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# Evidence that Progesterone Modulates Anterior Pituitary Neuropeptide Y Levels During the Progesterone-induced Gonadotropin Surge in the Estrogen-primed Intact Immature Female Rat

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In a previous study we reported that *in vivo* estrogen-priming alone, without subsequent progesterone-treatment, was sufficient to maximize NPY potentiation of gonadotropin hormone-releasing hormone responsiveness exhibited *in vitro* by the rat anterior pituitary. This observation suggests that the necessity, as reported by others, for both estrogen-priming and progesterone-treatment to maximize NPY potentiation of GnRH responsiveness *in vivo* may be due to progesterone acting primarily at the hypothalamus. Consequently, the current study was performed to determine whether progesterone facilitates gonadotropin secretion *in vivo* by acting to stimulate hypothalamic synthesis of NPY and the subsequent elevation of anterior pituitary tissue levels of NPY. Intact immature female rats were injected with estradiol at 1700 h on days 27 and 28. On day 29 at 0900 h, the animals received an injection of progesterone (2 mg/kg) or vehicle and were subsequently sacrificed at 1200, 1330 and 1500 h. Rats which received only estradiol injections were used as controls. Surge levels of serum LH and FSH were observed at 1330 and 1500 h. Hypothalamic levels of NPY mRNA at 1200 h on day 29 were higher ($P < 0.01$) in estradiol-primed rats which received progesterone; there was no accompanying statistically significant change in hypothalamic NPY content. NPY content in the anterior pituitary was significantly increased ($P < 0.01$) at 1200 h on day 29 in estradiol-primed rats which received progesterone; there was no accompanying significant change in anterior pituitary NPY mRNA levels. Hypothalamic GnRH mRNA content was significantly increased ($P < 0.01$) at 1330 h on day 29 concomitant with the peak of the gonadotropin surge in the estradiol-primed, progesterone-treated rat. The data indicate that progesterone modulates hypothalamic NPY mRNA and anterior pituitary NPY levels as well as GnRH mRNA levels and that modulation of NPY levels in the hypothalamic–pituitary axis occurs prior to modulation of GnRH gene expression. These studies support the hypothesis that in the estrogen-primed rat, progesterone facilitates the induction of the gonadotropin surge by maintaining hypothalamic synthesis of NPY as well as by modulating anterior pituitary NPY tissue levels.

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## INTRODUCTION

Neuropeptide Y (NPY) acts at both the hypothalamus and the anterior pituitary (AP) to modulate gonadotropin secretion. NPY has been shown to stimulate hypothalamic gonadotropin hormone releasing hormone (GnRH) secretion from intact and estrogen-primed ovariectomized rats [1, 2] and at the first pubertal preovulatory surge [3, 4]. Several studies have also suggested that NPY may regulate the potentiation of GnRH responsiveness at the AP [5–11]. Although it

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has been established that gonadal steroids are involved in modulating the effects of NPY on gonadotropin secretion [2, 12], the relative roles of estrogen and progesterone and the sites at which they act are unresolved. A previous study [13] utilizing ovariectomized, pentobarbital (PB)-blocked adult female rats reported that NPY potentiated GnRH-induced luteinizing hormone (LH) release in rats which had been treated with both estrogen and progesterone but not in those treated with estrogen alone. The study concluded that progesterone must be acting at the AP since hypothalamic neurosecretion had been presumably blocked by PB-treatment. However, because PB has been reported to incompletely block hypothalamic neurosecretion in the ovariectomized rat [14, 15], the relative role of progesterone at the hypothalamus as opposed to the AP remains questionable. In a previous study [16], we examined the effect of progesterone administered *in vivo* to estradiol-primed rats on the subsequent *in vitro* AP sensitivity to GnRH in the presence and absence of NPY. In that study we did not observe any difference in the *in vitro* NPY potentiation of GnRH responsiveness in the AP from estradiol-primed rats which had received progesterone and those which had not [16]. Thus the action of progesterone in inducing NPY potentiation of GnRH responsiveness *in vivo* [13] might be explained by the possibility that progesterone is acting at the hypothalamus rather than at the AP. Progesterone has been reported to increase GnRH and NPY content of the hypothalamus in the estrogen-primed ovariectomized rat [17–19]. Hypothalamic secretion of NPY and GnRH into the portal system has been shown to be elevated at the preovulatory surge [5] and progesterone has been shown to stimulate hypothalamic GnRH secretion both *in vitro* [20] and *in vivo* [21]. Thus progesterone could act at the hypothalamus by stimulating NPY secretion.

The objective of the current studies was to determine if progesterone stimulates hypothalamic synthesis of NPY mRNA and elevation of AP NPY tissue levels during the progesterone-induced gonadotropin surge in the estrogen-primed intact immature rat.

## MATERIALS AND METHODS

### *Description of animal model*

The intact immature estrogen-primed, progesterone-treated female rat utilized in the current study was chosen for several reasons. This model exhibits a gonadotropin surge which is similar in amplitude and duration to the preovulatory surge exhibited by the normally cycling rat [22–24]. The model does not exhibit the profound alterations in AP histology, gonadotropin synthesis and secretion, and GnRH secretion dynamics which have been observed in the adult ovariectomized rat [25]. In addition, the immature intact rat has low endogenous levels of gonadal steroids thereby facilitating the experimental manipulation of steroid status [26].

Immature female virus-free Holtzman rats were obtained from Harlan Company (Madison, WI) at 25 days of age. The animals were maintained in environmentally controlled quarters under 14–10 h light–dark conditions and supplied with tap water and rat chow *ad libitum*. On days 27 and 28 at 1700 h, rats received subcutaneous (s.c.) injection of either 2 $\mu$g estradiol-17$\beta$ or 200 $\mu$l 25% ethanol/saline vehicle. On day 29 at 0900 h, the rats received s.c. injection of either 2 mg/kg progesterone or 200 $\mu$l ethanol/saline vehicle. The animals were sacrificed at 1200 h on days 27 and 28 and at 1200, 1330 and 1500 h on day 29, at which times AP and hypothalami were collected. AP tissue was processed for determination of NPY mRNA and NPY tissue content, while hypothalami were processed for determination of NPY and GnRH mRNA as well as for NPY tissue content. Trunk blood was collected for determination of serum levels of LH and FSH. The current experiments required considerable sample handling and processing at the time of sacrifice. In order to facilitate sample processing, the estradiol-primed, progesterone-treated group and the estradiol-primed, vehicle-treated group were initiated on consecutive days. Therefore, even though all animals had been subjected to only one estradiol injection prior to 1200 h on day 28, there are nonetheless two data points plotted at this time point in Figs 2–7. One of the data points was collected preceding the sacrifice of the estradiol-primed, progesterone-treated group while the other was collected preceding the sacrifice of the estradiol-primed, vehicle group on the following day. All protocols involving live animals were reviewed and approved by the institutional Committee on Animal Use for Research and Education (CAURE) prior to performing any studies.

### *Radioimmunoassays (RIA)*

*Gonadotropin RIAs.* LH and FSH levels in rat serum were estimated by a double antibody RIA utilizing reagents supplied by the National Pituitary Hormone Program as described previously [16, 27]. Iodination was by the chloramine-T method and the LH standard curve ranged from 0.6–160 ng/tube while the FSH standard curve ranged from 3.9–500 ng/tube. The second antibody (goat anti-rabbit serum) was purchased from Arnell, Inc (Brooklyn, NY). Inter-assay and intra-assay coefficients of variation did not exceed 10% at any point on either curve. LH and FSH levels were expressed in terms of RP-1.

*Neuropeptide Y RIA.* NPY levels in rat hypothalamic and AP tissues were estimated by a double antibody RIA. Porcine NPY purchased from Bachem California (# PNPE70; Torrance, CA) was utilized for standard curve preparation and for iodination. The antibody (rabbit anti-porcine NPY) was purchased from Peninsula Laboratories (# RAS-7172; Belmont, CA). The

second antibody was purchased from Arnell, Inc. Porcine NPY was iodinated according to a modified chloramine-T method as previously described [28].

*Extraction of samples.* In order to remove peptide-degrading activity, AP and hypothalamic tissues were extracted immediately upon collection. APs were individually collected and homogenized in 0.5 ml ice cold 0.1 N HCl; entire hypothalamic blocks (including that area from the rostral end of the POA to the pituitary stalk to a depth of ~3 mm bordered laterally by the hypothalamic sulci) were rapidly isolated as previously described and individually homogenized in 1.0 ml 0.1 N HCl [29, 30]. The samples were microfuged for 10 min and the supernatants placed in new tubes; the samples were then titrated to pH 7.0 with 1.0 N NaOH. Following a second microcentrifugation for 5 min, the supernatants were stored at −20°C until NPY RIA was performed.

*Assay procedure.* All assay constitutents (standards, tracer, and antibody) were diluted in 0.01 M sodium phosphate buffered saline containing 1% bovine serum albumin; rabbit gamma globulin (ICN # 824551) was added to the antibody dilution buffer (4 mg/100 ml). Assay tubes were run in triplicate containing 100 μl each of sample (or standard), antibody (final in-assay dilution of 60,000) and ~20,000 CPM iodinated NPY (the standard curve tracer was added in a buffer prepared identically to that in which the samples were added). Following a 24 h incubation at 4°C, 200 μl second antibody (goat anti-rabbit serum diluted 250-fold) was added; subsequent to a second 24 h incubation, bound and free tracer were separated by centrifugation at 4°C. Pelleted bound counts were determined by counting in a gamma spectrometer. Unknowns were read from a logit transformation of the standard curve data and expressed in reference to porcine NPY. Increasing volumes of NPY exhibited parallel displacement of iodinated procine NPY binding whether extracted from hypothalamic or pituitary tissue. The actual standard curve points employed were 3.9, 7.6, 15.6, 31.3, 62.5, 125, 250, 500, 1000 and 2000 pg/tube. The interassay variability at each of these points was 1.6, 1.8, 2.2, 2.3, 3, 3.3, 3.3, 3.7, 2.5 and 2.3%, respectively. Data on variability were collected from 6 individually constructed standard curves.

*Northern analysis procedure*

Total RNA was extracted from AP and hypothalamic tissues utilizing RNAzol (Biotecx Labs, Houston, TX) according to the manufacturer-supplied protocol. Northern analysis was conducted as described previously [24, 31–34]. The cDNA for the NPY mRNA was a gift from Dr S. Sabol [35]. The cDNA probe for the GnRH mRNA (GnRH-C) was a gift from Dr K. Mayo [36]. A representative autoradiogram is presented (Fig. 1) in order to demonstrate that signals were of sufficient intensity to quantitatively measure differences between samples.

*Statistical analysis*

The data were generated utilizing three animals per group and are expressed as mean ± SEM. One way ANOVA was used to determine if differences existed within treatment groups; Duncan's multi-range test was used as a post-test to determine which points within a treatment group were significantly different. Student's $t$-test was used to compare two data points across groups; $P < 0.05$ was considered significantly different. The effects of progesterone or vehicle treatment on day 29 were analyzed for statistically significant differences by comparison to both day 28 estradiol-treated data points.

## RESULTS

*Gonadotropin secretion*

There was no statistically significant difference in gonadotropin secretion exhibited by the two estradiol-treated groups on day 28 at 1200 h, [LH and FSH, Fig. 2(A) and (B), respectively] and no significant differences between these two groups were observed in any of the other parameters tested (Figs 3–7). In estradiol-primed, progesterone-treated animals, highly significant ($P < 0.01$) gonadotropin surges were observed at 1330 and 1500 h on day 29 [LH and FSH, Fig. 2(A) and (B) respectively]. Estradiol-priming without subsequent progesterone treatment did not result in changes in serum levels of LH and FSH.

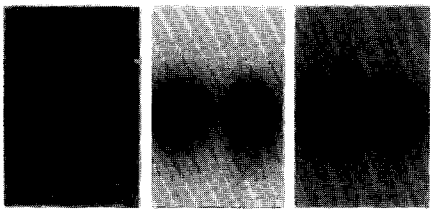


**Fig. 1. Representative Northern analysis signals obtained with the cDNA probe for NPY mRNA in the AP and hypothalamus (panels A and B, respectively; both 800 bp) and the cDNA probe for GnRH mRNA in the hypothalamus (panel C; 500 bp). Panels A, B and C utilized 6, 20 and 20 μg RNA, respectively. Total RNA was extracted utilizing RNAzol (Biotecx Laboratories, Houston, TX) and was subsequently electrophoresed on 1.5% agarose gels. Following alkaline transfer of RNA from agarose gels to membranes, the blots were hybridized overnight to $^{32}$P-dCTP-labeled cDNA probes at 42°C, washed and exposed to Kodak X-ray film. Exposures of 7 days were utilized for AP NPY mRNA (panel A) and for hypothalamic GnRH mRNA (panel C); hypothalamic NPY (panel B) typically required exposures of 24–48 h. Autoradiograms were scanned with an LKB/Pharmacia Ultroscan laser densitometer to determine relative levels of mRNA; data were expressed as arbitrary densitometric units (ADU) following standardization based on the relative amount of 28S rRNA in each lane [33].**

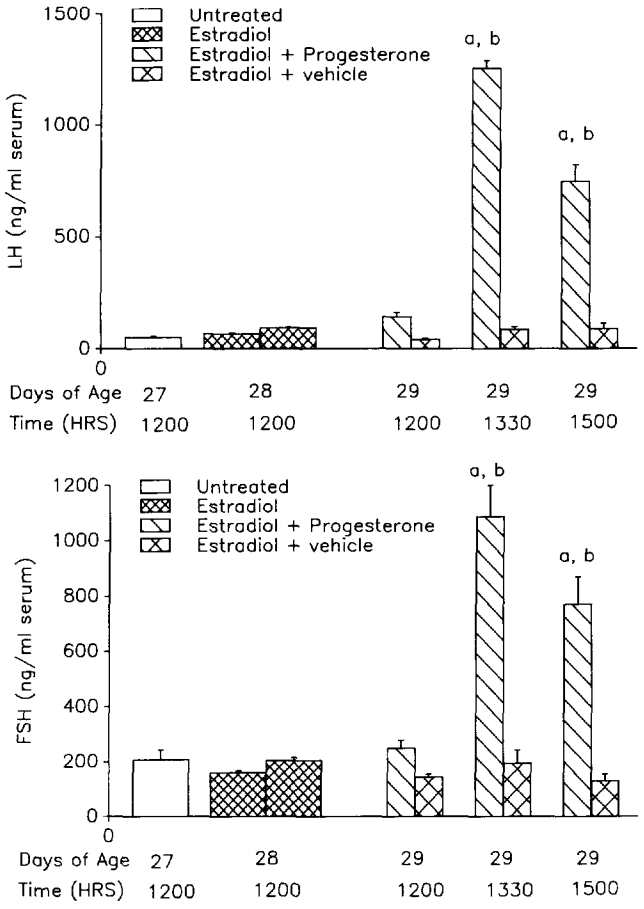


Fig. 2. Effect of estradiol-priming alone (small cross hatch), or estradiol-priming followed by progesterone (diagonal hatch) or vehicle (large cross hatch) treatment on LH (A) and FSH (B) secretion from the 27–29-day-old immature intact female rat. The animals were given 2 $\mu$g estradiol by s.c. injection at 1700 h on days 27 and 28. On day 29 at 0900 h, the animals received s.c. injection of either progesterone (2 mg/kg) or 200 $\mu$l saline/ethanol vehicle. The animals were sacrificed at 1200 h on days 27, 28 and 29 and at 1330 and 1500 h on day 29 and trunk blood was assayed for LH and FSH secretion. Three animals were used per time point ($n = 3$) and the data were expressed in terms of RP-1 as the mean ($\pm$SE) a, significantly greater ($P < 0.01$) than either estradiol-treated group at 1200 h on day 28; b, significantly greater ($P < 0.01$) than estradiol + vehicle at 1200 h on day 29.

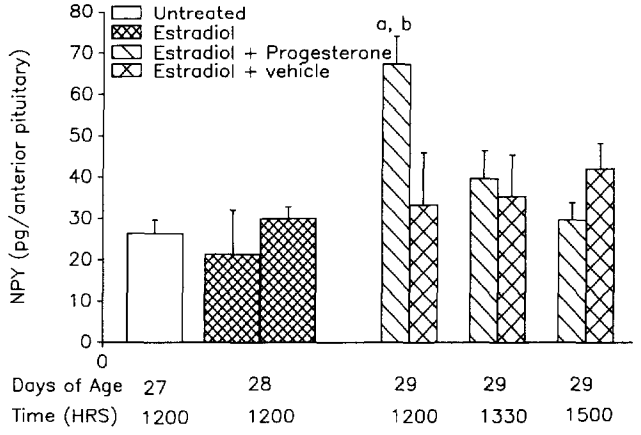


Fig. 3. Effect of estradiol-priming alone (small cross hatch), or estradiol-priming followed by progesterone (diagonal hatch) or vehicle (large cross hatch) treatment on the AP content of NPY in the 27–29-day old immature intact female rat. The animals were prepared as described in Fig. 2 and NPY RIA employed to determine NPY content in individually homogenized pituitaries subsequent to acidification and neutralization of the samples. Data points represent the mean ($\pm$SE) of 3 individual APs and are expressed in terms of porcine NPY (pg/AP). a, significantly greater ($P < 0.01$) than either estradiol-treated group at 1200 h on day 28; b, significantly greater ($P < 0.01$) than estradiol + vehicle at 1200 h on day 29.

### *Anterior pituitary and hypothalamic NPY and NPY mRNA levels*

In estradiol-primed, progesterone-treated animals, AP NPY content was significantly increased at 1200 h on day 29 when compared to either group of estradiol-treated animals on day 28 at 1200 h as well as 1200 h on day 29 in estradiol-primed, vehicle-treated animals (both $P < 0.01$; Fig. 3). There were no statistically significant differences in hypothalamic NPY content within the estradiol-primed, vehicle-treated group or within the estradiol-primed, progesterone-treated group (Fig. 4). In addition, there were no statistically significant differences in hypothalamic NPY content between the two groups on any of the days studied (Fig. 4).

Hypothalamic levels of NPY mRNA (Fig. 5) were significantly decreased ($P < 0.01$) at 1200 h on day 29 in estradiol-primed animals which did not receive progesterone when compared to either group of estradiol-treated animals at 1200 h on day 28. However, in

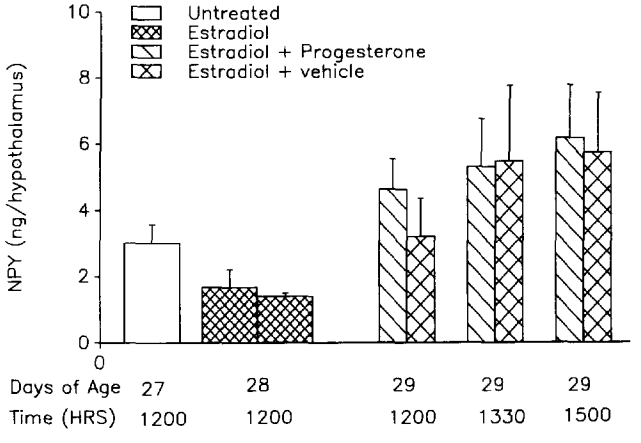


Fig. 4. Effect of estradiol-priming alone (small cross hatch), or estradiol-priming followed by progesterone (diagonal hatch) or vehicle (large cross hatch) treatment on the hypothalamic content of NPY in the 27–29-day-old immature intact female rat. The animals were prepared as described in Fig. 2 and NPY RIA used to determine NPY content in individually homogenized hypothalami subsequent to acidification and neutralization of the samples. Data points represent the mean ($\pm$SE) of 3 individual hypothalami and are expressed in terms of porcine NPY (ng/hypothalamus).

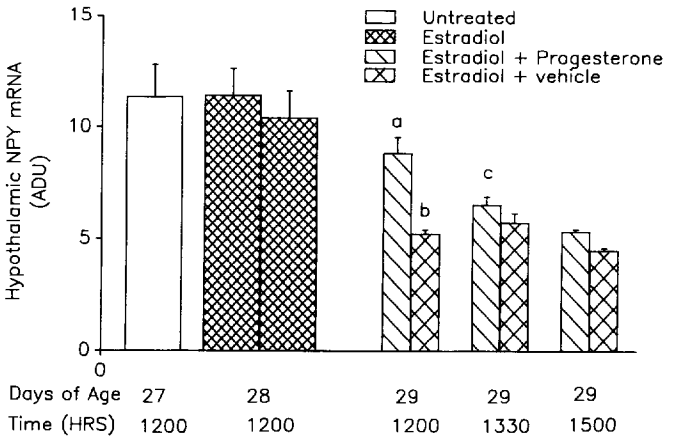


Fig. 5. Effect of estradiol-priming alone (small cross hatch), or estradiol-priming followed by progesterone (diagonal hatch) or vehicle (large cross hatch) treatment on the hypothalamic content of NPY mRNA in the 27–29-day-old immature intact female rat. The animals were prepared as described in Fig. 2. Total hypothalamic RNA was extracted from individual hypothalami and 20 $\mu$g aliquots of RNA were utilized to detect the relative levels of hypothalamic NPY mRNA according to the methods described in Fig. 1. Data Points represent the mean ($\pm$SE) of 3 individual hypothalami and are expressed as ADU. a, significantly greater ($P < 0.01$) in comparison to estradiol + vehicle at 1200 h on day 29; b, significantly less than ($P < 0.01$) either estradiol-treated group at 1200 h on day 28; c, significantly less than ($P < 0.05$) estradiol + progesterone at 1200 h on day 29.

estradiol-primed, progesterone-treated animals, hypothalamic NPY mRNA levels were not significantly decreased at 1200 h on day 29 when compared to either group of estradiol-treated animals at 1200 h on day 28.

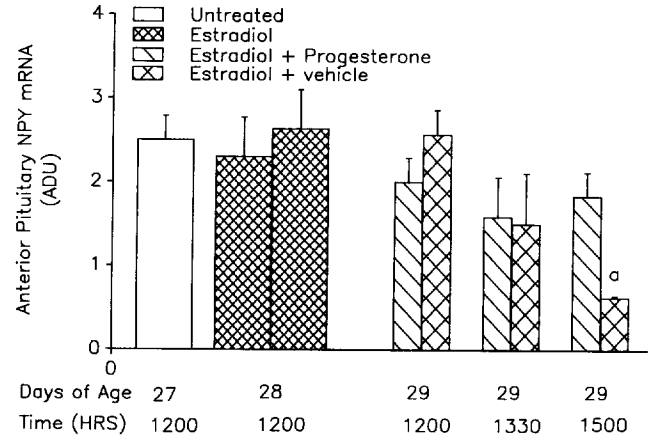


Fig. 6. Effect of estradiol-priming alone (small cross hatch), or estradiol-priming followed by progesterone (diagonal hatch) or vehicle (large cross hatch) treatment on the AP content of NPY mRNA in the 27–29-day-old immature intact female rat. The animals were prepared as described in Fig. 2. Total AP RNA was extracted and 6 $\mu$g aliquots of RNA were utilized to detect relative levels of AP NPY mRNA according to the methods described in Fig. 1. Data points represent the mean ($\pm$SE) of 3 individual AP and are expressed as ADU. a, significantly greater ($P < 0.01$) in comparison to estradiol + vehicle at same time point.

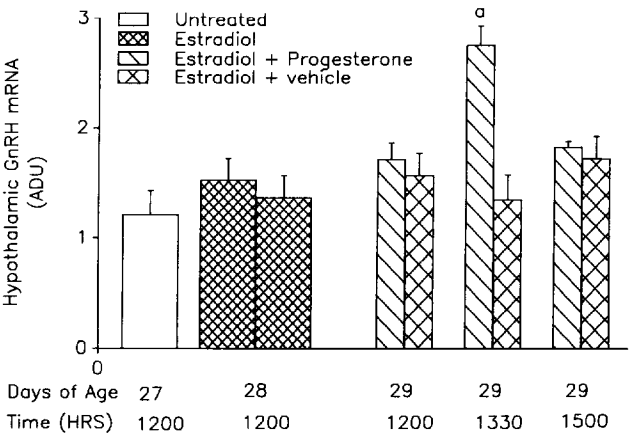


Fig. 7. Effect of estradiol-priming alone (small cross hatch), or estradiol-priming followed by progesterone (diagonal hatch) or vehicle (large cross hatch) treatment on the hypothalamic content of GnRH mRNA in the 27–29-day old immature intact female rat. The animals were prepared as described in Fig. 2. Total hypothalamic RNA was extracted from individual hypothalami and 20 $\mu$g aliquots of RNA were utilized to detect relative levels of hypothalamic GnRH mRNA according to the methods described in Fig. 1. Data points represent the mean ($\pm$SE) of 3 individual hypothalami and are expressed as ADU. a, significantly greater ($P < 0.01$) in comparison to all other points.

In addition, hypothalamic NPY mRNA content was significantly higher ($P < 0.01$) at 1200 h on day 29 (immediately preceding the surge) in the estradiol-primed, progesterone-treated animals when compared to the same time point in the estradiol-primed, vehicle-treated animals. Hypothalamic NPY mRNA levels in estradiol-primed animals which received progesterone were significantly decreased ($P < 0.05$) at 1330 h on day 29 and were comparable to those levels observed at the same time point in estradiol-primed, vehicle-treated animals. AP NPY mRNA levels were not significantly altered immediately preceding and accompanying the surge in either estradiol-primed animals which received progesterone or those which did not (Fig. 6). However, at 1500 h on day 29, AP NPY mRNA levels in estradiol-primed, vehicle-treated animals were significantly decreased ($P < 0.01$) when compared to estradiol-primed, progesterone-treated animals.

### *Hypothalamic GnRH mRNA levels*

In estradiol-primed animals, progesterone-treatment induced a significant increase ($P < 0.01$) in GnRH mRNA levels at 1330 h on day 29 (Fig. 7) when compared to GnRH mRNA levels observed in the estradiol-primed group which did not receive progesterone.

## DISCUSSION

In a previous study [16], we examined the effect of progesterone administered *in vivo* to estradiol-primed rats on the subsequent *in vitro* AP gonadotropin

responsiveness to GnRH in the presence and absence of NPY. From this study, we reported that NPY potentiation of GnRH responsiveness was not significantly greater in AP tissue from estradiol-primed animals which had received progesterone than in those which had not. Because the experimental design employed in the study eliminated any contribution of hypothalamic neurosecretory activity, we concluded that the necessity for progesterone to induce NPY potentiation of GnRH-induced LH release *in vivo* [8, 13] was probably due to actions exerted by progesterone at a site other than the AP. It has been demonstrated that progesterone acts at the hypothalamus to stimulate increase tissue levels [17–19] and secretion [20, 21] of GnRH. The currently reported studies were designed to determine whether progesterone facilitates the induction of the gonadotropin surge *in vivo* by acting at the hypothalamus to stimulate the synthesis of NPY as well as an increase in AP tissue content of NPY.

In estradiol-primed, vehicle-treated animals which did not receive progesterone, hypothalamic NPY mRNA levels were significantly decreased at 1200 h on day 29 when compared to either group of estradiol-treated animals at 1200 h on day 28, thereby indicating an apparent negative effect of estradiol-priming on hypothalamic NPY mRNA steady-state levels. Although a negative effect of estrogen-priming on hypothalamic NPY tissue levels in the adult ovariectomized rat has been previously reported [17], this is the first reported observation that estradiol-priming has a negative effect on hypothalamic NPY mRNA levels. However, in estradiol-primed animals which received progesterone, there was no such significant decrease in hypothalamic NPY mRNA at 1200 h on day 29; further, hypothalamic NPY mRNA levels were significantly higher in estradiol-primed, progesterone-treated animals at 1200 hr on day 29 when compared to estradiol-primed, vehicle-treated animals. Hypothalamic NPY mRNA levels in the estradiol-primed, progesterone-treated group did not decrease significantly until 1330 h on day 29 at which time point hypothalamic NPY mRNA levels had declined to the levels observed in the estradiol-primed, vehicle-treated group. Therefore, progesterone-treatment appeared to have the effect of maintaining hypothalamic NPY mRNA steady-state levels at 1200 h on day 29 by overriding an apparent negative effect of estradiol-priming. Whether progesterone maintained these levels by stimulating the transcription rate of the NPY gene or by stabilizing the half-life of NPY mRNA cannot be determined by Northern analysis alone. The half-life of most mammalian mRNA species is 3–6 h [37, 38]; therefore the ability of progesterone to override the negative effects of estradiol-priming after only 3 h treatment and to maintain hypothalamic NPY mRNA levels 2-fold higher than those observed in estradiol-primed, vehicle-treated animals is highly unlikely to be due solely to a slower rate of degradation and increased stability of the NPY mRNA. Therefore, it is logical to suggest that progesterone was able to maintain steady-state levels of hypothalamic NPY mRNA in estradiol-primed animals at least in part by maintaining transcription of the NPY gene.

The higher level of hypothalamic NPY mRNA at 1200 h on day 29 in progesterone-treated rats was not accompanied by significantly higher hypothalamic NPY tissue levels; however, at the same time point, AP NPY tissue levels were significantly increased while AP NPY mRNA levels did not change. These observations suggest that the NPY found in the AP probably originated from the hypothalamus. This possibility is supported by the previous demonstration of elevated levels of NPY in the hypophysial portal system at the time of the preovulatory surge [5]. In addition, although hypothalamic NPY mRNA levels at 1200 h on day 27 and day 28 (either group) were not statistically different when compared to estradiol-primed, progesterone-treated animals at 1200 h on day 29, NPY tissue levels in the AP were not significantly increased until 1200 h on day 29. Therefore, it appears that progesterone-treatment was necessary not only to maintain hypothalamic NPY mRNA steady-state levels, but also to induce an increase in AP NPY tissue levels. Taken together, it is tempting to speculate that in the current study, progesterone-treatment induced hypothalamic synthesis of NPY mRNA and the secretion of NPY into the portal system resulting in increased AP tissue content of NPY. This is the first report of elevated AP NPY content and higher hypothalamic NPY mRNA levels in conjunction with the steroid-induced gonadotropin surge in the immature intact rat. An increase in hypothalamic NPY mRNA levels in conjunction with the LH surge in the adult ovariectomized estrogen-primed, progesterone-treated rat was recently reported [39]. However, in that study, hypothalamic NPY mRNA levels were elevated immediately before and throughout the duration of the LH surge, whereas in the current study, hypothalamic NPY mRNA levels were higher prior to the onset of the LH surge and declined during the surge. Nonetheless, both studies observed an elevation of NPY mRNA prior to the onset of the gonadotropin surge in two diverse animal models.

In estradiol-primed animals, progesterone-treatment induced a highly significant increase in hypothalamic GnRH mRNA levels at 1330 h on day 29 which coincided with the peak of the gonadotropin surge in the same treatment group and was preceded by the above described changes in hypothalamic NPY mRNA and AP NPY content. This is the first time such an observation has been reported during the progesterone-induced gonadotropin surge in the estradiol-primed immature intact rat model. Kim *et al.* [40] reported a similar observation in the prepubertal ovariectomized estrogen-primed, progesterone-treated rat. In the adult

cycling rat, GnRH mRNA levels have been reported to be elevated at the time of the preovulatory surge, a time when endogenous progesterone levels are maximal [36, 41]. Park *et al.* [36] did not observe a progesterone-induced increase in GnRH mRNA in the adult ovariectomized estrogen-primed, progesterone-treated rat. However, the time point utilized in that study was 9 h following progesterone administration by which time the gonadotropin surge had declined and it is most likely (as surmized by the authors) that elevated GnRH mRNA levels had also returned to basal by that time.

In conclusion, in the current study, estradiol-primed intact immature rats which received progesterone treatment exhibited preovulatory-like gonadotropin surges, higher levels of hypothalamic NPY mRNA and GnRH mRNA and elevated AP NPY content. None of these characteristics were observed in the absence of progesterone treatment. Higher levels of hypothalamic NPY mRNA and elevation in AP NPY content occurred prior to the elevations in GnRH mRNA and gonadotropin secretion. This study supports the hypothesis that in the estradiol-primed immature intact rat, progesterone facilitates the induction of the gonadotropin surge in at least two major ways. First, progesterone appears to maintain hypothalamic NPY mRNA levels by overriding an apparent negative effect of estradiol on hypothalamic NPY gene expression. Second, progesterone appears to facilitate the elevation of NPY levels in the AP, possibly by stimulating the secretion of NPY from the hypothalamus.

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