Differential effects of aging on estrogen negative and positive feedback

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Shaw ND, Srouji SS, Histed SN, Hall JE. Differential effects of aging on estrogen negative and positive feedback. Am J Physiol Endocrinol Metab 301: E351–E355, 2011. First published May 10, 2011; doi:10.1152/ajpendo.00150.2011.—Recent studies have demonstrated an age-related decline in gonadotropins and a decrease in pituitary responsiveness to GnRH, indicating that aging influences the neuroendocrine components of the female reproductive axis independently of changes in ovarian function. To determine whether aging might also affect the luteinizing hormone (LH) negative and positive feedback responses to gonadal steroids, we administered a controlled, graded sex steroid infusion to 11 younger (45–56 yr) and nine older (70–80 yr) postmenopausal women (PMW) in whom endogenous ovarian steroids and peptides are uniformly low. The doses of estradiol (E2) and progesterone (P) were chosen to mimic levels across the normal follicular phase and have been shown previously to induce negative followed by positive feedback on LH. Similar E2 and P levels were achieved in younger and older PMW (P = 0.4 and 0.3, respectively) and produced a biphasic LH response in all subjects. The early decline in LH to 53% of baseline was not different in older vs. younger PMW. However, the positive feedback effect was attenuated in older compared with younger PMW (peak LH 144.4 ± 19.5 vs. 226.8 ± 22.3 IU/l, respectively, P = 0.01). In conclusion, these studies in PMW demonstrate preservation of short-term steroid negative and positive feedback in response to exogenous E2 and P with aging. Attenuation of positive feedback in older compared with younger PMW is consistent with previous reports of declining GnRH responsiveness with aging.

We have addressed this question in younger and older PMW, in whom endogenous estrogen and inhibin are uniformly low (4), by administering a controlled intravenous steroid infusion that mimics estradiol (E2) and progesterone (P) levels across the follicular phase and results in negative followed by positive feedback in reproductive-aged women (20, 31). This study demonstrates that, whereas short-term estrogen negative and positive feedback are retained after the menopausal transition, estrogen positive feedback is attenuated with aging.

MATERIALS AND METHODS

Subjects. Young (45–56 yr; n = 11) and old (70–80 yr; n = 9) PMW were studied. All subjects were healthy and had experienced their last menstrual period ≥18 mo previously, and therefore, they were postmenopausal according to the Stages of Reproductive Aging Workshop criteria (28). Subjects were not on any medication known to interact with the neuroendocrine reproductive axis, including over-the-counter medications or herbal remedies. Prolactin, thyroid-stimulating hormone, complete blood count, and renal function tests were normal. Electrocardiograms and mammograms were normal in all subjects, and none had any contraindications to hormone replacement therapy (HRT). Factor V Leiden mutations were absent in all patients. Subjects took 324 mg/day ferrous gluconate beginning 1 mo prior to the inpatient protocol until 1 mo after the study.

The study was approved by the Human Research Committee of the Massachusetts General Hospital, and signed informed consent was obtained from each subject before participation. The study was registered at ClinicalTrials.gov (NCT 00455741).

Experimental protocol. Subjects were admitted to the Clinical Research Center of the Massachusetts General Hospital for 5 days for a graded intravenous E2 and P infusion protocol that has previously been shown to result in negative followed by positive feedback in normal and PMW (20, 22, 31). A stepwise infusion of E2 was initiated at 1500 on the day of admission and continued for 96 h, simulating the rise in E2 that occurs over the course of the follicular phase (Fig. 1). An infusion of P was started 48 h after the onset of the E2 infusion and continued for 48 h to mimic the low concentrations of P that are present in normal cycles prior to ovulation. Three blood samples were obtained from each subject at 15-min intervals before initiation of the E2 infusion, and samples were then drawn every 4 h throughout the infusions and for 24 h after the infusions were discontinued.

Steroid infusions. The E2 infusion was constituted from a stock of crystalline E2 dissolved in propylene glycol at 17.34 mmol/l, and the P stock solution was similarly prepared with micronized P in propylene glycol at 31.8 mmol/l, as described previously (31). Both stock solutions were passed through a 0.22-μm filter. Five hundred microliters of stock solution was then mixed with 4.5 ml of 25% human serum albumin and added to 500 ml of normal saline in glass bottles. The final concentration of the E2 solution was 0.5 μg/ml; the final concentration of the P solution was 10 ng/ml. The infusions were administered using a Micro 965 Volumetric Infusion Pump (IMED, San Diego, CA) via a closed system and non-PVC fluid-path IV tubing (Accuset; IMED) that reduces steroid binding to its surface. The infusion rates were adjusted to produce E2 and P levels within the normal follicular phase range. For these studies, E2 was infused at a
The intra-assay coefficients of variation (CVs) for LH and FSH are 0.25 and 0.3, respectively. The negative feedback effect of E2 on LH was assessed by identifying the LH nadir between subjects, the area under the curve (AUC) of the LH response for each subject was calculated as the geometric area above the LH value just prior to the onset of positive feedback until the completion of the study.

Unpaired t-tests were used for comparisons between younger and older PMW. Results are expressed as means ± SE unless otherwise indicated. P < 0.05 was considered significant.

RESULTS

Baseline characteristics. Older and younger PMW were separated by age but did not differ in body weight or BMI (Table 1). The majority of subjects (n = 17) had undergone natural menopause, whereas one younger and two older subjects had undergone bilateral oophorectomy. Menopausal status was confirmed in all subjects at baseline by low E2 and elevated gonadotropins. Gonadotropins were lower in older compared with younger PMW at baseline, as seen previously (11), whereas no difference in E2 levels was seen between younger and older PMW. Seventy-three percent (8 of 11) of the younger women and 44% (4 of 9) of the older women had never taken HRT; those older women with a history of HRT had been off replacement for a median of 2.0 yr (range 1–10 yr). The younger women were a median of 5.1 yr [interquartile range (IQR) 3.3–9.5] postmenopause and 3.6 yr (IQR 1.6–5.1) postmenopause or HRT, whereas the older women were a median of 22 yr (IQR 19.4–24.1) postmenopause and 11.2 yr (IQR 8.9–13.4) postmenopause or HRT (P < 0.001 for both comparisons).

Steroid infusion. E2 levels rose within several hours of the start of the graded E2 infusion (Fig. 1), reaching a mean plateau of 351 ± 7 pg/ml (1,290 ± 25 pmol/l) from 48 to 96 h, which was not statistically different from levels observed in normally cycling women during the late follicular phase (1). There was no difference in mean E2 from 0 to 96 h between younger and older women [285 ± 18 vs. 306 ± 19 pg/ml (1,050 ± 66 vs. 1,120 ± 71 pmol/l), respectively, P = 0.4], nor was there a difference in mean E2 during the 24 h preceding the LH peak [332 ± 36 vs. 309 ± 34 pg/ml (1,220 ± 133 vs. 1,130 ± 123 pmol/l), P = 0.6]. Mean P levels during the infusion were 1.3 ± 0.2 ng/ml (4,130 ± 636 pmol/l), consistent with what is seen prior to ovulation in the late follicular phase of normal cycles (1), and did not differ between younger and older PMW [1.0 ± 0.1 vs. 0.9 ± 0.1 ng/ml (3,180 ± 318 vs. 2,860 ± 318 pmol/l), respectively, P = 0.3].

Estradiol negative feedback. In response to the E2 infusion, LH initially decreased to a lower absolute nadir of 36.3 ± 4.3

Table 1. Characteristics of younger and older postmenopausal subjects

<table>
<thead>
<tr>
<th></th>
<th>Younger (n = 11)</th>
<th>Older (n = 9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>49.9 ± 1.1</td>
<td>75.2 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.0 ± 5.6</td>
<td>67.0 ± 3.2</td>
<td>0.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.0 ± 1.3</td>
<td>26.1 ± 1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
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<tr>
<td>Estradiol, pg/ml*</td>
<td>26 ± 2.1</td>
<td>27 ± 2.3</td>
<td>0.8</td>
</tr>
<tr>
<td>LH, IU/l</td>
<td>121.9 ± 12.5</td>
<td>81.5 ± 7.8</td>
<td>0.02</td>
</tr>
<tr>
<td>FSH, IU/l</td>
<td>184.0 ± 19.4</td>
<td>122.0 ± 13.3</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. P values represent younger vs. older comparisons. Gonadotropins are expressed in IU/l as equivalents of the Second International Reference Preparation of human menopausal gonadotropins. Please see text for conversion to IU/l expressed in relation to pituitary standards for LH and FSH. *To convert pg/ml to pmol/l, multiply by 3.67.
IU/l in older compared with 58.6 ± 4.5 IU/l in younger PMW (P = 0.002; Fig. 1). However, as in previous studies, baseline LH levels were lower in older than in younger PMW, and the decrease in LH was not different when expressed as a percent decrease from baseline LH (53.2 ± 2.3 and 52.8 ± 3.0% for older and younger PMW, respectively, P = 0.9). These data suggest that aging affects baseline LH without altering sensitivity to short-term E2 negative feedback in women.

**Estrogen positive feedback.** With continued steroid infusion, LH levels increased above baseline in all subjects to levels consistent with those observed in normal women at the time of the endogenous LH surge (1). Although the time of onset of the surge was not influenced by aging (58.7 ± 6.1 vs. 64.4 ± 4.8 h in older and younger PMW, respectively, P = 0.5), peak LH was 36% lower in older compared with younger PMW (144.4 ± 19.5 vs. 226.8 ± 22.3 IU/l, respectively, P = 0.01; Figs. 1 and 2). Peak LH was not significantly different between older and younger PMW when adjusted for baseline LH (121.7 ± 30.7% increase in older vs. 114.8 ± 15.9% in younger, P = 0.8). However, the AUC for LH, which takes into account both the pattern and the peak of the LH increase and controls for differences in the absolute nadir between older and younger PMW, was reduced in older compared with younger PMW (2,885.5 ± 316.7 IU·l⁻¹·h vs. 4,544.6 ± 636.3 IU·l⁻¹·h, respectively; P = 0.03) (Fig. 2). There was no difference in the peak LH response in women with a history of HRT use compared with those without hormone exposure (P = 0.3). Taken together, these results suggest that although steroid positive feedback is retained after menopause, it is attenuated by 0.3). Taken together, these results suggest that although steroid positive feedback is retained after menopause, it is attenuated by aging.

**Discussion**

There is now ample evidence that aging of the neuroendocrine components of the reproductive axis occurs in women (12), with a decline in gonadotropin-releasing hormone (GnRH) pulse frequency (13) and pituitary responsiveness (27) but a concomitant increase in overall GnRH quantity (8). The current studies add estrogen positive feedback to this list of age-related changes. In studies in which E2 and P were carefully controlled and mimicked values achieved during normal menstrual cycles, we have now demonstrated a differential effect of aging on steroid negative and positive feedback such that the magnitude of the positive feedback LH response to ovarian steroids is attenuated with aging in women, whereas the magnitude of estrogen negative feedback is unaffected.

These studies used a validated protocol that is based on the known importance of both dose and duration of estrogen exposure for generation of positive feedback on LH (16, 40). In this protocol, recreation of physiological follicular phase E2 and P results in negative followed by positive feedback on LH in reproductive-aged women (20, 31), with peak LH levels mimicking those at the time of the endogenous LH surge in normally cycling women (1). By manipulating the time at which the estrogen infusion is stopped, previous studies have shown that the increase in LH using this infusion paradigm reflects positive feedback rather than merely a release from negative feedback (31). The biphasic LH response to this steroid infusion protocol in PMW is similar to that in reproductive-aged women, suggesting that it is a valid model with which to examine the effects of aging on estrogen negative and positive feedback.

The subjects in this study were separated by both age and time from estrogen exposure, and thus it is possible that the effect of aging may be mediated by time from estrogen exposure rather than chronological age itself. However, in a previous study demonstrating decreased pituitary responsiveness to GnRH with aging (27), we found no direct relationship between years from estrogen exposure (i.e., menopause or HRT) and pituitary responsiveness.

We have reported previously that 1 mo of estrogen administration produces decreases in mean LH (7), hypothalamic GnRH secretion (8), and direct inhibition of LH at the pituitary (26) that do not differ between younger and older PMW. Similarly, in the current study, the negative feedback response to short-term E2 administration was not influenced by aging. Interestingly, the E2 levels achieved and the percent suppression of LH were greater than in our longer-term studies in a similar subject population, suggesting that the effect of aging on estrogen negative feedback on LH is true across a range of low doses of E2. Studies of short-term estrogen negative feedback by other groups have been less consistent, demonstrating either enhanced or attenuated negative feedback with aging. However, these studies have had methodological limitations such as the inclusion of perimenopausal women in whom endogenous estrogen exposure may influence the results (33), the use of different estrogen doses in the two groups being compared (32), or the use of clomiphene citrate as an estrogen agonist (24) since clomiphene citrate may have both agonist and antagonist properties, depending on the endogenous estrogen milieu.

It is intriguing that the neurophysiological pathways responsible for generation of the LH surge in response to exogenous but physiological steroid administration appear to be intact in women into the eighth decade of life despite years without endogenous ovarian steroids. The absolute LH levels achieved in PMW were comparable with those in young reproductive-aged women during both endogenous (1) and induced (31) surges, although the percent increase relative to baseline LH levels was less due to the long-term lack of steroid negative feedback on baseline LH levels in PMW. In an observational study of regularly cycling women in their 30s to 50s, Lee at al. (19) found no change in midcycle LH profiles with aging, and...
perimenopausal women have ovulatory cycles for several years after initially entering into the menopause transition (5), consistent with the preservation of positive feedback. These findings are also consistent with the results of a large observational study in perimenopausal women in which fewer than 4% of women failed to ovulate in the face of periovulatory estrogen levels (34). Taken together, these data suggest that the decreased LH positive feedback response demonstrated in the current studies is a relatively late event in reproductive aging.

These findings in women contrast with those in other animal species, in which diminished estrogen positive feedback is a relatively early event in the process of reproductive aging. In intact middle-aged rats, the proestrous LH surge is blunted by ~40% compared with young rats prior to any overt changes in estrous cyclicity (37). The observation that these findings were replicated in ovariectomized rats treated with E2 (25, 38) suggests that this effect is independent of other changes in ovarian secretion that accompany aging in rodents analogous to the current study.

Attenuation of estrogen positive feedback in rodents is attributed to the effects of aging at the hypothalamus secondary to an imbalance between stimulatory (glutamate, norepinephrine) and inhibitory (GABA, opioids) inputs (reviewed in Refs. 3, 6, and 39), a diminished diurnal rhythm of suprachiasmatic nucleus input (17), and reduced responsiveness to kisspeptin (18). However, there is considerable evidence that estrogen positive feedback in women is mediated primarily at the pituitary, with hypothalamic GnRH secretion playing only a permissive role (14, 15, 21). Neuroanatomic studies in PMW undergoing a graded steroid infusion similar to that used in the current studies indicate that negative feedback is associated with a decrease in metabolic activity in the medial basal hypothalamus followed by increased pituitary, but not hypothalamic, activity associated with positive feedback (22), suggesting that in PMW, as in reproductive-aged women, the positive feedback effect resides primarily at the pituitary.

We have shown previously that pituitary responsiveness to GnRH is decreased with aging (27) and hypothesize that attenuation of the LH surge with aging in response to a controlled steroid infusion may be an additional manifestation of pituitary aging. The mechanisms through which aging may alter pituitary responsiveness to GnRH and estrogen positive feedback are unknown. Recent studies demonstrate a decrease in pituitary volume with aging in women (10), but it is unclear whether this translates into changes in gonadotrope number, morphometry, or function. Additional possibilities include decreased expression of estrogen receptors or changes in intracellular signaling as a function of aging.

In summary, the current studies in PMW, in which the rising physiological steroid levels of the normal follicular phase have been reproduced, now demonstrate that the neuroendocrine mechanisms responsible for estrogen feedback remain intact well after the menopause. Whereas estrogen negative feedback remains robust with aging, estrogen positive feedback is attenuated. These studies add to an increasing body of work that demonstrates that aging impacts the central components of the reproductive axis in women and provide support for differential effects of aging on the hypothalamus and pituitary.

GRANTS

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DISCLOSURES

The authors have nothing to declare.

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