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Changes in Mediobasal Hypothalamic Gonadotropin-Releasing Hormone Messenger Ribonucleic Acid Levels Induced by Mating or Ovariectomy in a Reflex Ovulator, the Ferret*

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ABSTRACT

The ferret is a reflex-ovulating species in which receipt of an intromission induces a prolonged (±12 h) preovulatory LH surge in the estrous female. This LH surge is probably stimulated by a large release of GnRH from the mediobasal hypothalamus (MBH). In Exp 1 we asked whether GnRH messenger RNA (mRNA) levels increase in response to mating so as to replenish the MBH GnRH stores needed to sustain the preovulatory LH surge. Estrous females were killed 0, 0.25, 0.5, 1, 3, 6, 14, or 24 h after the onset of a 10-min intromission from a male. Coronal brain sections ranging from the rostral preoptic area caudally to the posterior hypothalamus were processed for *in situ* hybridization using a ^{35}S-labeled oligoprobe complementary to the human GnRH-coding region. We found no evidence of increased MBH GnRH mRNA levels during the ferret's mating-induced preovulatory LH surge. Instead, the number of GnRH mRNA-expressing cells dropped significantly in the arcuate region beginning 6 h after onset of intromission and remained low thereafter. Furthermore, cellular GnRH mRNA levels decreased in the arcuate region toward the end of the preovulatory LH surge. In Exp 2 we asked whether ovarian hormones regulate MBH GnRH mRNA levels in the female ferret. Ovariectomy of estrous females significantly reduced the number of GnRH mRNA-expressing cells in the arcuate region. This decrease was probably not due to the absence of circulating estradiol. Gonadally intact anestrous females had levels of MBH GnRH mRNA similar to those in estrous females even though plasma estradiol levels were equally low in anestrous females and ovariectomized females. Ovarian hormones other than estradiol may stimulate MBH GnRH mRNA levels in anestrous and estrous females. (*Endocrinology* **140:** 595–602, 1999)

IN THE FERRET, a reflex ovulator, receipt of an intromission induces a preovulatory LH surge in the estrous female (1, 2). This elevation in circulating LH begins around 1.5 h after the onset of intromission, peaks approximately 6 h later, and is sustained for at least 12 h (2). The preovulatory LH surge in the female ferret is probably stimulated by a large, sustained release of GnRH from the mediobasal hypothalamus (MBH) into the pituitary portal vessels. It was previously found that the *in vitro* release from perifused MBH slices and MBH tissue content of GnRH were significantly reduced in estrous females killed 0.25 h after receipt of an intromission (3). Also, fewer GnRH-immunoreactive perikarya were detected in the MBH of ovariectomized, estradiol-primed female ferrets killed 20 min after receiving mechanical vagino-cervical stimulation (4). In the vole, another reflex ovulating species, a similar depletion in hypothalamic GnRH content was found in females 5 min after mating (5). These findings suggest that in these species mating induces a large release of GnRH from the MBH that initially depletes GnRH neuronal terminals of peptide. Interestingly, no decrease in the MBH release of GnRH was observed in estrous female ferrets killed 1 or 2.6 h after the receipt of an intromission (3), suggesting that releasable GnRH stores in the MBH are replenished as early as 1 h after mating. This replenishment could reflect a mating-induced increase in the biosynthesis of GnRH peptide as a result of increased GnRH gene expression. In Exp 1, we addressed this question by comparing GnRH messenger RNA (mRNA) levels in MBH neurons of estrous female ferrets killed at different times during the course of the mating-induced preovulatory LH surge.

In spontaneous ovulators such as rat, hamster, sheep, and human, estrogens exert both positive and negative feedback actions on the hypothalamus and/or pituitary gland to control LH secretion. In the ferret, there is only evidence of a negative feedback action of estrogen (1). Female ferrets in estrus have high levels of circulating estrogen coupled with low or undetectable levels of LH (6). Ovariectomy caused a gradual rise in plasma LH in ferrets (6), which was suppressed by administering estradiol (7). One might expect that the hypersecretion of LH observed after ovariectomy is driven by increased GnRH release from the MBH. However, a body of evidence from the rat (reviewed in Ref. 8) suggests that GnRH release, measured in the MBH using either *in vitro* or *in vivo* methods, is actually diminished after ovariectomy. Likewise, ovariectomy of estrous ferrets caused a decrease in the *in vitro* release and content of GnRH peptide in the MBH (3). This decrease could reflect a decrease in the biosynthesis of GnRH peptide in response to a reduction in GnRH gene expression. In Exp 2, we addressed this question by com-

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paring GnRH mRNA levels in MBH neurons of ovariectomized female ferrets as well as gonadally intact estrous and anestrous females. In both experiments, neuronal GnRH mRNA levels were measured using isotopic *in situ* hybridization.

Materials and Methods

Animals and experimental design

Adult, gonadally intact, European male and female ferrets in breeding condition were purchased from Marshall Farms (North Rose, NY). Subjects were housed individually in modified rabbit cages under a long day photoperiod (16 h of light, 8 h of darkness; lights on at 0700 h). All ferrets were fed moistened Purina ferret chow (Ralston Purina Co., St. Louis, MO) once a day. Water was available *ad libitum*.

In Exp 1, estrous females received a 10-min intromission from a male in breeding condition. This mating stimulus reliably provokes a preovulatory LH surge (2). Mated females were killed 0.25, 0.5, 1, 3, 6, 14, or 24 h after the onset of intromission. Additional estrous females were taken directly from their home cage and killed (0 h; unmated controls). All estrous females had fully swollen vulvas, and all mated females showed high levels of behavioral receptivity. In Exp 2, estrous females were ovariectomized via a single midline incision and killed 22 days later when plasma LH levels were expected to be high (7). Additional gonadally intact females in estrus or anestrus were taken directly from their home cage and killed.

Blood and brain collection

Ferrets were quickly anesthetized using CO_2 and decapitated, and the brains were removed and frozen in powdered dry ice before being stored at −80 C. Trunk blood was collected in heparinized tubes. Blood samples were spun down, and plasma was collected and stored at −20 C before being shipped elsewhere on dry ice for hormone assays.

Hormone assays

Plasma LH levels were quantified in duplicate in a RIA using the GDN 15 antiovine LH antiserum (6). The minimum detection level of the assay was 0.45 ng/ml. The LH assay was performed by Dr. Kathleen Ryan (Magee-Womens Research Institute, Pittsburgh, PA). Plasma estradiol levels were measured in duplicate using a double antibody RIA kit (Diagnostic Products Corp., Los Angeles, CA). The minimum detection level of the assay was 2 pg/ml. The estradiol assay was performed by Dr. Geralyn Messerlian Lambert (Womens and Infants Hospital, Providence, RI).

In situ hybridization for GnRH mRNA

Frozen brains were sectioned coronally at 14 μm using a cryostat and mounted onto Vectabond-coated slides. Brain sections were collected beginning rostrally at the level of the organum vasculosum of the lamina terminalis and extending caudally to the posterior hypothalamus. Slides were stored in boxes containing desiccant at −80 C until *in situ* hybridization was performed.

Every fourth brain section was used for *in situ* hybridization, which was carried out at the Tufts University Center for Reproductive Research using a 48-base synthetic oligonucleotide probe complementary to the GnRH-coding region (bases 102–149) of the human complementary DNA (9). This oligoprobe has previously been used successfully in the rat (10) and ferret (11). An initial batch of the oligoprobe was provided by Dr. Cheryl Sisk of Michigan State University (East Lansing, MI). Then, additional amounts of the oligoprobe were synthesized at the Department of Physiology, Tufts Medical School (Boston, MA). The GnRH oligoprobe was 3′-end labeled by incubation with [^{35}S]deoxy-ATP (75 pmol; New England Nuclear, Boston, MA) and terminal deoxynucleotidyl transferase (25 U; Boehringer Mannheim, Indianapolis, IN) to a specific activity of approximately 10^6 cpm/μl. The size and the relative purity of the labeled oligoprobe were determined by gel electrophoresis (Phast system, Pharmacia, Uppsala, Sweden).

The hybridization protocol was modified slightly from the method used by Tang *et al.* (11). Prehybridization treatment consisted of warming the sections to room temperature, fixing in 4% paraformaldehyde for 10 min, acetylating with 0.25% acetic anhydride, dehydrating through a series of ethanols (70%, 80%, 95%, 100%, and 95%), defatting in chloroform, and air-drying at room temperature for at least 1 h. The ^{35}S-labeled GnRH oligoprobe was mixed with 2 × SSC, 1 × Denhardt's solution, 10% dextran sulfate, 25 μg/ml yeast transfer RNA, and 0.5 mg/ml salmon sperm DNA to a specific activity of 6×10^3 cpm/μl. The resulting hybridization solution was heated to 65 C, quenched in ice, and applied to the sections (20 μl/section). Slides were coverslipped and placed in humid hybridization chambers overnight at 41 C. After hybridization, sections were desalted using decreasing concentrations of SSC (2, 1, and 0.5 ×) containing 1 M dithiothreitol (DTT), followed by a 30-min wash in 0.1 × SSC containing 1 M DTT at 41 C. After a final wash in 0.1 × SSC containing 1 M DTT at room temperature, sections were dehydrated through a series of ethanols (50%, 60%, 95%, and 100%) and air-dried overnight in slide boxes. Slides were dipped into photographic emulsion (Kodak NTB-3, Eastman Kodak Co., Rochester, NY; diluted 1:1 with distilled water) and exposed for 10 days at 4 C. Then slides were developed in Kodak D-19, fixed with Kodak general purpose fix, counterstained lightly with 0.1% toluidine O blue, and coverslipped using Permount (Fisher Scientific, Fairlawn, NJ). Addition of an excess of unlabeled probe to the hybridization solution completely abolished labeling.

Data analysis

Cell counts. Brain sections from 44 ferrets distributed over 9 *in situ* hybridization runs were analyzed. Each run contained brain sections from an unmated estrous female and a subset of brain sections from different treatment groups. All slides were coded so that the treatment of the ferret was unknown to the investigator analyzing the slides. First, all hybridized cells detected in every section run for *in situ* hybridization were counted in each brain region (preoptic area, anterior hypothalamus, arcuate region, and median eminence). Only cells with more than 5 times the number of silver grains in the adjacent background were considered labeled. Then the mean number of GnRH mRNA-expressing cells per section was computed for each of these 4 brain regions by dividing the total numbers of hybridized cells by the number of sections included in each brain region. Then, 4 anatomically matched brain sections (containing at least 2 hybridized cells/section) from the anterior hypothalamus and arcuate region and 3 anatomically matched brain sections (containing at least 1 hybridized cell/section) from the rostral preoptic area and the median eminence were selected for image analysis. GnRH mRNA-positive cells were identified by the presence of silver grains overlying a counterstained cell body.

Image analysis. All GnRH mRNA-positive neurons in the three or four anatomically matched sections from four brain regions were digitized for image analysis. Digital images were taken at ×1000 magnification using a Zeiss Axioscope (Bellingham, MA) and a Hamamatsu charge-coupled device video camera together with an 8-bit (256 gray scale levels) frame grabber board controlled by Bioscan, Inc.'s OPTIMAS image analysis software. A threshold intensity was set at the level of the underlying counterstained cell body so that only the silver grains overlying this cell were above this threshold. In addition, the same set of three GnRH mRNA-expressing cells from one animal was used as a standard to calibrate the system during each analysis session. For each hybridized cell, the cell body was circumscribed manually, and the total hybridization area per cell was estimated by computing the sum of areas occupied by silver grains. All hybridized cells found in three or four matched sections per region were analyzed for each subject. The average hybridization area per cell was calculated for each brain region for each animal, and these values were used to determine the group mean and SEM for each postcoital time point (Exp 1) or endocrine treatment (Exp 2).

Statistical analysis. Because of variability in the quantitative results, cell numbers and cellular GnRH mRNA values were compared using nonparametric two-tailed Mann-Whitney U tests. LH and estradiol levels in plasma were also analyzed using these tests.

Results

Distribution of neurons containing GnRH mRNA

Cells expressing GnRH mRNA were recognized as a cluster of silver grains overlying a counterstained cell. Examples of GnRH mRNA-positive neurons in the arcuate region from an unmated estrous female (A) and an ovariectomized female (B) are shown in Fig. 1. GnRH mRNA-expressing cells were widely distributed across the MBH (Fig. 2). Only a few GnRH mRNA cells were detected in the rostral preoptic area and septal area. Larger numbers of GnRH mRNA cells were found near the base of the anterior hypothalamus and in the ventral arcuate region, and only a few GnRH mRNA cells were seen in the median eminence (Fig. 2). The distribution and number of cells hybridized for GnRH mRNA were similar to those reported in the male ferret (11) and those found to be immunoreactive for GnRH protein in ferrets of both sexes (12–14).

Effect of mating on GnRH mRNA cell numbers

The mean number of GnRH mRNA-expressing cells in the arcuate region decreased significantly during the course of the preovulatory LH surge (Fig. 3). Significantly fewer hybridized cells were detected in the arcuate region of mated females killed 6 or 14 h after onset of intromission compared with those in unmated females ($P = 0.03$ and $P = 0.04$, respectively). There was also a trend for cell numbers in the preoptic area ($P = 0.06$) in females killed 6 or 24 h after onset of intromission and in the anterior hypothalamus ($P = 0.07$) in females killed 6, 14, or 24 h after the onset of intromission to decline over the course of the preovulatory LH surge (Fig. 3).

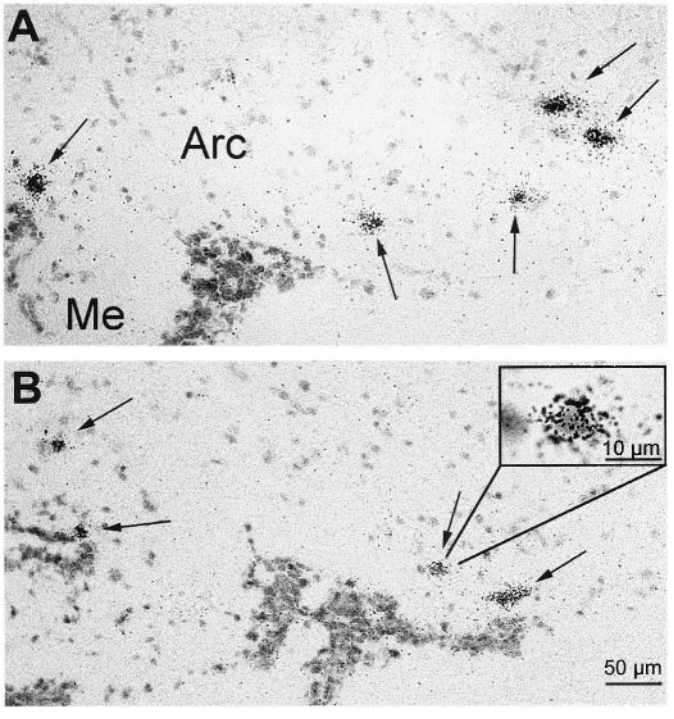

FIG. 1. Photomicrographs of cells expressing GnRH mRNA in the arcuate region (Arc) and the median eminence (Me) of an unmated estrous female ferret (A) and an ovariectomized female ferret (B). The high magnification *inset* in B shows a labeled cell with silver grains over a counterstained cell body. *Arrows* indicate labeled cells.

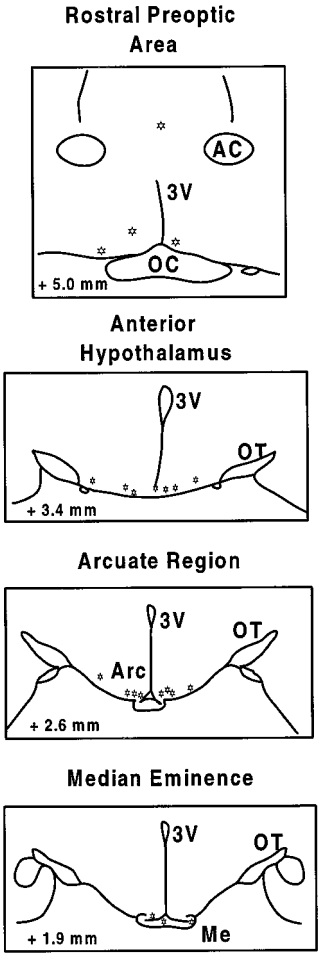

FIG. 2. Camera lucida drawings of coronal sections through the rostral preoptic area (+5.0 mm anterior to the interaural line), anterior hypothalamus (+3.4 mm), arcuate region (+2.6 mm), and median eminence (+1.9 mm) showing the distribution of GnRH mRNA-containing neurons (☆) in a representative, unmated, estrous female ferret. 3V, Third ventricle; AC, anterior commissure; Arc, arcuate nucleus; Me, median eminence; OC, optic chiasm; OT, optic tract.

Effect of mating on cellular GnRH mRNA levels

Cellular GnRH mRNA levels decreased in the arcuate region over the course of the preovulatory LH surge (Table 1). Mean cellular levels of GnRH mRNA in hybridized cells of the arcuate region were significantly lower in mated estrous females killed 1 or 14 h after the onset of intromission compared with those in unmated estrous controls (Table 1). There were no significant mating-induced changes in cellular GnRH mRNA levels in the preoptic area, anterior hypothalamus, or median eminence (Table 1).

Effect of ovariectomy on GnRH mRNA levels

Ovariectomy significantly decreased the mean number of GnRH mRNA-expressing cells per section in the arcuate region compared with those in gonadally intact estrous females ($P = 0.03$; Fig. 4). The mean number of GnRH mRNA-

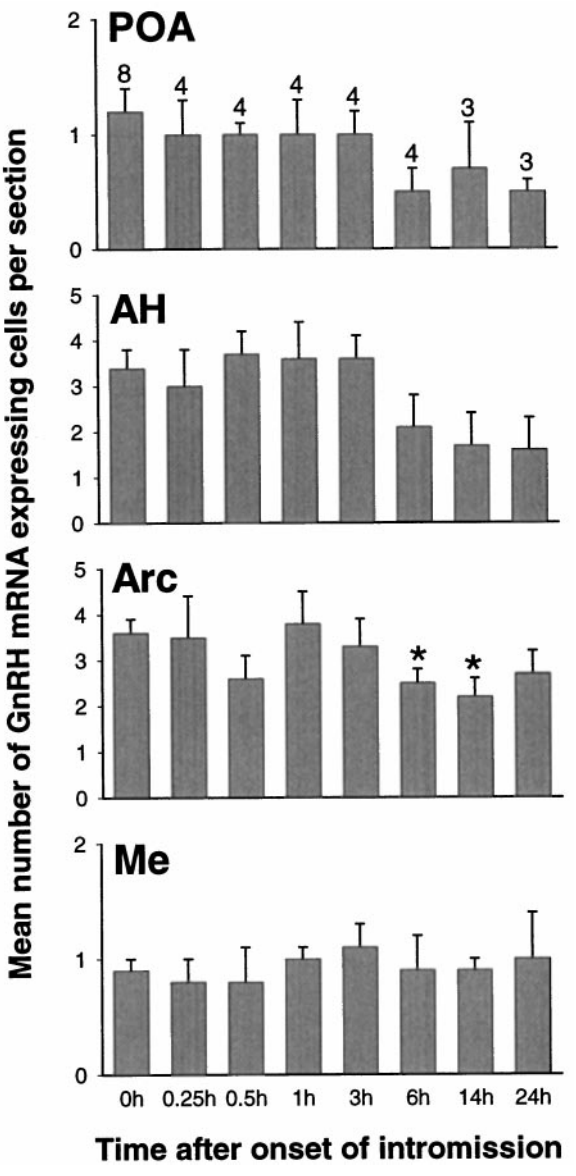

FIG. 3. Effect of receipt of an intromission from a male on the mean (±SEM) number of GnRH mRNA-expressing cells per brain section. The mean (±SEM) numbers of coronal brain sections within each region were: preoptic area (POA), 23 ± 1; anterior hypothalamus (AH), 17 ± 1; arcuate region (Arc), 26 ± 1; and median eminence (Me), 26 ± 1. *, $P < 0.05$, by two-tailed Mann-Whitney U comparisons with unmated (0 h) estrous females. The number of subjects per group is shown *above the bars* in the *top panel*.

expressing cells did not differ significantly between ovariectomized females and gonadally intact anestrous females. There was no effect of ovariectomy on cellular GnRH mRNA levels in any of the brain regions analyzed (Table 2). In addition, cellular GnRH mRNA levels did not differ between gonadally intact estrous and anestrous females.

Plasma LH and estradiol

There was evidence of a mating-induced preovulatory LH surge in estrous females (Fig. 5A). Mean plasma LH levels were significantly higher in mated females killed 0.25 h ($P = 0.008$), 0.5 h ($P = 0.004$), 1 h ($P = 0.02$), 14 h ($P = 0.01$), or 24 h ($P = 0.04$) after the onset of intromission than in unmated estrous females (0 h). Ovariectomy increased plasma LH levels to those seen in mated, estrous females during the preovulatory LH surge (Fig. 5A). Anestrous females had plasma LH levels that were intermediate between those of unmated estrous and ovariectomized females. Both ovariectomized females ($P = 0.004$) and anestrous females ($P = 0.005$) had significantly higher plasma LH levels than unmated estrous females, whereas ovariectomized females had higher plasma LH levels than anestrous females ($P = 0.009$). When the data from Exp 1 and 2 were combined, no significant correlation was found between plasma LH levels and the number of GnRH mRNA-expressing cells or cellular GnRH mRNA levels in any of the four brain regions analyzed.

Plasma estradiol levels were significantly higher in estrous females (unmated; 0 h) than in anestrous ($P = 0.05$) or ovariectomized females ($P = 0.01$; Fig. 5B). Plasma estradiol levels did not vary significantly among groups of estrous females killed at different times during the mating-induced preovulatory LH surge (Fig. 5B). Also, plasma estradiol levels were equally low in anestrous and ovariectomized females. When data from Exp 1 and 2 were combined, no significant correlation was found between plasma estradiol levels and the number of GnRH mRNA-expressing cells or cellular GnRH mRNA levels in any of the four brain regions analyzed.

Discussion

Relationship between GnRH mRNA levels and GnRH release after mating

We found no evidence of increased GnRH mRNA levels in the MBH during the ferret's mating-induced preovulatory LH surge. Instead, GnRH mRNA levels actually decreased in some MBH regions over the course of the preovulatory LH surge. In a previous study (3), we reported that the in *vitro* release of GnRH from perifused MBH slices was significantly reduced in estrous female ferrets killed 0.25 h after the onset of intromission, but was restored to unmated levels in females killed 1 or 2.6 h after the onset of intromission. These results suggest that there is a large release of GnRH immediately after mating that depletes releasable stores of the peptide in MBH nerve terminals. Replenishment of GnRH stores apparently occurs within 1 h after mating. We hypothesized that an increase in MBH GnRH gene expression contributes to this replenishment. However, in the present study MBH GnRH mRNA levels were not elevated during the first hour after the onset of intromission. The absence of any increase in GnRH mRNA levels suggests that posttranscriptional events, such as increased GnRH mRNA translation, increased conversion of the pro-GnRH peptide into the mature GnRH decapeptide, and/or increased transport of the peptide to the nerve terminals, contribute to the previously observed (3) replenishment of GnRH stores in the MBH.

GnRH mRNA levels began to decrease in the MBH during the peak phase of the preovulatory LH surge. Specifically, the number of GnRH mRNA-expressing cells was lower in the arcuate region of estrous females killed 6, 14, or 24 h after the onset of intromission. This decrease probably reflects a re-

TABLE 1. Effect of receipt of an intromission from a male on cellular GnRH mRNA levels in three or four anatomically matched sections from the mediobasal hypothalamus of estrous female ferrets

Time after onset of intromission (h)	n[a]	Rostral POA (n = 7)[b]	Anterior hypothalamus (n = 17)	Arcuate region (n = 19)	Median eminence (n = 7)
0	8	102 ± 13	97 ± 7	98 ± 7	72 ± 11
0.25	4	75 ± 13	96 ± 11	121 ± 16	80 ± 12
0.5	4	91 ± 19	115 ± 20	99 ± 14	74 ± 9
1	4	108 ± 18	120 ± 13	63 ± 8[c]	72 ± 11
3	4	101 ± 14	109 ± 22	94 ± 6	72 ± 13
6	4	155 ± 22	93 ± 10	91 ± 14	53 ± 7
14	3	81 ± 17	59 ± 12	68 ± 7[c]	76 ± 4
24	3	74 ± 14	76 ± 7	76 ± 10	73 ± 9

Data are expressed as the mean ± SEM hybridization area (square microns) per cell.
[a] Number of subjects per group.
[b] Mean number of hybridized cells analyzed per region in each subject.
[c] Significantly lower ($P < 0.05$) than unmated female value (0 h).

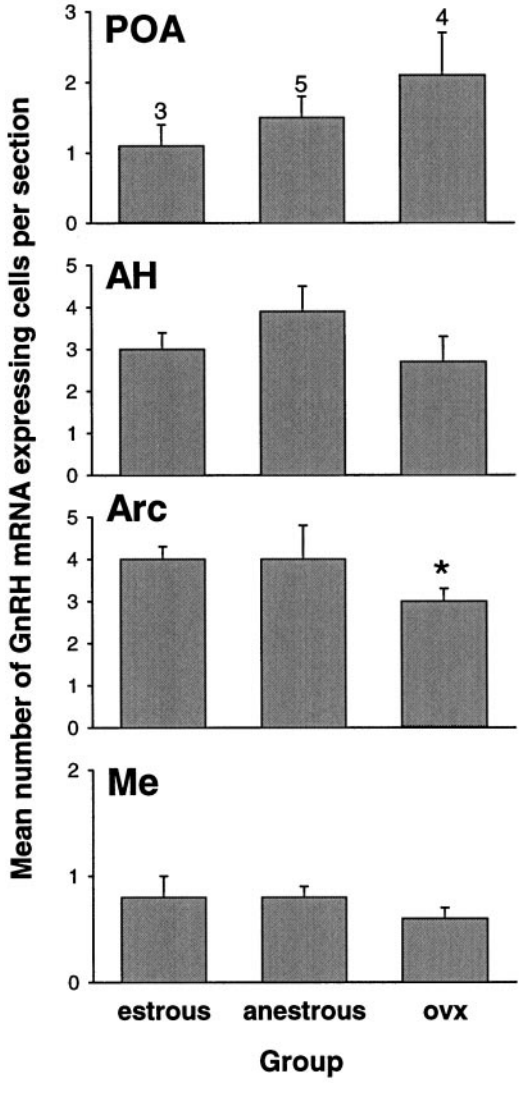

FIG. 4. Effect of ovariectomy on the mean (±SEM) number of GnRH mRNA-expressing cells per brain section. The mean (±SEM) numbers of coronal brain sections within each region were: preoptic area (POA), 19 ± 0; anterior hypothalamus (AH), 20 ± 1; arcuate region (Arc), 27 ± 1; and median eminence (Me), 27 ± 1. *, $P < 0.05$, by two-tailed Mann-Whitney U comparisons with estrous females. The number of subjects per group is shown *above the bars* in the *top panel*.

duction in cellular mRNA content, so that cells expressing decreased levels of mRNA were no longer detected with the *in situ* hybridization method used. Indeed, cellular levels of GnRH mRNA in the arcuate region decreased significantly in estrous females killed 14 h after the onset of intromission. In addition, there was a trend in the preoptic area and anterior hypothalamus for cellular GnRH mRNA levels and the number of GnRH mRNA-expressing cells to decline over the course of the preovulatory LH surge.

The decrease in MBH GnRH mRNA levels during the preovulatory LH surge may have been caused by reductions in GnRH gene transcription and/or GnRH mRNA stability. In rats, GnRH mRNA levels appear to be regulated across the estrous cycle at the level of gene transcription and mRNA stability (15, 16). For example, GnRH mRNA levels repeatedly exhibit two significant peaks in cycling rats, one on diestrus and another on the afternoon of proestrus (15). The increase in GnRH mRNA levels on diestrus is probably due to an increase in mRNA stability, whereas the increase on proestrus is accompanied by an increase in gene transcription (15). However, the decrease in GnRH mRNA levels during the mating-induced preovulatory LH surge in estrous ferrets is most likely not the result of changes in ovarian steroid secretion. Plasma progesterone concentrations do not rise until 5 days after receipt of an intromission (17), and plasma estradiol concentrations did not fluctuate significantly during the mating-induced preovulatory LH surge (present study) and only began to drop 4 days after receipt of an intromission (17).

In the present study, mean plasma LH levels were significantly elevated in most groups of mated estrous females, suggesting that they experienced a mating-induced preovulatory LH surge. This confirms our previous observations that intromissions that last longer than 2 min always suffice to induce a preovulatory LH surge in estrous female ferrets (1–3). However, there was considerable variability in the data, with relatively low levels of plasma LH measured in females killed 1, 3, or 6 h after the onset of intromission. Carroll *et al.* (2) demonstrated that the mating-induced preovulatory LH surge is characterized by increases in pulse frequency and amplitude. As we took a single trunk blood sample when subjects were killed for *in situ* hybridization, we probably measured LH in both peaks and valleys of these

TABLE 2. Effect of ovariectomy on cellular GnRH mRNA levels in three or four anatomically matched sections from the mediobasal hypothalamus of female ferrets

Group	n[a]	Rostral POA (n = 9)[b]	Anterior hypothalamus (n = 18)	Arcuate region (n = 20)	Median eminence (n = 8)
Estrous	3	111 ± 25	100 ± 12	108 ± 13	91 ± 25
Anestrous	5	78 ± 7	99 ± 3	94 ± 15	67 ± 9
Ovariectomy	4	111 ± 11	101 ± 14	85 ± 14	65 ± 10

Data are expressed as the mean ± SEM hybridization area (square microns) per cell.
[a] Number of subjects per group.
[b] Mean number of hybridized cells analyzed per region in each subject.

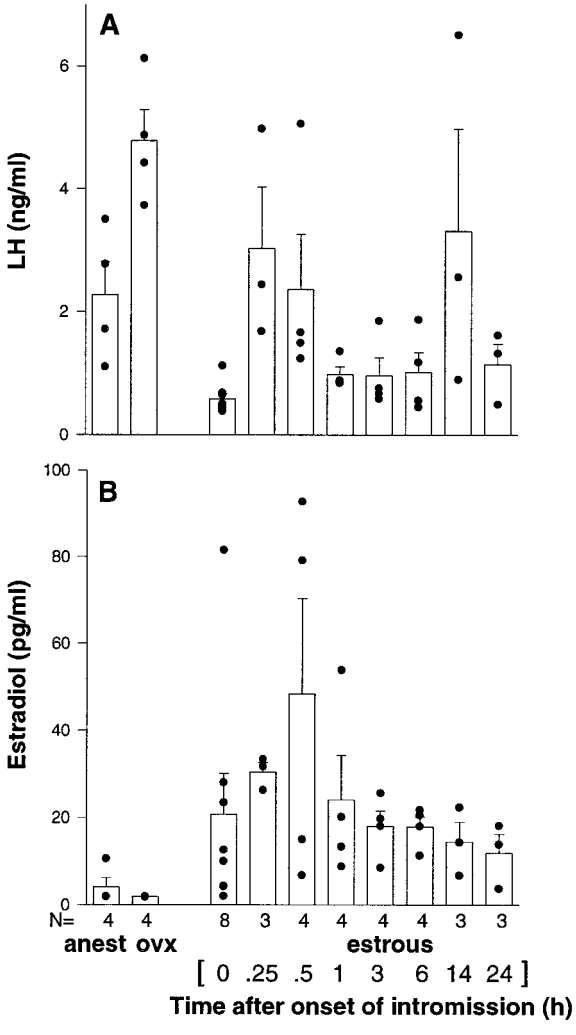

FIG. 5. LH (A) and estradiol (B) levels in trunk plasma taken when female ferrets were killed to obtain brains for GnRH mRNA *in situ* hybridization. Individual values (*solid circles*) and group means (*bars*) ± SEM are shown. Anest, Anestrous; ovx, ovariectomized. The number (N) of subjects per group is given at the *bottom* of B. Note that five of eight unmated estrous females had plasma LH levels that were below the detection limit of the assay (0.45 ng/ml) and that all ovariectomized females and three of four anestrous females had plasma estradiol levels that were below the detection limit of the assay (2 pg/ml).

pulses. Wersinger and Baum (14) also found relatively low plasma LH levels in female ferrets killed 1.5 or 3 h after the onset of a 5-min intromission, suggesting that synchronized phases of reduced LH secretion occur during the ferret's preovulatory LH surge. Even so, Wersinger and Baum (14) found appreciable numbers of MBH Fos/LHRH double-labeled neurons in all mated females, including those with low LH levels at the time of death. This suggests that MBH GnRH neurons were activated in all estrous females that received an intromission.

Ovarian regulation of GnRH mRNA levels

There appears to be a relationship between levels of GnRH mRNA in the ferret's MBH (present study) and the *in vitro* release of GnRH peptide from the MBH (3). In estrous female ferrets, GnRH mRNA (present study) and basal *in vitro* release and content of GnRH peptide (3) in the MBH are high. After ovariectomy, GnRH mRNA levels (present study) and basal *in vitro* release and content of GnRH peptide (3) in the MBH are reduced. These data suggest that ovarian hormones stimulate GnRH gene expression as well as GnRH release. This stimulatory action of ovarian hormones on GnRH gene expression and release are in contrast to the negative feedback action of ovarian hormones on LH release from the pituitary (7, 18). There is evidence that estrogen promotes GnRH gene transcription in the rat (16). Estradiol, but not progesterone, treatment increased levels of GnRH RNA primary transcript in the organum vasculosum of the lamina terminalis/rostral preoptic area of ovariectomized rats, which points to an estrogenic induction of GnRH gene transcription (16). Also, GnRH primary transcript levels were elevated on proestrus in cycling female rats, a finding consistent with an estrogen-induced increase in GnRH transcription (15). However, the present study provides no indication that estradiol is the ovarian hormone that promotes MBH GnRH mRNA levels in the female ferret. We found equivalent MBH GnRH mRNA levels in gonadally intact female ferrets that were in estrus or anestrus even though plasma estradiol levels were significantly higher in estrous females. Also, there was no positive correlation between plasma estradiol levels and GnRH mRNA levels in any brain region studied. The absence of significant differences in GnRH mRNA levels between estrous and anestrous females suggests that the reduced function of the hypothalamic-pituitary-gonadal axis during anestrus might reflect a reduced release of GnRH from the MBH or reduced sensitivity of the pituitary to GnRH, as opposed to a down-regulation of MBH GnRH mRNA levels. The reduction in MBH GnRH mRNA levels after ovariectomy suggests that ovarian hormones other than estradiol may facilitate MBH GnRH mRNA levels in anestrous and estrous females.

Species comparisons

Our finding that the mating-induced preovulatory LH surge is accompanied by progressively decreasing levels of GnRH mRNA is comparable to earlier findings in sheep. In the ovariectomized ewe, the estrogen-induced LH surge was associated with decreased levels of GnRH mRNA in the preoptic area (19, 20). In the rat, however, in those studies in which changes were reported (reviewed in Ref. 21), increases in either cellular GnRH mRNA levels or GnRH mRNA cell numbers occurred in the organum vasculosum of the lamina terminalis/rostral preoptic area during the estrogen-induced LH surge (10, 15, 22, 23). There is a species difference in the temporal relationship between the changes observed in GnRH mRNA levels and the LH surge. In the ferret, in which the preovulatory LH surge is induced by mating, MBH GnRH mRNA levels started to decrease during the peak phase of the surge and remained low until its end. In the sheep, GnRH mRNA levels in the preoptic area decreased in advance of the onset of the estrogen-induced LH surge (20). In the rat, GnRH mRNA levels in the organum vasculosum of the lamina terminalis/rostral preoptic area increased in advance (15) or at the time of the LH surge (10, 22, 23). These species differences in the temporal relationship between changes in hypothalamic GnRH mRNA levels and LH release are one characteristic of the different mechanisms controlling the preovulatory LH surge in reflex *vs.* spontaneous ovulators.

We found that ovariectomy decreased the GnRH mRNA cell number in the arcuate region of the ferret. A similar decrease in GnRH mRNA levels has been reported in the rat (24, 25), although this finding is controversial (reviewed in Ref. 21). Other researchers found no effect (26) or an increase (27) in GnRH mRNA levels after ovariectomy in female rats. In the female ferret, estradiol alone appears not to stimulate MBH GnRH mRNA expression. However, estradiol may play a permissive role by increasing the sensitivity of MBH GnRH neurons to activation by somatosensory or olfactory stimuli associated with mating.

Our finding that anestrous and estrous female ferrets have equivalent levels of MBH GnRH mRNA agrees with earlier findings in another long day seasonal breeder, the hamster (28, 29). Male hamsters chronically exposed to either long or short photoperiods had similar numbers of hypothalamic GnRH mRNA-expressing neurons. This suggests that the reduced function of the hypothalamic-pituitary-gonadal axis in both sexually quiescent male hamsters and anestrous female ferrets is mediated primarily by reductions in GnRH release or pituitary sensitivity to GnRH, as opposed to reduced hypothalamic GnRH mRNA levels.

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