of LHRH release and increases in mean LHRH release as well as pulse amplitude. Although we did not measure NPY in perfusate samples in the present study, based on the findings in previous studies that NPY pulses precede LHRH pulses and an increase in the LHRH pulse frequency was accompanied by an increase in NPY pulse frequency after progesterone (9), we speculated that prolonged suppression of NPY release may alter the pattern of the progesterone-induced LHRH surge. The results of the second experiment indicate that AS NPY infusion indeed blocks the positive feedback action of progesterone. In contrast, SC NPY infusion did not have any significant effect on LHRH release. In fact, the pattern of mean LHRH release with SC NPY was similar to that seen in the absence of oligonucleotide infusion in the first experiment. The results of the third experiment further indicate that AS NPY infusion lowered NPY release in the S-ME, and that the effectiveness of AS NPY infusion in oil-treated animals tended to be more persistent than that in the progesterone-treated animals. Although we did not examine the effects of SC NPY in oil-treated animals, in a previous study we found that SC NPY does not alter the pulsatility of NPY release or LHRH release in OVX unprimed monkeys (14).

The results of this and previous (14) studies suggest that infusion of AS NPY into the S-ME consistently suppresses

both NPY and LHRH in rhesus monkeys. The infused AS NPY was presumably taken into NPY neurons in the S-ME and retrogradely transported from neuroterminals to the cell body, where AS NPY interferes with NPY synthesis, resulting in a reduction of NPY release and, subsequently, LHRH release. A study from this laboratory (17) further indicates that the diameter of diffusion in the S-ME with push-pull perfusion is approximately 700  $\mu\text{m}$ . Thus, the NPY neuroterminals affected by AS NPY infusion are in a relatively small area within the S-ME. Alterations of LH pulses (21), steroid-induced LH surges (22), and food intake behavior (23) after treatment with AS NPY in the hypothalamus have been reported in rats.

It has been extensively shown that NPY is a major modulator for pulsatile LHRH release. NPY infusion directly into the S-ME *in vivo* stimulates LHRH pulses (12, 13, 24, 25), whereas infusion of an antibody to NPY or an AS NPY into the ME suppresses LHRH pulses in rats, rabbits, and monkeys (13, 14, 21, 22, 26). Further, NPY stimulates LHRH release in ME fragments of rats (27–29). As NPY was directly infused into the S-ME in our studies (12, 24), where abundant LHRH neuroterminals, but only a few LHRH cell bodies, are present in primates (30, 31), and as the rat ME does not contain LHRH cell bodies (32), the stimulatory action of NPY appears to occur at the level of LHRH neuroterminals. In fact, a recent study using confocal microscopy with double labeled immunofluorescence has demonstrated colocalization

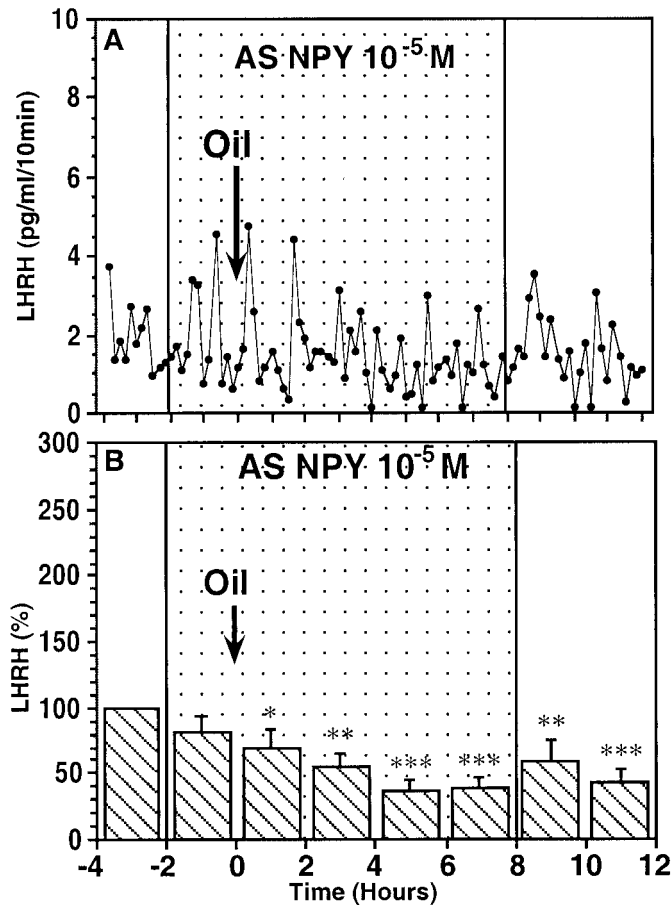


FIG. 5. Effects of AS NPY on LHRH release in oil-treated monkeys (n = 5). An example (A) and mean LHRH levels (B) are shown. Note that AS NPY suppressed mean LHRH release in oil-treated animals. \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001 [vs. data before AS (within group)].

of NPY Y<sub>1</sub> receptors on LHRH neuroterminals (33). However, LHRH cell bodies may also be involved in the stimulatory action of NPY, as NPY results in LHRH release in GT-1 cells in a dose-responsive manner (34), which is strikingly similar to that observed in the monkey S-ME *in vivo* (12, 24). Cell bodies of LHRH neurons are innervated by NPY neurons in rats and sheep (35, 36), but not in monkeys (37). Although there are several reports indicating that ventricular infusion of NPY suppresses LHRH/LH release (26, 38, 39), NPY infused into the ventricular system may diffuse elsewhere into the brain, resulting in inhibitory effects on LHRH release through interneurons. Nonetheless, a direct inhibitory role of NPY in LHRH release cannot be excluded, because NPY mRNA, measured by ribonuclease protection assay, decreased at the onset of puberty in castrated male monkeys concomitant with an increase in LHRH mRNA (40, 41). NPY is inhibitory to hippocampal and suprachiasmatic neurons via presynaptic or postsynaptic receptors (42–45), and studies in NPY knockout mice indicate that NPY is an endogenous antiepileptic agent through presynaptic inhibition of glutamate neurons (46).

NPY neurons are profoundly involved in the preovulatory LHRH surge or steroid-induced LHRH surge in rats (10, 11,

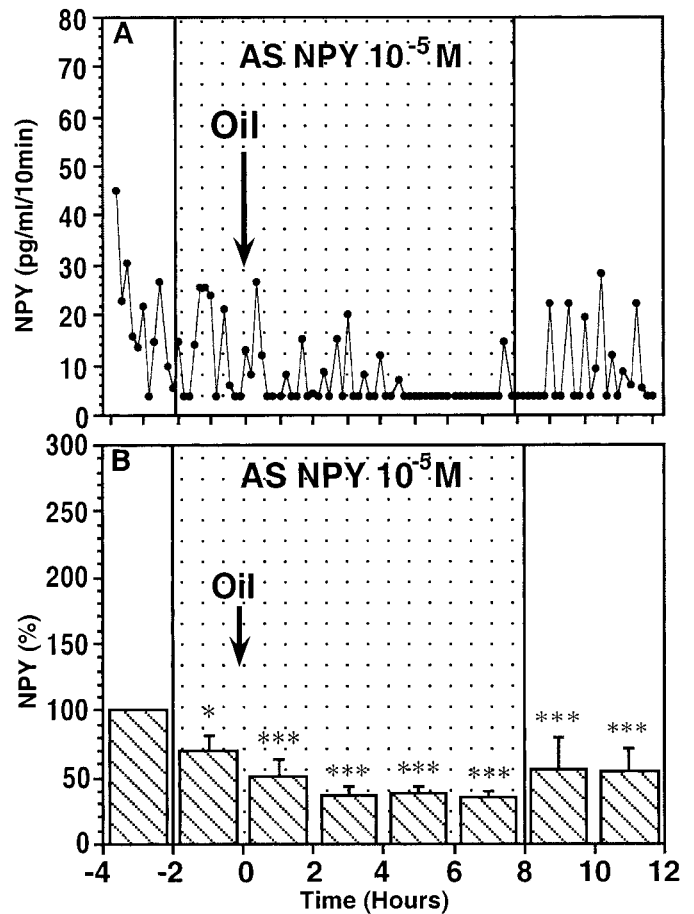


FIG. 6. Effects of AS NPY on NPY release in oil-treated monkeys (n = 5). An example (A) and mean NPY levels (B) are shown. Note that AS NPY suppressed mean NPY release in oil-treated animals. \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001 [vs. data before AS (within group)].

47). Expression of NPY mRNA, the tissue content of NPY in the ME, and NPY release from the ME increase before or coincident with the preovulatory and steroid-induced LH surges (48–50), whereas treatment with NPY antiserum or AS NPY suppresses the preovulatory or steroid-induced LH surge (22, 51). Further, the stimulatory effect of NPY on LHRH release is greatly enhanced by estrogen (24, 28, 52), suggesting the role of NPY in the positive feedback action of steroids. The previous studies in rats demonstrating that NPY neurons in the arcuate nucleus contain estrogen receptors (10, 53, 54) and that NPY neurons innervate LHRH cell bodies in the preoptic area (33, 35, 36), further suggest that NPY neurons are important for mediating the steroid action on the LHRH neuronal system. In the present study we found that inhibition of NPY synthesis by its antisense oligodeoxynucleotide blocks the progesterone-induced LHRH surge in estrogen-primed OVX monkeys.

The NPY neuronal system may not be the only contributor to the positive feedback effect of progesterone on LHRH release. The suppression of AS NPY on LHRH release in oil-treated monkeys tended to be more persistent than that in progesterone-treated monkeys, suggesting that progesterone may have stimulated other neuronal systems, such as norepinephrine and glutamate neurons, both of which re-

portedly contain estrogen receptors (53, 55). Further, preliminary observations in our laboratory suggest that both norepinephrine and glutamate levels increase during the progesterone-induced LHRH surge (Terasawa, E., and L. Luchansky, unpublished observation). The question of whether input from all of these neuronal systems is equally important for the positive feedback effects of ovarian steroids or whether one of them, perhaps NPY, plays a key role in the preovulatory LH surge remains to be answered.

In primates, it has been shown that an increase in serum estradiol precedes the preovulatory LH surge (1). However, a significant increase in progesterone has been observed several hours before the LH peak (2, 3), and administration of the antiprogestin RU486 interferes with the expected preovulatory gonadotropin surge and ovulation in women and monkeys (56, 57). These reports indicate that in primates, estrogen induces the preovulatory LH release, whereas progesterone facilitates or augments it. As the increase in circulating progesterone during the preovulatory phase originates from the primary follicle (58), full maturation of the Graafian follicle appears to give a signal to the hypothalamus to stimulate the release of LHRH, thereby setting the timing of the LH surge to synchronize with ovulation (7).

In primates, the positive feedback effects of progesterone differ from those of estrogen. The progesterone-induced LH surge (3, 5–7, 59) occurs with constant peak latency (6–9 h) and a relatively short duration (16–18 h), whereas both the estrogen-induced and spontaneous preovulatory LH surges (5, 60, 61) occur with a longer peak latency (36–48 h) and a longer duration (36–48 h). Although the progesterone-induced LH surge can be blocked with pentobarbital anesthesia (5), the estrogen-induced LH surge cannot (15). Although the difference with pentobarbital anesthesia could be explained by the fact that the positive feedback effects of estrogen do not require the presence of the hypothalamus (62), whereas the positive feedback effects of progesterone do (63), an increase in LHRH output during the estrogen-induced LH surge and preovulatory surge has been consistently reported in monkeys (64–67). Interestingly, similar differential effects of progesterone and estrogen on the LH surge are observed in OVX guinea pigs (68), *i.e.* the progesterone-induced LH surge was completely blocked by pentobarbital anesthesia, whereas the estrogen-induced LH surge was not blocked by either multiple injections of pentobarbital or a single injection of phenobarbital, a long-acting barbiturate. Because the circadian-dependent mechanism of estrogen action remained in this case, estrogen's action is not solely at the pituitary level (68); thus, there is a significant difference in the mechanisms of action of progesterone and estrogen (6). It is possible, therefore, that different neuronal substrates may be involved in estrogen and progesterone actions, and that our findings may not represent the entire mechanism of the preovulatory LH surge.

In summary, understanding the positive feedback mechanism of steroid hormones in primates is essential, as the preovulatory gonadotropin surge is the central event in reproduction. In the present study we have examined the role of NPY in the progesterone-induced LHRH surge. Our results demonstrate that suppression of NPY release impairs the progesterone-induced LHRH surge and further suggest

that NPY appears to play a pivotal role in the preovulatory gonadotropin surge.

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