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Hypertrophy and Increased Kisspeptin Gene Expression in the Hypothalamic Infundibular Nucleus of Postmenopausal Women and Ovariectomized Monkeys

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Context: Human menopause is characterized by ovarian failure, gonadotropin hypersecretion, and neuronal hypertrophy in the hypothalamic infundibular (arcuate) nucleus. Recent studies have demonstrated a critical role for kisspeptins in reproductive regulation, but it is not known whether menopause is accompanied by changes in hypothalamic kisspeptin neurons.

Objectives: Our objective was to map the location of neurons expressing kisspeptin gene (KiSS-1) transcripts in the human hypothalamus and determine whether menopause is associated with changes in the size and gene expression of kisspeptin neurons. In monkeys, our objective was to evaluate the effects of ovariectomy and hormone replacement on neurons expressing KiSS-1 mRNA in the infundibular nucleus.

Subjects: Hypothalamic tissues were collected at autopsy from eight premenopausal and nine postmenopausal women and from 42 young cynomolgus monkeys in various endocrine states.

Methods: We used hybridization histochemistry, quantitative autoradiography, and computer-assisted microscopy.

Results: Examination of human hypothalamic sections revealed that KiSS-1 neurons were located predominantly in the infundibular nucleus. In the infundibular nucleus of postmenopausal women, there was a significant increase in the size of neurons expressing KiSS-1 mRNA and the number of labeled cells and autoradiographic grains per neuron. Similar to postmenopausal women, ovariectomy induced neuronal hypertrophy and increased KiSS-1 gene expression in the monkey infundibular nucleus. Conversely, in ovariectomized monkeys, estrogen replacement markedly reduced KiSS-1 gene expression.

Conclusions: The cynomolgus monkey experiments provide strong evidence that the increase in KiSS-1 neuronal size and gene expression in postmenopausal women is secondary to ovarian failure. These studies suggest that kisspeptin neurons regulate estrogen negative feedback in the human. (***J Clin Endocrinol Metab* 92: 2744–2750, 2007**)

THE MENOPAUSE SIGNALS a profound loss of reproductive function in aging women. Degeneration of ovarian follicles initiates a cascade of events that culminates with the loss of menstrual cyclicity, a reduction in ovarian hormones to castrate levels, and hypersecretion of gonadotropins from the anterior pituitary gland. Although this process has significant repercussions throughout the body and affects a large proportion of our society, the neuroendocrine control mechanisms that accompany menopause are poorly understood.

A pronounced enlargement of neurons occurs in the hypothalamic infundibular (arcuate) nucleus of postmenopausal women (1–4). This cellular hypertrophy is characterized by increased Nissl substance (indicative of increased protein synthesis) and enlarged nuclei and nucleoli, suggesting increased neuronal activity (3, 4). The neuronal hypertrophy does not appear to be a compensatory response to cell degeneration because there is neither cell loss nor signs of a pathological process in the infundibular nucleus of older women (2, 4). Hybridization histochemistry studies have shown that the hypertrophied neurons express estrogen receptor α (ERα) and neurokinin B (NKB) mRNA and that menopause is associated with a striking increase in NKB gene expression (2, 3). Remarkably, ovariectomy of young cynomolgus monkeys produces identical changes (5), providing strong evidence that the hypertrophy and increased NKB gene expression in postmenopausal women is secondary to the ovarian failure of menopause. Because the hypertrophy occurs in a subpopulation of ERα mRNA-expressing neurons in concert with estrogen withdrawal and gonadotropin hypersecretion, it has been proposed that these hypertrophied neurons participate in the hypothalamic circuitry regulating estrogen negative feedback (2).

In 2003, two reports documented that mutations in the G protein-coupled receptor 54 (GPR54) result in hypogonadotropic hypogonadism, a condition of reproductive immaturity secondary to low gonadotropin secretion (6, 7). Since these seminal reports, a wealth of information has accumulated showing that kisspeptins, the endogenous peptide ligands of GPR54, play an important role in the regulation of LH secretion (8) and the initiation of puberty (9). Kisspeptins are potent stimulators of LH secretion in mice, rats, monkeys, sheep, and men, and thus their effects are widely conserved among mammalian species (for reviews, see Refs. 8 and 10). Exogenous administration of kisspeptin activates GnRH

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Abbreviations: AVPV, Anteroventral periventricular; ERα, estrogen receptor α; GPR54, G protein-coupled receptor 54; MBH, medial basal hypothalamus; NKB, neurokinin B; OVX, ovariectomized.

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neurons (11) and stimulates GnRH secretion (12). GnRH neurons express GPR54 mRNA (11–13) and are closely apposed by kisspeptin-immunoreactive fibers (14, 15). Taken together, these data suggest that the kisspeptins act directly on GnRH neurons through GPR54 to stimulate gonadotropin release.

In laboratory rodents and sheep, kisspeptin neurons express ERα (16, 17), and kisspeptin gene (KiSS-1) expression is increased in the arcuate nucleus in response to ovariectomy (15, 16, 18, 19). Based on these data, we hypothesized that KiSS-1 gene transcripts would be localized within the hypertrophied neurons in postmenopausal women and KiSS-1 mRNA would be increased in association with the ovarian failure of menopause. In the present study, hybridization histochemistry was used to map the distribution of neurons expressing KiSS-1 mRNA in the human hypothalamus, evaluate whether KiSS-1 mRNA is expressed in the hypertrophied infundibular neurons, and determine whether menopause is associated with changes in KiSS-1 gene expression. Because comparisons of premenopausal and postmenopausal women are complicated by two variables (age and ovarian status), additional studies were conducted to determine the effects of ovariectomy and hormone replacement on KiSS-1 neurons in the infundibular nucleus of young cynomolgus monkeys. A finding of KiSS-1 mRNA within the hypertrophied neurons would lend strong support for the hypothesis that these neurons are involved in the regulation of estrogen negative feedback in the human.

Subjects and Methods

Experiment 1: localization of neurons expressing KiSS-1 mRNA in the human hypothalamus

The subjects were eight premenopausal women (32 ± 2.7 yr of age; range, 21–41 yr) and nine postmenopausal women (72 ± 2.7 yr of age; range, 59–86 yr) who died of sudden, unexpected causes (trauma, myocardial infarct, arrhythmia, or pulmonary embolus). The specimens were collected and data recorded in such a manner that subjects could not be identified in accordance with the guidelines set forth in Federal Register 46.101 and the Human Subjects Committee at the University of Arizona. The postmortem interval was not significantly different between the two groups (premenopausal, 13.2 ± 2.4 h; postmenopausal, 15 ± 2.3 h). There was no history of estrogen replacement, drug use, or chronic systemic illness other than atherosclerosis. Endometrial samples were examined to determine reproductive status. At autopsy, each brain was bisected in the midsagittal plane, and hypothalamic blocks were dissected and frozen in isopentane at −30 C. The hypothalami were then sagittally sectioned (20 μm thickness) in a cryostat, thaw mounted onto gelatinized slides, and stored at −80 C. Hybridization histochemistry was performed on every 10th section throughout the medial hypothalamus. Sections were systematically examined using both bright-field and dark-field microscopy to evaluate the distribution of labeled neurons.

Experiment 2: quantitative analysis of neurons expressing KiSS-1 mRNA in the infundibular nucleus of premenopausal and postmenopausal women

Sections from the hybridization histochemistry procedure (above) were matched to Fig. 4-4 from Nauta and Haymaker (20). At this level of the hypothalamus, the infundibular nucleus has a well-defined border that is clearly delineated from the ventromedial nucleus. Quantitative computer microscopy was used to determine the number of infundibular neurons labeled with the KiSS-1 probe, the size of labeled neurons, and the number of autoradiographic grains per neuron.

Experiment 3: effects of ovariectomy on neurons expressing KiSS-1 mRNA in the infundibular nucleus of young cynomolgus monkeys

Sections were selected from the hypothalami of nine intact and nine ovariectomized (OVX) young-adult, cynomolgus monkeys (*Macaca fascicularis*, 8–10 yr of age). The endocrine status of these monkeys has been described previously (5). Animal treatments were carried out in compliance with state and federal laws, standards of the Department of Health and Human Services, and the guidelines of the Institutional Animal Care and Use Committee at the Wake Forest School of Medicine and the University of Arizona. Animals were fed (*ad libitum*) a diet low in phytoestrogens and were killed 10–16 months after ovariectomy. Intact monkeys were killed in the follicular phase. Hypothalamic tissue blocks were snap-frozen and coronally sectioned in a cryostat (12 μm thickness). The sections were mounted on gelatinized slides and stored at −80 C. Hybridization histochemistry was performed on two sections from each animal matched to plate 840 of a monkey hypothalamic atlas (21). At this level, the infundibular nucleus has a distinct arcuate shape and is well delineated from the ventromedial nucleus. Quantitative computer microscopy was used to determine the number of infundibular neurons labeled with the KiSS-1 probe, the somatic size of labeled neurons, and the number of autoradiographic grains per neuron.

Experiment 4: effects of hormone replacement on neurons expressing KiSS-1 mRNA in the infundibular nucleus of young OVX cynomolgus monkeys

Sections were selected from hypothalami of 24 young-adult, female, cynomolgus macaques (5–13 yr of age). These monkeys had been OVX and 2 yr later received no treatment (OVX), continuous estrogen treatment (conjugated equine estrogen; Wyest Ayerst Laboratories, Radnor, PA), or continuous estrogen plus progesterone (conjugated equine estrogen plus medroxyprogesterone; ESI Lederle, Philadelphia, PA). The hormone treatments were administered orally in amounts calculated to be equivalent to a daily clinical dose of 0.625 mg conjugated equine estrogen or 2.5 mg medroxyprogesterone. Hormone replacement was given for a total of 30 months before animals were killed. For detailed information on the experimental design and hormone replacement regimens of these animals, see Ref. 22. Processing of hypothalamic sections was as described in experiment 3. Quantitative computer microscopy was used to analyze the number of neurons labeled with the KiSS-1 probe in the infundibular nucleus. Because of the scant numbers of neurons detected in the hormone-replaced groups, additional analysis of cell size and grain number was not performed.

Hybridization histochemistry

Both human and monkey sections were hybridized as previously described (23) with a synthetic ^{35}S 48-base radiolabeled cDNA probe complementary to bases 638–685 of the human KiSS-1 gene (GenBank accession no. NM_002256). GenBank searches showed this sequence to have no significant homology to other mammalian central nervous system genes. All slides were processed within the same hybridization procedure. After stringent washes, the slides were dried, dipped into Kodak NTB nuclear emulsion (diluted 1:1 with water), and stored in the dark at 4 C. Test slides were developed at different times to determine the optimal exposure length of 4 wk. Sections were counterstained with toluidine blue. Control sections using a radiolabeled KiSS-1 sense probe yielded no labeling in both human and monkey sections. Additional support for probe specificity was the identical pattern of labeling in the cynomolgus monkey infundibular nucleus to that previously described in rhesus monkeys using a [^{35}S]UTP-labeled cRNA KiSS-1 probe (9).

Quantitative analysis

Labeled neurons (silver grains more than five times background) were manually marked using an image-combining computer microscope equipped with a Lucivid miniature cathode ray tube, a motorized stage, and Neurolucida software (Microbrightfield, Colchester, VT). All labeled neurons within the selected sections of infundibular nucleus were marked and counted. Unbiased stereological procedures were used (Stereo Investigator software; Microbrightfield) to randomly select

approximately 40 (human) or 20 (monkey) KiSS-1 neurons in each section for measurements of cell perimeter and grain number. Images of these neurons were captured at ×60 with an Optronics camera and imported into Simple PCI software for analysis (Compix Inc., Cranberry Township, PA). The cell perimeters were manually digitized for calculations of cell area, and image analysis was used to quantify the number of autoradiographic grains per neuron. For statistical comparisons between groups, the average numbers of labeled cells per section, cell areas, or numbers of autoradiographic grains per neuron were calculated for each individual human or monkey subject. These values were used to compute group means and SE. Student's *t* tests were used to compare two groups (experiments 2 and 3). Comparisons among three groups (experiment 4) were analyzed using one-way ANOVA with a Tukey's *post hoc* test ($\alpha = 0.05$). Values are expressed as the mean ± SEM.

Results

Experiment 1: neurons expressing KiSS-1 mRNA were localized predominantly in the infundibular nucleus of the human hypothalamus

Systematic examination of serial sections through the medial hypothalamus of premenopausal and postmenopausal women revealed that KiSS-1 neurons were found predominantly in the infundibular nucleus (Fig. 1). Rare, sparsely labeled neurons were scattered elsewhere within the hypothalamic sections including the medial preoptic area, but these neurons were not grouped in discrete foci. Extensive study of medial hypothalamic sections did not reveal a focus of KiSS-1 neurons in a distribution homologous to the anteroventral periventricular (AVPV) nucleus of the rodent (16).

Qualitative inspection revealed a marked increase in the number of labeled KiSS-1 mRNA-expressing neurons in the infundibular nucleus of postmenopausal women (Fig. 1). As previously described (1, 2, 4), numerous neurons in the infundibular nucleus of postmenopausal women displayed morphological features of hypertrophy (Fig. 2), and most of these hypertrophied neurons were labeled with the KiSS-1 probe. No changes in the number or morphology of labeled KiSS-1 neurons were observed in any hypothalamic region other than the infundibular nucleus.

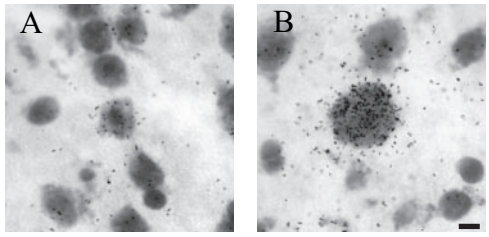

FIG. 2. Representative photomicrographs of KiSS-1 neurons in the infundibular nucleus of a premenopausal (A) and postmenopausal (B) woman. The grains mark the location of KiSS-1 mRNA, and the sections are counterstained with toluidine blue. Most of the neurons expressing KiSS-1 mRNA in the postmenopausal group were hypertrophied and associated with increased numbers of autoradiographic grains. *Scale bar*, 10 μm.

Experiment 2: hypertrophy and increased gene expression of KiSS-1 neurons in the infundibular nucleus of postmenopausal women

Quantitative analysis revealed that the average number of neurons expressing KiSS-1 mRNA in the infundibular nucleus more than doubled in the postmenopausal women (premenopausal, 159.0 ± 38.9 neurons per section, n = 8; postmenopausal, 367.9 ± 53.3 neurons per section, n = 9; $P < 0.01$; Fig. 3). The mean profile area of KiSS-1 mRNA-expressing neurons was also significantly increased in the postmenopausal infundibular nucleus (premenopausal, 221.9 ± 11.6 μm^2, n = 7; postmenopausal, 280.9 ± 17.4 μm^2, n = 7; $P < 0.05$). Finally, there was more than a 2-fold increase in the average number of autoradiographic grains per KiSS-1 neuron in the postmenopausal women (premenopausal, 41.9 ± 5.0 grains per neuron, n = 7; postmenopausal, 101.1 ± 21.9 grains per neuron, n = 7; $P < 0.05$).

Experiment 3: ovariectomy of young cynomolgus monkeys resulted in hypertrophy and increased gene expression of KiSS-1 neurons in the infundibular (arcuate) nucleus

KiSS-1 mRNA-expressing neurons were concentrated within the infundibular nucleus of intact and OVX cynomolgus monkeys (Fig. 4). Qualitative inspection revealed a marked increase in the number of labeled KiSS-1 neurons in

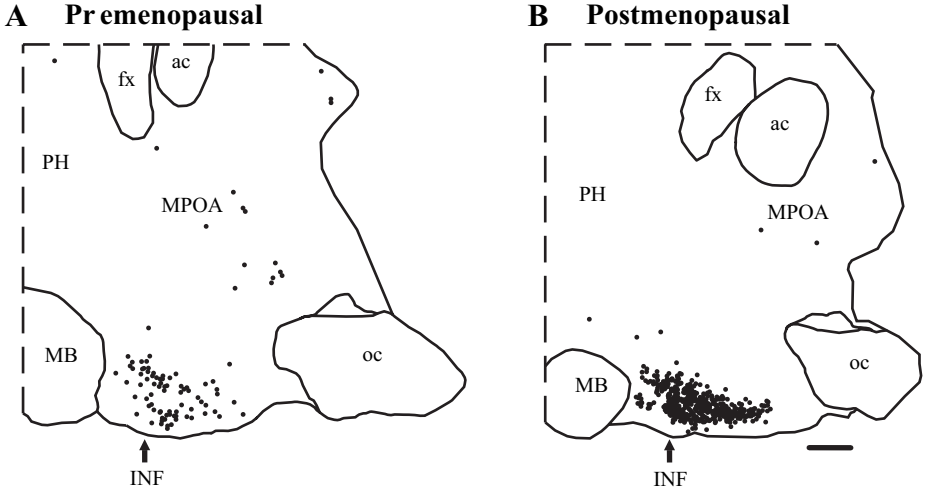

FIG. 1. Computer-assisted maps showing the distribution of neurons expressing KiSS-1 mRNA in representative sagittal sections from a premenopausal (A) and a postmenopausal (B) woman. Neurons expressing KiSS-1 gene transcripts were predominantly located in the infundibular nucleus of both groups. A marked increase in the number of neurons labeled with the KiSS-1 probe was observed in the infundibular nucleus of postmenopausal women. Each *symbol* represents one labeled neuron. The *arrow* indicates the location of the infundibular nucleus. ac, Anterior commissure; fx, fornix; INF, infundibular nucleus; MB, mammillary body; MPOA, medial preoptic area; oc, optic chiasm; PH, posterior hypothalamus. *Scale bar*, 2 mm.

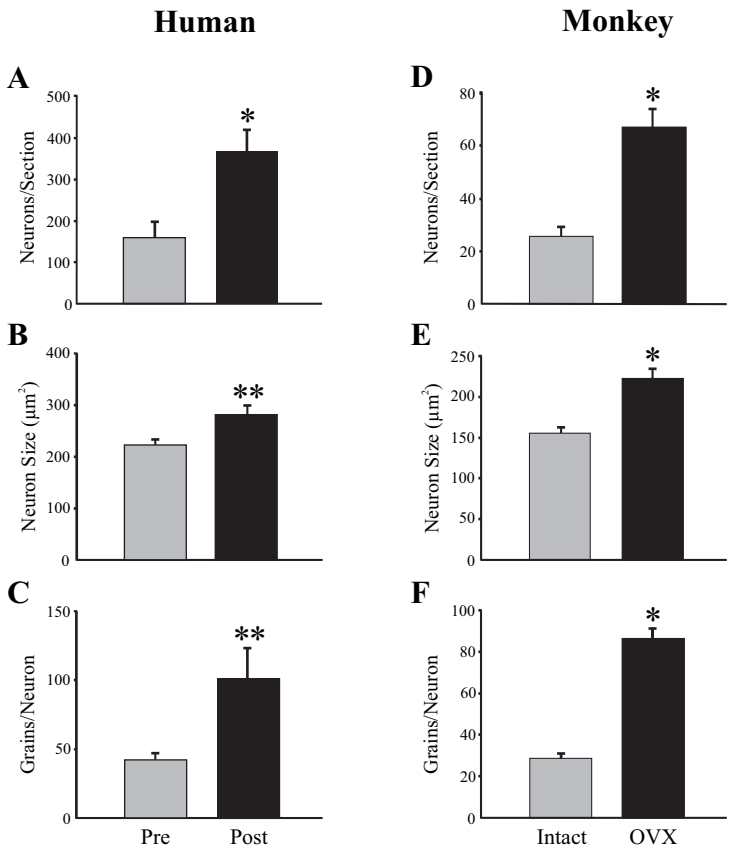

FIG. 3. Changes in neuronal morphology and KiSS-1 gene expression in the infundibular nucleus of premenopausal and postmenopausal women (A–C) or the infundibular nucleus of intact and OVX young cynomolgus monkeys (D–F). A and D, Mean number of neurons expressing KiSS-1 mRNA in sagittal human sections (A) and mean number of labeled neurons in unilateral coronal sections in the monkey (D); B and E, mean profile area (μm^2) of KiSS-1 neurons; C and F, mean number of autoradiographic grains per labeled neuron. KiSS-1 mRNA-expressing neurons in postmenopausal women were increased in size, and there were increased numbers of labeled KiSS-1 neurons and autoradiographic grains per cell. These changes were remarkably similar to those induced by ovariectomy of young cynomolgus monkeys. Values are expressed as mean ± SEM. *, Significantly different from premenopausal women (A) or intact monkeys (D–F), $P < 0.001$; **, significantly different from premenopausal women (B and C), $P < 0.05$.

the OVX group, and many of these neurons exhibited a hypertrophied morphology (Figs. 4 and 5). This observation was confirmed by quantitative analysis. The detection of neurons expressing KiSS-1 mRNA more than doubled in the infundibular nucleus of OVX monkeys (intact, 25.7 ± 3.4 neurons per section, n = 8; OVX, 66.8 ± 7.1 neurons per section, n = 8; $P < 0.001$; Fig. 3), and the mean neuronal profile area of KiSS-1 neurons was significantly increased (intact, 155.4 ± 6.9 μm^2, n = 8; OVX, 222.2 ± 11.7 μm^2, n = 8; $P < 0.001$). The average number of autoradiographic grains per KiSS-1 neuron tripled in the OVX monkeys (intact, 28.4 ± 1.8 grains per neuron, n = 8; OVX, 86.4 ± 4.8 grains per neuron, n = 8; $P < 0.001$).

Experiment 4: estrogen or estrogen plus progesterone replacement suppressed KiSS-1 gene expression to near undetectable levels in the infundibular nucleus of OVX cynomolgus monkeys

The distribution, cell number, size, and grain number of KiSS-1 mRNA-expressing neurons in the infundibular nucleus of the OVX monkeys in this experiment was similar to

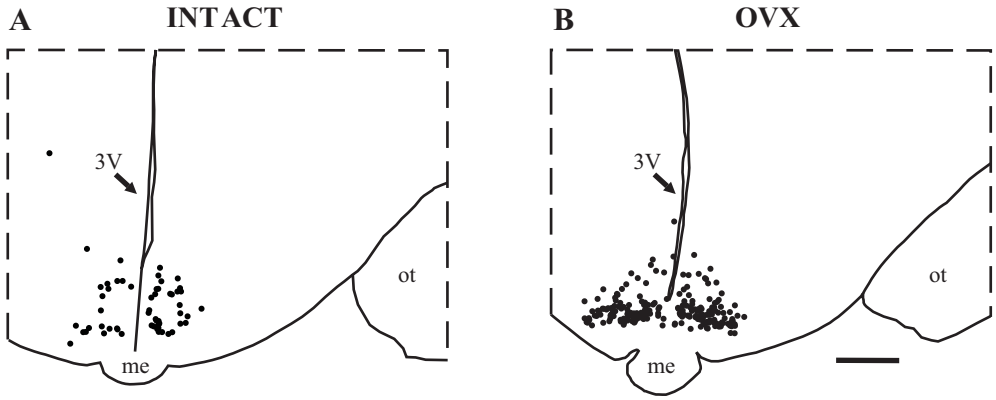

FIG. 4. Computer-assisted maps showing the distribution of neurons expressing KiSS-1 mRNA in representative coronal sections from an intact (A) and an OVX (B) monkey. The number of neurons labeled with the KiSS-1 probe was significantly increased in the infundibular nucleus of the OVX group. Each *symbol* represents one labeled neuron. me, Median eminence; ot, optic tract; 3V, third ventricle. *Scale bar*, 1 mm.

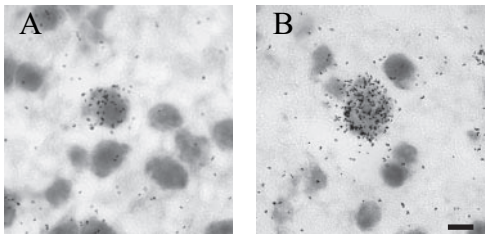

FIG. 5. Representative photomicrographs of neurons in the infundibular nucleus of intact (A) or OVX (B) cynomolgus monkeys labeled with the KiSS-1 probe. The autoradiographic grains mark the location of KiSS-1 mRNA, and the sections have been counterstained with toluidine blue. Similar to the postmenopausal women, the KiSS-1 neurons in the OVX group were hypertrophied and displayed an increased number of autoradiographic grains. *Scale bar*, 10 μm.

the OVX group described in experiment 3. In contrast, only a few neurons were detected in the OVX animals treated with estrogen or estrogen plus progesterone (OVX, 60.4 ± 18.9 neurons per section, n = 8; OVX+E, 2.1 ± 0.8 neurons per section, n = 7; OVX+EP, 2.1 ± 0.7 neurons per section, n = 7; $P < 0.001$; Fig. 6). Further analysis of cell size and grain number was not conducted because of the inadequate numbers of neurons in both of the hormone replaced groups.

Discussion

Menopause is associated with ovarian failure, gonadotropin hypersecretion, and hypertrophy of neurons within the hypothalamic infundibular nucleus. In the present study, we identify KiSS-1 gene transcripts within the hypertrophied neurons and show a marked increase in the number of labeled KiSS-1 neurons and autoradiographic grains per cell in the infundibular nucleus of postmenopausal women. Ovariectomy of young cynomolgus monkeys resulted in hypertrophy of KiSS-1 neurons and increased KiSS-1 gene expression that was remarkably similar to that seen in postmenopausal women. Conversely, estrogen or estrogen plus progesterone replacement given to OVX monkeys reduced the detection of KiSS-1 mRNA-expressing neurons in the infundibular nucleus to near undetectable levels. Our studies of cynomolgus monkeys provide strong evidence that the changes in KiSS-1 neuronal morphology and gene expression in the infundibular nucleus of postmenopausal women are secondary to loss of ovarian estrogen.

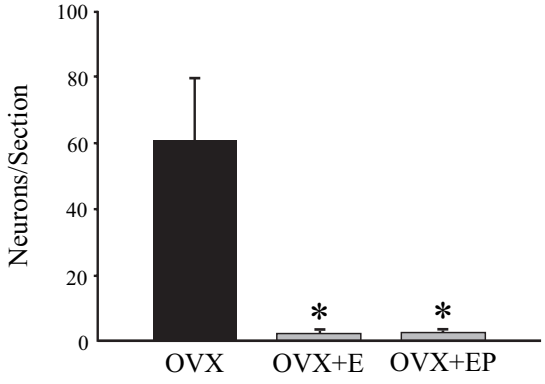

FIG. 6. Effects of estrogen or estrogen plus progesterone replacement on the number of neurons expressing KiSS-1 mRNA in unilateral coronal sections of the infundibular nucleus of young cynomolgus monkeys. The graph shows a reduction in the number of labeled KiSS-1 neurons to near undetectable levels after hormone replacement. OVX+E, OVX plus estrogen; OVX+EP, OVX plus estrogen and progesterone. Values are expressed as mean ± SEM. *, Significantly different from OVX, $P < 0.001$.

The localization of the changes in KiSS-1 gene expression to the human infundibular nucleus supports the concept that the medial basal hypothalamus (MBH) is the control center for reproduction in the primate. Classic studies in rhesus monkeys showed preservation of both the positive and negative feedback effects of estrogen after removal of all neural input to the MBH (24). Furthermore, multiunit volleys of electrical activity that are synchronized with pulses of LH in the peripheral circulation have been recorded within the monkey infundibular nucleus (25). The cellular origin of this GnRH pulse generator is not known, but the number of volleys increases after ovariectomy over a time course consistent with cellular remodeling (26), and it is profoundly inhibited by estrogen replacement (27). The importance of the MBH in human reproduction is also supported by the identification of GnRH neurons in this location (23, 28). GnRH neurons in the MBH exhibit increased levels of gene expression in postmenopausal women (29), and these changes are duplicated by ovariectomy of young cynomolgus monkeys (5). The kisspeptin receptor (GPR54) has also been identified in the monkey MBH (9), but the location of GPR54 in the human hypothalamus has not yet been described.

Examination of serial sections throughout the human hypothalamus showed the distribution of neurons expressing KiSS-1 mRNA to be relatively restricted to the infundibular nucleus. In particular, a focus of kisspeptin neurons was not identified in a distribution homologous to the AVPV of the rodent (11, 16). We cannot exclude the possibility that the failure to reveal significant numbers of kisspeptin neurons in the AVPV is due to a limitation in the sensitivity of our methods. However, using either hybridization histochemistry or immunocytochemical methods, kisspeptin neurons have not been identified in the AVPV of the ewe (17, 19). Thus, there may be limited conservation among mammalian species in the localization of kisspeptin-expressing neurons in the AVPV.

KiSS-1 mRNA was identified in the majority of the hypertrophied neurons in the infundibular nucleus of postmenopausal women. In our previous studies, we demonstrated that the majority of hypertrophied neurons expressed ERα and NKB mRNA (2, 3). Furthermore, the distribution and morphology of ERα and NKB neurons in the human infundibular nucleus was identical to the kisspeptin neurons described here (2, 3). These data provide indirect evidence that kisspeptin, NKB, and ERα are colocalized within the same subpopulation of hypothalamic neurons. Our findings are consistent with the descriptions of ERα colocalization with kisspeptin or NKB in the arcuate nucleus of other species (16, 17, 30). Moreover, estrogen suppresses both KiSS-1 (16, 19) and NKB (22, 31, 32) gene expression in the arcuate nucleus of OVX animals, and this effect is eliminated in ERα-knockout mice (16, 33). Interestingly, NKB neurons in the primate infundibular nucleus are modulated by gonadal steroids in a fashion identical to the KiSS-1 neurons. In post-

menopausal women, there is increased detection of NKB mRNA-expressing neurons, neuronal hypertrophy, and increased numbers of autoradiographic grains (3). Similarly, ovariectomy results in increased size and gene expression of NKB neurons in the infundibular nucleus of young cynomolgus monkeys (5). Finally, estrogen or estrogen plus progesterone replacement suppresses NKB gene expression to below detectable levels in OVX monkeys (22). NKB has also been implicated in the control of GnRH secretion in experimental animals (30, 34–37). Thus, kisspeptin and NKB could modulate the reproductive axis in a synergistic manner.

The present study contributes to the understanding of the neuroendocrine control mechanisms in postmenopausal women. After the menopause, there are age-related changes in the reproductive neuroendocrine axis (38), but in contrast to the complete failure of the ovaries, many aspects of hypothalamic function appear preserved. Negative feedback effects of estrogen and progesterone on gonadotropin pulses and GnRH secretion are maintained in older postmenopausal women (39). Furthermore, the quantity of GnRH secreted (estimated by competitive receptor blockade) is increased after the menopause (40), consistent with the elevated GnRH gene expression observed in the MBH of postmenopausal women (29). These data provide evidence that the neuroendocrine axis in postmenopausal women responds to removal of steroid negative feedback by increasing the secretion of GnRH from the hypothalamus. Based on the present studies of cynomolgus monkeys, the hypertrophy and elevation of KiSS-1 gene expression in postmenopausal women also appears to reflect a compensatory response to loss of ovarian steroids. Given the stimulatory effects of kisspeptin on GnRH secretion and the essential role of GPR54 in the regulation of reproduction, kisspeptin neurons in the infundibular nucleus are prime candidates for mediating estrogen negative feedback in the human. Taken together, our studies suggest that hypothalamic kisspeptin neurons play an important role in the elevation in gonadotropin secretion that occurs in response to the ovarian failure of menopause.

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