Published in final edited form as:
Peptides. 2009 January ; 30(1): 111–122. doi:10.1016/j.peptides.2008.05.016.

Menopause and the Human Hypothalamus: Evidence for the Role of Kisspeptin/Neurokinin B Neurons in the Regulation of Estrogen Negative Feedback

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Abstract

Menopause is characterized by depletion of ovarian follicles, a reduction of ovarian hormones to castrate levels and elevated levels of serum gonadotropins. Rather than degenerating, the reproductive neuroendocrine axis in postmenopausal women is intact and responds robustly to the removal of ovarian hormones. Studies in both humans and non-human primates provide evidence that the gonadotropin hypersecretion in postmenopausal women is secondary to increased gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus. In addition, menopause is accompanied by hypertrophy of neurons in the infundibular (arcuate) nucleus expressing KiSS-1, neurokinin B (NKB), substance P, dynorphin and estrogen receptor α (ERα) mRNA. Ovariectomy in experimental animals induces nearly identical findings, providing evidence that these changes are a compensatory response to ovarian failure. The anatomical site of the hypertrophied neurons, as well as the extensive data implicating kisspeptin, NKB and dynorphin in the regulation of GnRH secretion, provide compelling evidence that these neurons are part of the neural network responsible for the increased levels of serum gonadotropins in postmenopausal women. We propose that neurons expressing KiSS-1, NKB, substance P, dynorphin and ERα mRNA in the infundibular nucleus play an important role in sex-steroid feedback on gonadotropin secretion in the human.

Keywords

steroid feedback; GnRH; estrogen; progesterone; aging; reproduction; pituitary; ovary

1. Introduction

Menopause marks the cessation in reproductive cycles of middle-aged women. It is heralded by depletion of ovarian follicles leading to the loss of ovarian hormones with repercussions throughout the body. The removal of sex-steroid negative feedback results in a marked increase in serum luteinizing hormone (LH) and follicle stimulating hormone (FSH). This open-loop condition is accompanied by increased GnRH gene expression and cellular hypertrophy of a subpopulation of neurons within the human infundibular nucleus, the homologue of the arcuate

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The author has nothing to disclose

nucleus in other species [80,81]. Although postmenopausal neuronal hypertrophy was first described in 1966 [96], a major breakthrough in our understanding of this phenomenon occurred in 2007, when KiSS-1 mRNA was identified within the hypertrophied neurons [86]. To place this discovery in perspective, this article will review aging of the reproductive axis in women and the marked changes in hypothalamic morphology and gene expression in the postmenopausal human hypothalamus. As it will become apparent, the studies of kisspeptin and neurokinin B gene expression in the human hypothalamus provide considerable insight into reproductive neuroendocrine regulation in postmenopausal women.

2. Ovarian aging and the menopausal transition

Ovarian failure is the critical determinant of menopause in women. At birth, there are approximately 500,000 to 1,000,000 primordial ovarian follicles. Recent stereological studies have shown that degeneration of non-growing ovarian follicles continually accelerates from the time of birth to menopause [43]. Although it has been argued that the loss of follicles accelerates after the age of 37 [25,82], this conclusion has not been supported by mathematical modeling studies [60]. Regardless of the rate of decline, the ovary is virtually depleted of follicles after the menopause [6,60,82]. Thus, the postmenopausal phase of life is dominated by the hormonal milieu of ovarian failure.

Guidelines for the classification of stages of reproductive life are shown in Figure 1 [101]. The reproductive stage is followed by the menopausal transition, which marks the onset of irregular cycle lengths [101]. However, before the onset of irregular cycles, fertility begins to decline and ovarian hormone levels change. The first hormone alteration is an elevation in FSH in the early follicular phase, accompanied by declining levels of inhibin B [50,51,97,107]. Inhibin B, secreted by granulosa cells in small antral follicles, is a useful marker of ovarian reserve [85,103]. Because inhibin B is the dominant form of inhibin suppressing FSH in the early follicular phase [85], the early rise in FSH secretion is likely due to decreased secretion of inhibin B. Anti-Mullerian hormone is another marker of ovarian function that decreases in the late reproductive stage [38]. Thus, the decline in inhibin B and anti-Mullerian hormone are signs of ovarian follicle depletion preceding the menopausal transition. It has been proposed that hypothalamic signals could trigger the transition [111], but this hypothesis is controversial because much of it is based on a rat model that exhibits significant ovarian function after the loss of reproductive capacity [67]. While altered frequency of gonadotropin pulses can have deleterious effects upon ovarian follicle development [75], there is currently conflicting evidence on whether this mechanism could contribute to the loss of ovarian follicles in the human [39].

During the early menopausal transition, when cycles are irregular, many women exhibit elevated levels of FSH throughout the cycle and lower levels of inhibin A, inhibin B, and progesterone [37]. Serum estrogen levels of the early menopausal transition are either preserved or increased. A rise in follicular phase FSH may lead to accelerated follicular development with shortening of the length of the menstrual cycle [49]. The late menopausal transition is marked by periods of amenorrhea, frequent anovulatory cycles, and substantial variability in ovarian hormone levels and cycle lengths [37,59]. In a subgroup of perimenopausal women, anovulatory cycles are characterized by normal estrogen and an LH surge but inadequate formation of corpora lutea [106]. In others, a rise in follicular phase estrogen is seen, but this is not accompanied by an LH surge [106]. These findings and others [104] have been interpreted as indicative of insensitivity to estrogen positive feedback, but carefully controlled studies to test this hypothesis are not available [40]. In any case, the marked fluctuation of ovarian hormones in the late menopausal transition gives rise to many of the clinical symptoms of the perimenopausal period. Erratic levels of serum estrogen, rather than

the absolute level of estrogen, is a major factor in the occurrence of hot flashes in the perimenopausal phase [77].

3. The reproductive neuroendocrine axis in postmenopausal women

By the time of the postmenopausal period, the degeneration of ovarian follicles is complete and circulating estrogen and progesterone are reduced to castrate levels [4,9,66,105]. The profound deficiency in ovarian hormones results in elevated levels of serum gonadotropins due to the removal of steroid negative feedback and loss of the restraining action of inhibin on FSH secretion. In addition, ovarian failure results in a shift in the composition of the gonadotropins to acidic isoforms, retarding the clearance of LH from the peripheral circulation [94,108]. Due to the long duration of the postmenopausal phase, there may also be alterations in biological rhythms, energy homeostasis and the secretion of adrenal and thyroid hormones and growth hormone [58,100]. As chronological age advances after the menopause, there are also changes in the reproductive neuroendocrine axis. Between 50 and 80 years of age, mean levels of serum LH and FSH decline and LH pulse frequency decreases [88,91]. Decreased GnRH pulse frequency has also been documented using pulsatile gonadotropin free α-subunit pulses as a marker [41]. These studies show that after the menopausal transition, there is aging of the hypothalamic-pituitary axis independent from the loss in gonadal function.

Despite the continued aging of the central nervous system, there is compelling evidence that many aspects of reproductive neuroendocrine function remain intact after menopause. The hypothalamic/pituitary axis of postmenopausal women is capable of responding to steroid positive feedback signals with increased LH secretion [69,71]. In addition, studies using indirect pharmacological methods provide evidence that GnRH secretion is increased in postmenopausal women compared to premenopausal women [39]. Importantly, the ability of estrogen feedback to decrease GnRH secretion and gonadotropin secretion is not diminished by age [30,31]. Similarly, administration of progesterone will still suppress serum gonadotropin levels, GnRH secretion and free α-subunit pulse frequency in older postmenopausal women pretreated with estrogen [30]. Because the hypothalamus has been shown to be the major site of steroid negative feedback in the human [8,31], these studies demonstrate intact hypothalamic function in the postmenopausal period. Interestingly, the quantity of GnRH secretion continues to increase with age after the menopause [31]. Although it is not understood why GnRH secretion would increase in the postmenopausal period, this finding provides clear evidence that GnRH neurons in older women remain capable of releasing significant quantities of GnRH. Overall, these studies indicate that removal of steroid negative feedback in postmenopausal women is linked to increased GnRH secretion from the hypothalamus.

Autopsy studies of premenopausal and postmenopausal women provide additional evidence that the reproductive neuroendocrine axis in postmenopausal women responds to the removal of ovarian steroids. The content of the GnRH decapeptide is decreased in the hypothalamus of postmenopausal women [72]. A similar change is observed in young oophorectomized women [72], suggesting that the decrease in hypothalamic GnRH content in postmenopausal women is a consequence of ovarian failure and may represent decreased storage due to increased release of decapeptide into the portal circulation [110]. Moreover, GnRH mRNA is increased in the hypothalamus of postmenopausal women, as would be expected with removal of steroid negative feedback [80]. The elevation of GnRH gene expression occurs within a subpopulation of neurons scattered in the ventral preoptic region, retrochiasmatic area and the infundibular nucleus but not within the dorsal preoptic area or septal region [80]. Combined with the studies cited in the preceding paragraph, these data reinforce the concept that removal of steroid negative feedback leads to increased GnRH synthesis and secretion, leading to the gonadotropin hypersecretion characteristic of the postmenopausal state.

Experiments using non-human primates support the hypothesis that loss of steroid negative feedback in postmenopausal women leads to increased GnRH gene expression and secretion. Removal of the ovaries in young rhesus monkeys results in increased GnRH secretion into the portal capillary system [10]. Ovariectomy of young cynomolgus monkeys also results in increased GnRH gene transcripts and these changes are similar in distribution and magnitude to the changes in GnRH gene expression in postmenopausal women [90]. Conversely, in ovariectomized monkeys, estrogen replacement decreases GnRH release into the stalk-median eminence [11,64] and GnRH gene expression in the ventral hypothalamus [22,54]. Moreover, estrogen markedly suppresses the bursts of multiunit activity within the primate medial basal hypothalamus that are correlated with pulses of LH in peripheral plasma (the GnRH pulse generator) [47]. These data provide evidence that estrogen negative feedback occurs at the level of the GnRH neurons in the primate, although they do not address whether these effects on GnRH neurons are direct or indirect. They also indicate that increased GnRH gene expression in ovariectomized primates is linked with increased GnRH secretion from the hypothalamus. Taken together, these studies provide compelling evidence that the rise in hypothalamic GnRH gene expression and gonadotropin hypersecretion in postmenopausal women is secondary to ovarian failure, with withdrawal of estrogen being an important factor [80].

Similar to humans, menopause in non-human primates is accompanied by ovarian failure and gonadotropin hypersecretion [112]. However, menopause occurs very late in the lifespan of the monkey compared to the mid-life menopausal transition of humans [5]. In a recent study, GnRH secretion was compared in young and aged rhesus monkeys using push-pull perfusion [34]. The older monkeys exhibited low estrogen levels characteristic of ovarian follicle depletion and would be considered postmenopausal or within the late menopausal transition by the STRAW (Stages of Reproductive Aging Workshop) classification [101]. Remarkably, the amount of pulsatile GnRH secretion was dramatically increased in the aged monkeys, while GnRH pulse frequency was not significantly different between groups [34]. These findings correlate well with the increase in GnRH gene expression and secretion observed in postmenopausal women [31,80] and demonstrate remarkable preservation of GnRH neuronal function in the non-human primate, even in very advanced age.

4. Changes in morphology and NKB gene expression in the infundibular nucleus of postmenopausal women

More than four decades ago, Sheehan and Kovacs described pronounced differences in hypothalamic neuronal morphology between pre- and postmenopausal women [96]. The neurons were larger in postmenopausal women, in a subregion of the infundibular (arcuate) nucleus which they named the subventricular nucleus [68,96]. The enlarged neurons exhibited other signs of hypertrophy, including increased nuclear size, larger nucleoli and prominent Nissl substance. There was no evidence of increased storage material, chromatolysis, swelling or any other pathological changes that explained the change in neuron size. The hypertrophied neurons were identified in women over the age of 50 and in women with a history of post-partum hypopituitarism, but were inconspicuous in men of any age [96]. Because the neuronal hypertrophy was strongly correlated with uterine atrophy in patients with post-partum hypopituitarism, Sheehan proposed that the hypertrophy of neurons in postmenopausal women was related to loss of ovarian estrogen secretion [95].

Subsequent analysis using computer microscopy showed a 30 to 40% increase in the size of neurons in the infundibular nucleus of postmenopausal women (Fig. 2, [2,79]). These studies also demonstrated that the neuronal hypertrophy occurred in a subpopulation of neurons within the infundibular nucleus [2,79]. Stereological studies revealed no neuronal cell loss in the

infundibular nucleus of older women [2]. Thus, the neuronal hypertrophy is not a compensatory response to adjacent neuronal cell death.

The development of *in situ* hybridization allowed characterization of mRNA expression in the hypertrophied neurons of postmenopausal women. The hypertrophied neurons express ERα mRNA but do not express GnRH [79]. The increase in GnRH gene expression in postmenopausal women occurs in a separate subpopulation of neurons scattered diffusely in the ventral hypothalamus and these GnRH neurons do not exhibit changes in cell size [80]. Hybridization of hypothalamic sections with a variety of cDNA probes revealed that the majority of hypertrophied neurons express neurokinin B (NKB) and substance P (SP) gene transcripts [81]. In addition to the increase in cell size, there are increased amounts of NKB and SP mRNA per cell and a striking increase in the number of cells expressing tachykinin gene transcripts in postmenopausal women. Ovariectomy of young, cynomolgus monkeys produces NKB neuronal hypertrophy and increased gene expression that is nearly identical to that seen in postmenopausal women [90]. Conversely, the expression of NKB mRNA in the infundibular nucleus of young ovariectomized cynomolgus monkeys is markedly reduced by estrogen replacement therapy [3]. These studies strongly support the hypothesis that the hypertrophy and increased NKB gene expression in the infundibular nucleus of older women is secondary to ovarian failure.

Reciprocal changes in neuropeptide Y (NPY) and proopiomelanocortin (POMC) gene expression occurs within separate subgroups of neurons in the hypothalamus of older women [1,23]. Specifically, the number of neurons expressing POMC gene transcripts decreases in the infundibular nucleus of postmenopausal women [1] whereas the gene expression of NPY neurons increases in both the infundibular nucleus and retrochiasmatic region [24]. However, unlike the NKB and ERα mRNA expressing neurons in the infundibular nucleus, NPY and POMC neurons do not exhibit changes in cell size. Furthermore, the changes in NPY and POMC gene expression in postmenopausal women are not mimicked by ovariectomy of young cynomolgus monkeys [23,90]. Thus, not all of the changes in gene expression observed within the hypothalamus of older women can be explained by ovarian failure.

5. Evidence in animal models that NKB neurons in the infundibular/arcuate nucleus play a role in the sex-steroid feedback on gonadotropin secretion

In postmenopausal women and ovariectomized monkeys, the hypertrophy and increased gene expression of NKB/ERα neurons occurs in association with removal of ovarian steroids. These changes are accompanied by increased hypothalamic GnRH gene expression and elevated levels of serum gonadotropins consistent with removal of steroid negative feedback (see sections 3 and 4). These findings suggest that NKB neurons in the human infundibular nucleus play a role in the hypothalamic circuitry regulating steroid negative feedback [79,81]. Multiple lines of evidence in experimental animals provide support for this hypothesis. Similar to humans, virtually all the NKB neurons in the arcuate nucleus of sheep and rats colocalize ERα [7,36] and estrogen replacement suppresses NKB gene expression in rat, mouse, sheep and monkeys, indicating that this circuit is highly conserved [3,15,17,73]. ERα is essential for estrogen negative feedback [19,45] and for the suppressive effects of estrogen on NKB gene expression [17]. Arcuate NKB neurons are sexually dimorphic [12,36] and NKB gene expression varies with the rat estrous cycle [78]. Finally, LH secretion is modulated by central injections of senktide, an agonist for the NK_3 receptor (the preferential receptor for NKB). Initial studies showed a negative effect of senktide injection on LH secretion in ovariectomized rats with very low levels of exogenous estrogen [89]. However, in the ewe, central injection of senktide dramatically stimulates LH secretion (more than 15 fold) in the follicular phase, but not in the luteal phase [61]. Thus, the outcome of NK_3 receptor activation on LH secretion

depends on the hormonal milieu. Taken together, these data provide strong support for a role of NKB in the sex-steroid feedback on gonadotropin secretion.

Morphological studies in the rat suggest that arcuate NKB neurons could influence LH secretion via projections to GnRH axons in the median eminence. Tract-tracing studies show that arcuate NKB neurons project to the median eminence as well as multiple hypothalamic sites [55]. Because arcuate NKB neurons fail to take up retrograde tracer after systemic injections, these projections do not link to the portal capillary system [56]. Within the median eminence, NKB and GnRH axons are closely apposed [36,56] and NK_3 receptors are identified on GnRH axons (Fig. 3)[56]. Ultrastructural studies show that NKB varicosities are in direct contact with GnRH axons without classical synapses [12]. These data suggest that NKB may modulate GnRH secretion through non-synaptic transmission, a common mechanism of peptide signaling [62]. The convergence of GnRH axons and terminals in the median eminence represents a final coordinating site for synchronization of pulsatile GnRH secretion [65]. NKB neurons in the arcuate nucleus could provide a sex-steroid responsive input to the GnRH neuronal network via NK_3 receptors in the median eminence (Fig. 4).

NKB is extensively colocalized with dynorphin within arcuate neurons of the ewe and rat [7, 12,26]. Because dynorphin modulates progesterone's effects on pulsatile GnRH secretion [32], the colocalization of NKB and dynorphin provides additional evidence that arcuate NKB neurons participate in the reproductive axis. Immunocytochemical studies reveal close apposition of dual-labeled NKB/dynorphin terminals on NKB/dynorphin neurons in the arcuate nucleus of the rat and ewe [7,26]. In the ewe, ultrastructural examination reveals synaptic contacts at the site of closely apposing dynorphin-immunoreactive terminals and dynorphin-immunoreactive somata [27]. Because the majority of dynorphin neurons in the rat arcuate nucleus express NK_3 receptor-immunoreactivity [7], a putative synapse between dynorphin/NKB fibers and dynorphin/NKB cell bodies could be mediated through the NK_3 receptor (Fig. 4). It is not known if these inputs represent recurrent innervation or synapses between different dynorphin/NKB neurons within the arcuate nucleus. Although speculative, these connections could provide a mechanism to synchronize neuronal activity among dynorphin/NKB neurons within the arcuate nucleus and thus regulate pulsatile secretion of GnRH [7,26]

6. Kisspeptin neurons in the human hypothalamus and changes in KiSS-1 gene expression in postmenopausal women

Numerous studies have recently documented the importance of kisspeptin, the endogenous ligand of the G protein-coupled receptor 54 (GPR54), in the regulation of reproduction and the initiation of puberty [16,74,76,83,93]. Inactivating mutations of GPR54 in the human results in a failure of pubertal development with low levels of circulating gonadotropins and low serum sex hormones [16,93]. Moreover, a GPR54-activating mutation has been shown to be associated with central precocious puberty [102]. GnRH neurons in experimental animals express GPR54 mRNA [42,46] and are closely apposed by kisspeptin-immunoreactive fibers [13,48]. Exogenous administration of kisspeptin excites GnRH neurons, stimulates GnRH secretion and raises levels of LH and FSH in peripheral plasma [42,63,76,92]. The stimulatory action of kisspeptin on the reproductive axis is conserved among a wide variety of mammalian species, including humans [18,20,92].

Studies in experimental animals reveal many similarities between kisspeptin and NKB neurons in the arcuate nucleus. Like NKB neurons [81], arcuate kisspeptin neurons have been proposed to play a role in estrogen negative feedback [35]. Arcuate kisspeptin neurons express ERα [29,99] and kisspeptin (KiSS-1) gene expression is increased in the arcuate nucleus after ovariectomy [48,78,84,90,98,99]. Similarly, both KiSS-1 and NKB gene expression in the

arcuate nucleus is suppressed by estrogen replacement [3,15,48,84,98,99] and ERα is required for this effect [17,99]. Based on these findings, it seemed likely that kisspeptin and NKB would be colocalized in arcuate neurons. If this hypothesis is correct, the hypertrophied neurons in the infundibular nucleus of postmenopausal women would express KiSS-1 mRNA, in addition to NKB and ERα mRNA.

Studies were initiated in our laboratory to map the location of KiSS-1 mRNA expressing neurons in serial sections throughout the medial hypothalamus of pre- and postmenopausal women (Fig. 5). These studies showed a preferential distribution of KiSS-1 mRNA-containing neurons in the infundibular nucleus with only a few scattered KiSS-1 mRNA cell bodies in the medial preoptic area [86]. Significantly, the hypertrophied neurons in the infundibular nucleus of postmenopausal women were strongly labeled by the KiSS-1 probe, with a distribution and morphology identical to the hypertrophied NKB and ERα-containing neurons described earlier (see Section 4). Quantitative analyses revealed that the mean cross-sectional area of neurons expressing KiSS-1 mRNA increased in the infundibular nucleus of postmenopausal women, accompanied by an increase in the number of autoradiographic grains per neuron (Fig. 6). In addition, there was a marked increase in the number of neurons expressing KiSS-1 mRNA in the infundibular nucleus of postmenopausal women. Nearly identical changes in cell size and KiSS-1 gene expression occurred in young cynomolgus monkeys in response to ovariectomy (Fig. 6). Conversely, estrogen replacement of young ovariectomized cynomolgus monkeys reduced the number of KiSS-1 neurons in the infundibular nucleus to virtually undetectable levels [86]. These data provide strong evidence that the hypertrophy and increased gene expression of KISS-1 neurons in postmenopausal women are secondary to the loss of ovarian estrogen.

The changes in KiSS-1 neuronal morphology and gene expression in the human and monkey infundibular nucleus were virtually identical to those observed in NKB neurons [81,90]. Nearly 75% of the hypertrophied neurons express KiSS-1 mRNA, similar to the percentage of the hypertrophied neurons previously shown to express NKB, SP or ERα mRNA [81] providing indirect evidence that KiSS-1, NKB, SP and ERα mRNAs are colocalized within the human infundibular nucleus. Dynorphin mRNA has also been identified within the hypertrophied neurons, but the number of neurons expressing dynorphin mRNA is decreased in postmenopausal women [87]. These findings are in agreement with the colocalization of kisspeptin, NKB and dynorphin in neurons demonstrated within the arcuate nucleus of the ewe [33], and NKB, dynorphin and ERα colocalization in the arcuate nucleus of the rat and ewe [7,26]. Definitive proof of KiSS-1, NKB, SP, dynorphin and ERα colocalization in the human infundibular nucleus awaits multiple labeling studies. However, the identification of each of these mRNAs in the hypertrophied neurons provides strong evidence that this circuit is preserved among mammalian species. A future challenge will be to understand the mechanism of the differential effects of sex-steroids on neuropeptide gene expression within the human infundibular nucleus and the contribution of each of these neuropeptides to reproductive regulation.

Studies of GPR54 mutations document the essential nature of kisspeptin/GPR54 signaling in reproductive control mechanisms [16,93]. Therefore, the relatively restricted distribution of neurons expressing KiSS-1 mRNA in the infundibular (arcuate) nucleus of the human underscores the importance of this region in the regulation of the reproductive axis. They also agree with clinical studies showing a hypothalamic site of estrogen negative feedback in the human [8,31,44]. These findings are consistent with classic studies in rhesus monkeys implicating the medial basal hypothalamus as the reproductive control center. For example, surgical isolation of the arcuate nucleus, median eminence, and portions of the ventromedial nuclei and premammillary areas from the rest of the brain does not interfere with estrogen negative or positive feedback [57]. Moreover, destruction of the arcuate region in rhesus

monkeys abolishes pulsatile gonadotropin release [53]. Finally, electrodes placed in, or adjacent to, the arcuate nucleus will detect electrical activity synchronized with pulsatile release of LH (the GnRH pulse generator)[109]. The number of multiunit volleys increases after ovariectomy over a time course consistent with cellular remodeling and hypertrophy [70]. Conversely, the volleys are inhibited by estrogen replacement in ovariectomized monkeys [47], reminiscent of the dramatic inhibition of NKB and KiSS-1 gene expression by estrogen [86,90]. These data raise the intriguing possibility that the numerous KiSS-1/NKB neurons with the infundibular nucleus could contribute to the multiunit activity known as the GnRH pulse generator.

7: Summary and Conclusions

The hormonal milieu of postmenopausal women is characterized by the depletion of ovarian follicles, loss of ovarian steroid secretion and secondary gonadotropin hypersecretion from the anterior pituitary gland. Rather than showing signs of degeneration, the reproductive neuroendocrine axis in postmenopausal women responds robustly to the removal of ovarian hormones. Throughout the postmenopausal period, administration of exogenous sex steroids is still effective in reducing GnRH and LH secretion. Moreover, postmenopausal women exhibit increased hypothalamic GnRH gene expression and indirect evidence suggests that this is linked to increased GnRH secretion. These findings, combined with studies of non-human primates, provide strong evidence that the hypersecretion of gonadotropins in postmenopausal women is secondary to the increased synthesis and secretion of GnRH. In the infundibular nucleus of postmenopausal women, hypertrophy occurs in a subpopulation of neurons expressing KiSS-1, NKB, SP, dynorphin and ERα mRNA. Postmenopausal neuronal hypertrophy is accompanied by increased KiSS-1, NKB and substance P gene transcripts and decreased expression of dynorphin mRNA. Ovariectomy of young experimental animals induces nearly identical findings [28,86,90], providing evidence that the changes in GnRH, KiSS-1, NKB and dynorphin gene expression in the infundibular nucleus of postmenopausal women reflects a compensatory response to ovarian failure. Conversely, estrogen replacement reduces GnRH, KiSS-1 and NKB gene expression in ovariectomized cynomolgus monkeys [3,54,86]. Because GnRH and KiSS-1/NKB neurons in postmenopausal women exhibit changes similar to those in young monkeys after ovariectomy, these studies support the concept that reproductive hypothalamic function is preserved after menopause. The anatomical site of the hypertrophied neurons, the colocalization of ERα, as well as the extensive data implicating NKB, kisspeptin, and dynorphin in the regulation of GnRH secretion provide compelling evidence that the hypertrophied neurons are part of the neural network responsible for the increased levels of serum gonadotropins in postmenopausal women (Fig. 7).

We hypothesize that the stimulatory effects of kisspeptin and NKB, combined with a reduction in the inhibitory effects of dynorphin, ultimately results in increased GnRH gene expression and secretion in postmenopausal women. While it is well recognized that GnRH neurons are influenced by multiple converging inputs, studies of GPR54 mutations in humans [16,93] and transgenic mice emphasize the essential nature of this circuitry in the regulation of reproduction. ERα is a critical component because estrogen negative feedback on GnRH gene expression and secretion will not occur in ERα knockout mice [14,19]. Similarly, ERα is essential for the suppressive effects of estrogen on KiSS-1 and NKB gene expression in the arcuate nucleus [17,99]. GPR54 receptor signaling is required for initiation of puberty [93], basal secretion of gonadotropins [93], the postovariectomy rise in LH [21] and the stimulatory effects of kisspeptin on GnRH neurons [63]. Further studies will be necessary to determine if NKB or dynorphin signaling are also critical factors in the reproductive axis. Another challenge will be to characterize putative connections between the hypertrophied neurons and GnRH neurons in the human hypothalamus. Because there is a wealth of information showing the critical role of kisspeptin signaling in reproduction, the identification of alterations in KiSS-1

gene expression in the infundibular of postmenopausal women sheds considerable light on our understanding of human reproductive neuroendocrine regulation. These studies provide strong evidence that a subpopulation of neurons in the infundibular nucleus coexpressing kisspeptin, NKB, SP, dynorphin and ERα mediates estrogen negative feedback on GnRH secretion in the human.

Acknowledgements

This research was supported by a grant from the NIH (AG-09214). The author gratefully acknowledges the comments and support from Dr. Nathaniel McMullen, Adonna Rometo, Penny Dacks and Sally Krajewski.

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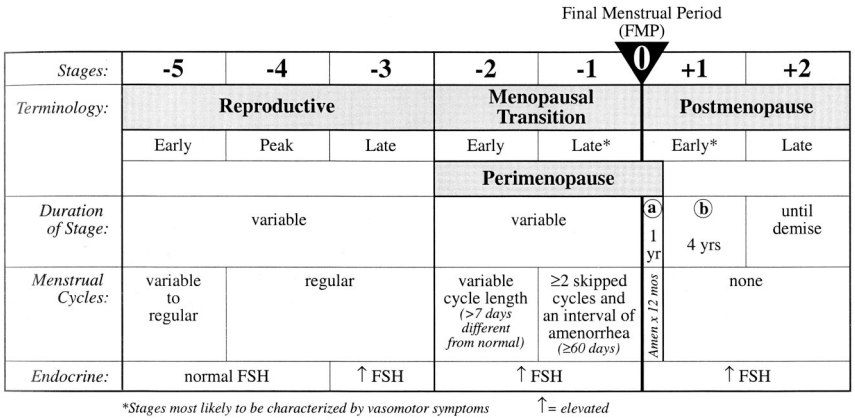

Figure 1.

The STRAW (Stages of Reproductive Aging Workshop) staging system. The menopause is defined as the time of the final menstrual period. The onset of variable cycle lengths characterizes the menopausal transition. Note that the earliest change is increased FSH secretion before cycles become irregular. This increase in FSH secretion is inversely correlated with decreased levels of inhibin B.

From Soules, et al. [101], reproduced with permission from Elsevier.

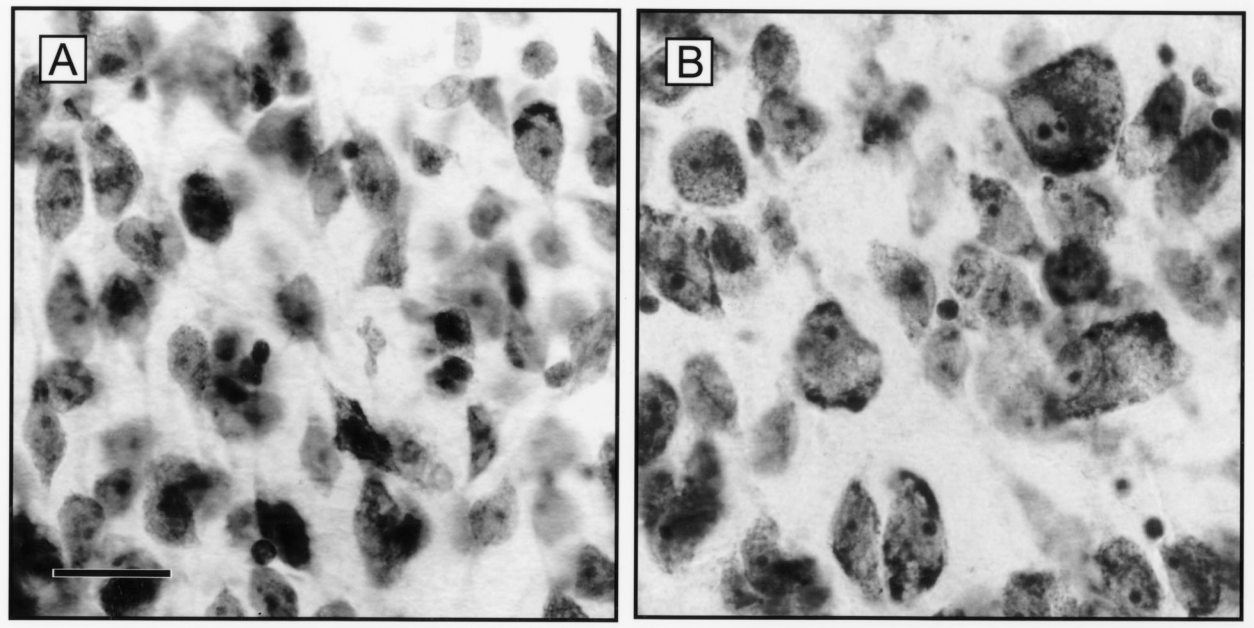

Figure 2.
Representative photomicrographs of cresyl violet-stained sections of the infundibular nucleus of young, premenopausal (**A**) and older, postmenopausal (**B**) women. Note the considerably enlarged neurons in the older subject with increased size of nuclei and nucleoli as well as increased Nissl substance. Scale bar = 25 microns in A (applies to A,B). From Abel and Rance [2], reproduced with permission from Wiley-Liss Inc.

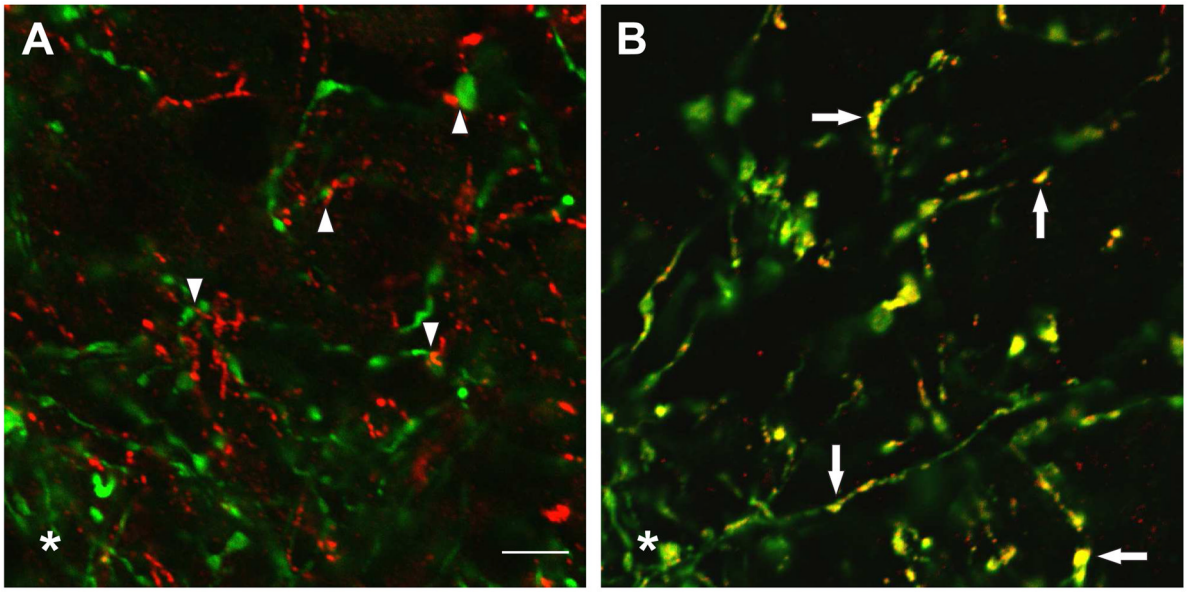

Figure 3.

Confocal microscopy of the rat median eminence. The images were captured at a single focal plane of approximately 0.80 μm in thickness. **A:** Color-combined image of GnRH (green) and proNKB (red)- immunofluorescence showing dense intermingling and multiple foci of close apposition (arrowheads). **B:** In contrast, combined images of GnRH (green) and NK_3R (red)-immunofluorescence show punctate colocalization of NK_3R on GnRH fibers (yellow, arrows). The asterisks in A and B mark the edge of the lateral palisade zone. These studies provide morphological evidence that NKB modulates GnRH secretion at the level of the rat median eminence. Scale bar = 5μm in A (applies to A, B). From Krajewski et al., [56] reproduced with permission Wiley-Liss Inc.

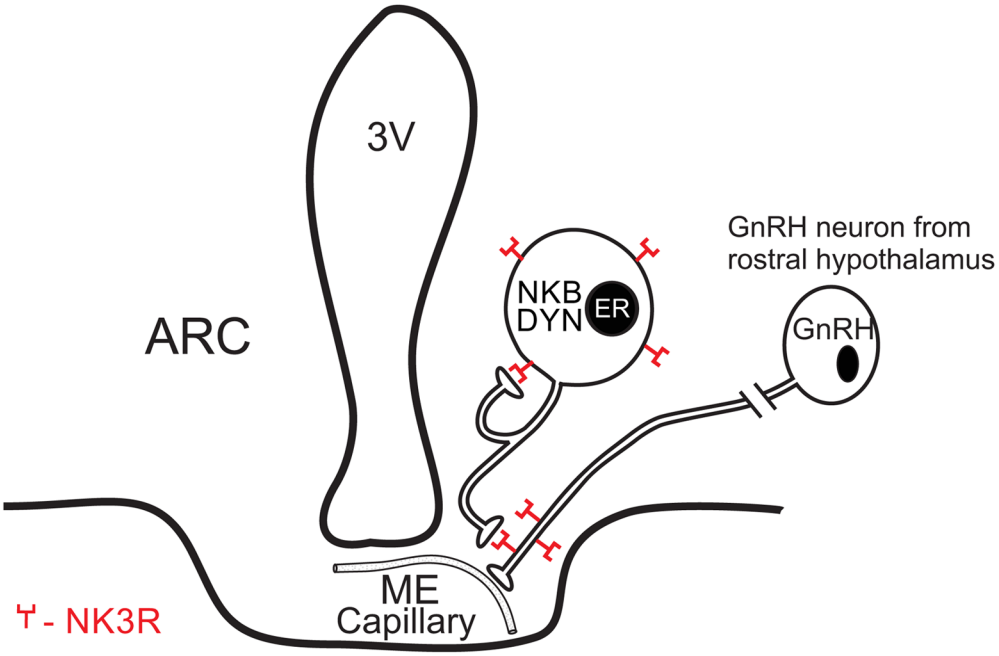

Figure 4.
Schematic diagram of the relationship between NKB (neurokinin B), Dynorphin (DYN), ER (estrogen receptor α) and GnRH (gonadotropin releasing-hormone) in the arcuate nucleus and median eminence of the rat. Although the presence of NK_3 receptors is shown as an autofeedback loop, it is not known if these connections represent recurrent collaterals or synapses between NKB/dynorphin neurons. This diagram is based on immunocytochemical and tract-tracing studies from several sources [7,55,56].

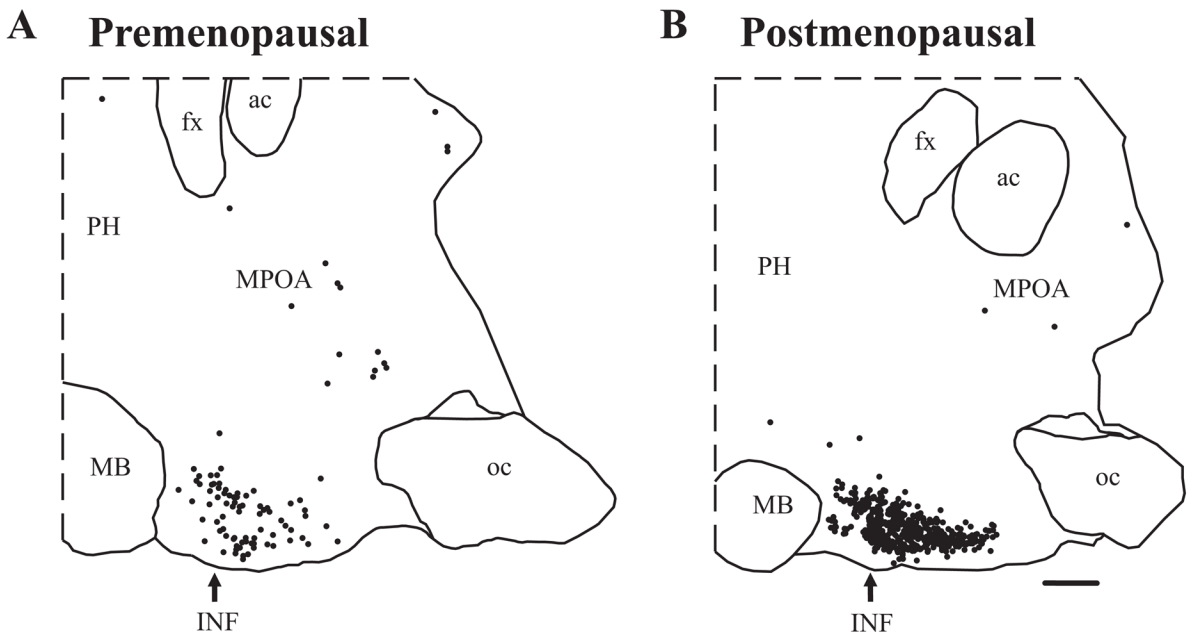

Figure 5.

Computer-assisted maps showing the distribution of neurons expressing KiSS-1 mRNA in representative parasagittal sections from a premenopausal (A) and a postmenopausal (B) woman. Each filled circle represents one labeled neuron. Neurons expressing KiSS-1 mRNA were predominantly located in the infundibular nucleus of both groups. A marked increase in the number of neurons expressing KiSS-1 mRNA was observed in the infundibular nucleus of postmenopausal women. The arrow indicates the location of the infundibular nucleus. Abbreviations: 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.

From Rometo et al. [86], reproduced with permission from The Endocrine Society.

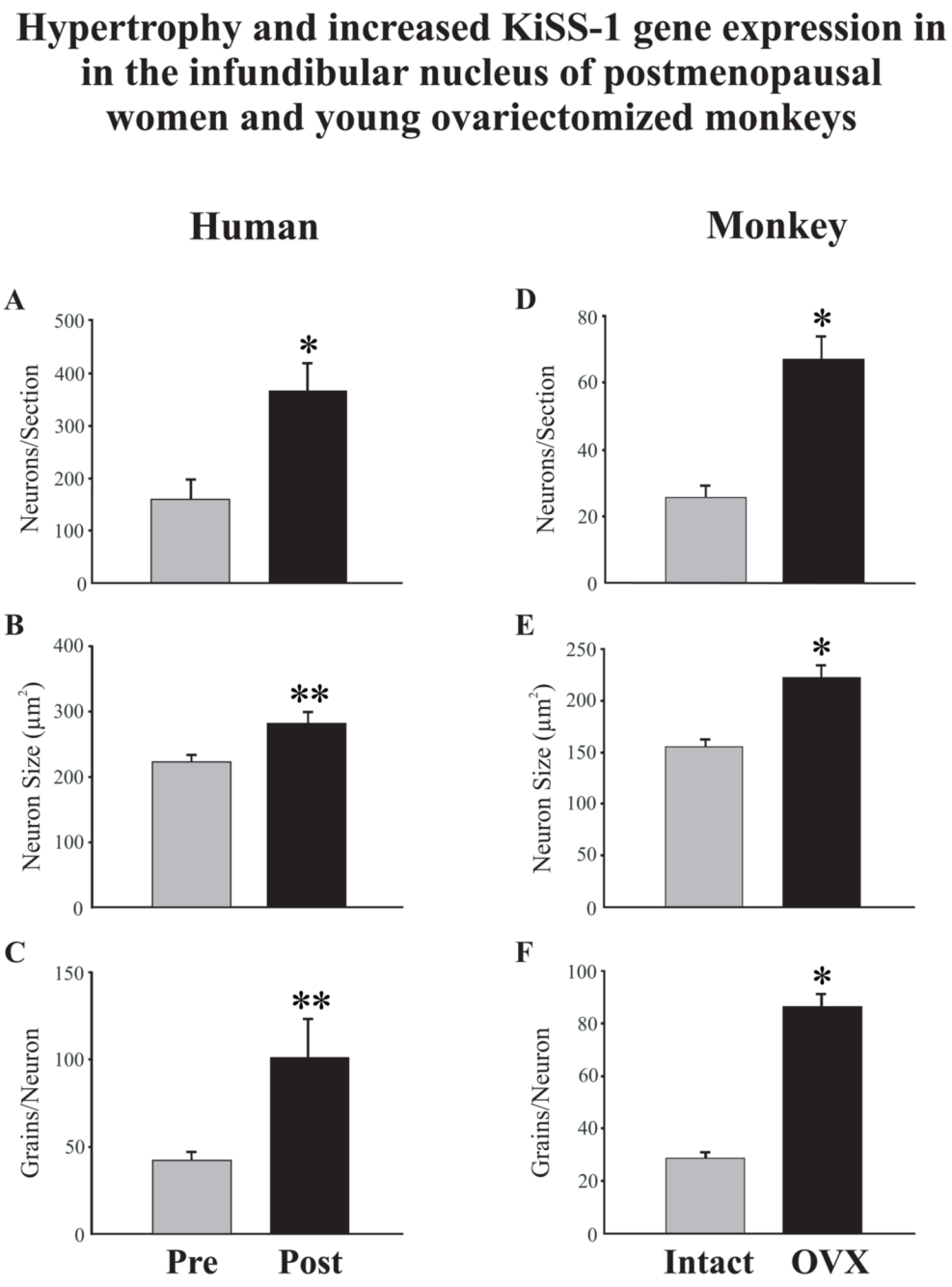

Figure 6.

Changes in neuronal morphology and KiSS-1 gene expression in the infundibular nucleus of premenopausal and postmenopausal women (A,B,C) or the infundibular nucleus of young, intact and ovariectomized cynomolgus monkeys (D, E, F). Figure 6A shows the mean number of neurons expressing KiSS-1 mRNA in sagittal human sections and 6D shows the mean number of neurons in unilateral coronal sections in the monkey. 6B and E show the mean profile area (μm^2) of KiSS-1 neurons and C and F show the mean number of autoradiographic grains for each labeled neuron. Postmenopausal women exhibited increased number, size and gene expression of KiSS-1 neurons that was similar to that seen in young, ovariectomized cynomolgus monkeys. Values are expressed as mean ± SEM. * Significantly different from

premenopausal (A) or intact (D, E, F), $p < 0.001$. ** Significantly different from premenopausal (B, C), $p < 0.05$.
From Rometo et al. [86], reproduced with permission from The Endocrine Society.

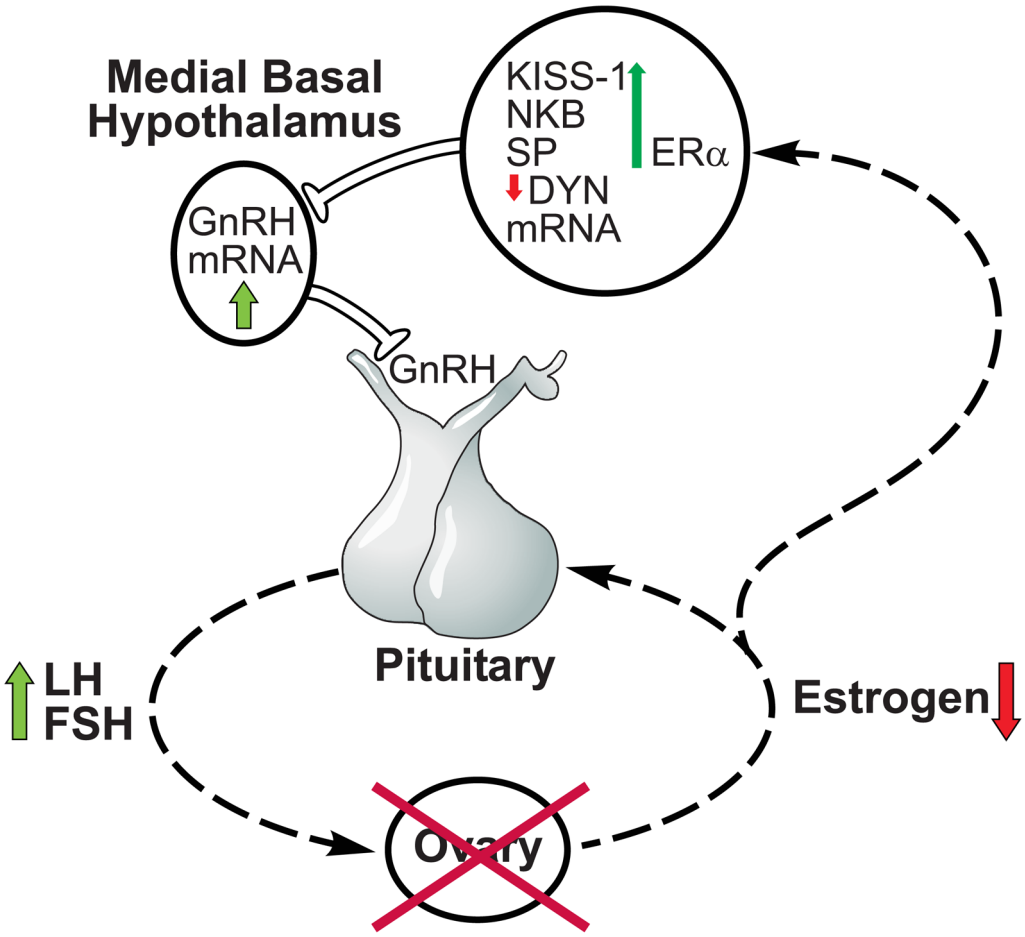

Figure 7.

Schematic diagram of neuroendocrine regulation of LH secretion in postmenopausal women. The hallmark of menopause is ovarian aging with follicle depletion resulting in castrate levels of ovarian hormones. Removal of estrogen leads to hypertrophy of a subpopulation of infundibular neurons expressing KiSS-1, NKB, substance P, dynorphin and ERα mRNA in the human infundibular nucleus. Within these neurons, there is increased expression KiSS-1, NKB and substance P gene transcripts and the decreased gene expression of dynorphin mRNA. We hypothesize that stimulatory effects of kisspeptins and NKB, combined with a reduction in the inhibitory effects of dynorphin, ultimately results in increased GnRH gene expression. Increased GnRH secretion leads to the gonadotropin hypersecretion characteristic of the postmenopausal period. These studies provide evidence that neurons expressing KiSS-1, NKB, substance P, dynorphin and ERα mRNA play a role in the regulation of steroid negative feedback in the human. See text for justification of this model.