Gonadotropin Hormone-Releasing Hormone Induced Luteinizing Hormone Responses in Young and Old Female C57BL/6J Mice (43651)

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Abstract. **The estrogen-induced luteinizing hormone (LH) surge is markedly attenuated in old (24 months) female mice compared with young (6 months) females. To test whether or not this attenuated LH response is due to a diminished capacity of the pituitary to respond to hypothalamic gonadotropin hormone-releasing hormone (GnRH), the pituitary response to exogenous GnRH was measured in young (5–6 months), normally cycling ($n = 6$) and old (24 months), acyclic constant diestrus ($n = 6$) C57BL/6J female mice. Mice were ovariectomized and estrogen-treated for 7 days. After intrajugular catheterization on Day 6, serial blood samples were taken at 15-min intervals for 165 min on Day 7. Serum LH was measured in samples obtained before and after infusion of either saline or GnRH (5 μg/5 μl saline/kg body wt) and 15 μg/15 μl saline/kg body wt 1 hr apart). Saline-treated animals demonstrated no LH response in either young (0.09 ± 0.02 ng/ml baseline) or old (0.11 ± 0.01 ng/ml baseline) females. However, a significant release of LH was obtained in response to each challenge of GnRH in both young (0.3 ± 0.04 ng/ml first challenge, 0.69 ± 0.1 ng/ml second challenge) and old (0.78 ± 0.1 ng/ml first challenge, 1.76 ± 0.2 ng/ml second challenge) mice. The LH response in the aged group was significantly greater ($P < 0.05$, analysis of variance) than in the young group. These results show that pituitaries of old female mice were at least as capable of responding to exogenous stimulation by GnRH as those of young. We conclude that alteration in the capacity of the pituitary to respond to GnRH is not likely to be a factor contributing to altered LH secretion with age in C57BL/6J mice.**

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An important event associated with the loss of estrous cyclicity in rodents is an alteration in the pattern of secretion of luteinizing hormone (LH) (1). In the female C57BL/6J mouse, lengthening and irregularity of estrous cycles is associated with a progressive impairment of the estrogen (E_2)-induced LH surge that occurs as early as middle age (12–14 months) in this strain. Eventually, the complete loss of estrous cycles (18–20 months) is accompanied by chronic anovulation and LH surges are absent (2, 3).

While the ovary appears to be a primary pacemaker for the loss of regular estrous cycles in rodents (4), the alteration in LH secretion is attributed, at least in part, to dysfunction of the hypothalamopituitary axis (1, 5). Pituitary LH production is dependent upon stimulation by hypothalamic gonadotropin hormone-releasing hormone (GnRH). Changes in the pituitary responsiveness to GnRH are known to occur during the normal estrous cycle in the rat (6). It has been suggested that an age-related change in the capacity of the pituitary to respond to GnRH may result in a decrease in LH release (7). The purpose of this study was to evaluate the capacity of the pituitary to respond to GnRH in young and old mice. Serum LH levels after a GnRH challenge were compared in young (5–6 months) and old (24 months) female C57BL/6J mice. The young mice were normally cycling; old mice were acyclic. Therefore, both age groups were ovariectomized (OVX) and administered tonic physiologic levels of E_2 (8).

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Method

Animals. Virgin female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were triply housed in a limited-access room restricted to aging mice. Bedding, food, and water (light:dark, 12:12-hr) were as described (9). Animal husbandry adhered to Canadian Council on Animal Care Standards and all protocols were approved by the McGill University and Royal Victoria Hospital Animal Care Utilization Committee. A sentinel program monitored parasitic, bacterial, and viral pathogens in the colony; none was detected during the period of study.

Two age groups were studied. Young mice ($n = 9$), which were 5–6 months old exhibited a minimum of three consecutive 4- to 5-day estrous cycles as evaluated by daily vaginal smears. Old mice ($n = 9$), which were 24 months old, exhibited leukocytic vaginal smears for a minimum of 12 consecutive days.

Animals were OVX and an E_2 capsule was implanted under the skin under 2,2,2-tribromoethanol anesthesia. E_2 capsules were prepared according to the method of Bronson (8) and contained estradiol 17-β mixed in Silastic adhesive (Type A, No. 891; Dow Corning Corp., Midland, MI) (0.5 mg/ml) packed in Silastic tubing (i.d. 0.04 in, o.d. 0.085 in; Dow Corning) (length 10 mm). This size of capsule produces physiologic levels of E_2 (8). On the sixth day, the mice received indwelling jugular catheters according to the method of Gibson *et al.* (10). Animals were allowed to recover for 24 hr and serial blood samples were taken the following day.

Blood Sampling. Serial blood samples were drawn at 15-min intervals for 165 min. Samples were centrifuged and plasma was stored at −70°C until assay. Blood cells were resuspended in an equal volume of charcoal-stripped, heparinized (2 units/ml) human serum albumin (11) and returned to the host via the indwelling catheter. Sampling was always performed between 13.30 and 16.30 hr. After a baseline sample, exogenous GnRH (Beckman Instruments Inc., Palo Alto, CA) was administered at 5 μg/5 μl saline/kg body wt followed by 15 μg/15 μl/kg body wt 1 hr later (12). Control animals ($n = 3$) in each age group received saline, 5 μl, followed by a second dose of 15 μl/kg body wt 1 hr later. At the end of the experiment, the animals were necropsied for the presence of pituitary tumors and uterine weights were recorded.

Radioimmunoassay. Blood samples were measured in duplicate for LH with the kit for rat LH (National Institute of Diabetes, Digestive, and Kidney Diseases) with rat LH RP-2 standard. Sensitivity of the assay was 0.1–0.4 ng/ml. The intra- and interassay coefficients of variation were 5.03% and 7.8%, respectively. Serum concentration of E_2 was determined using 16α-iodo,17β-[^{125}I]-estradiol and an antibody to E_2 (Radioimmunoassay Systems Laboratories Inc., Carson, CA). Sensitivity of the assay was 1.0 pg/ml E_2 and inter- and intra-assay coefficients of variation were 13.8% and 12.7%, respectively.

Statistical Analysis. Data are expressed as means ± SE. A significant LH response was identified according to the criteria of Saitoh *et al.* (13): (i) initial elevation of plasma LH greater than 20% of the value at 0 min, and (ii) SD of peak value during challenge test greater than twice the intra-assay coefficient of variation. LH responses to the two GnRH challenges were compared within and between age/treatment group by analysis of variance (ANOVA) and modified Bonferroni test used for post hoc comparisons using Instat version 1.13 (GraphPad Software, San Diego, CA). Differences with P-values < 0.05 were considered significant.

Results

The effects of saline and GnRH challenges on pituitary LH release in young and old females is shown in Figure 1. In the saline-treated group, there was no significant difference between the baseline serum LH levels in the young (0.09 ± 0.02 ng/ml) and old (0.11 ± 0.01 ng/ml) animals. No LH response was obtained as compared with baseline levels after saline administration in either the young (0.09 ± 0.05 ng/ml and 0.09 ± 0.04 ng/ml) or old (0.11 ± 0.02 ng/ml and 0.16 ± 0.01 ng/ml) group.

In the group challenged with GnRH, baseline levels of LH in the young (0.11 ± 0.02 ng/ml) and old (0.11 ± 0.02 ng/ml) were not significantly different. However, a significant and brisk release of LH was obtained as compared with baseline levels after each challenge of GnRH in both age groups. Peak serum LH values were observed within 15 min of challenge of both doses of GnRH in both young and old females (Fig. 1). A significant rise in serum LH was obtained in response to the first challenge of GnRH in both young (0.3 ± 0.04 ng/ml, $P < 0.01$) and old (0.78 ± 0.1 ng/ml, $P < 0.01$) animals and the LH response to the second challenge was significantly greater when compared with the first in young (0.69 ± 0.1 ng/ml, $P < 0.05$) as well as old (1.76 ± 0.2 ng/ml, $P < 0.01$) mice. The LH response to both challenges of GnRH in the aged group was significantly greater than that of the young group ($P < 0.05$ first challenge, $P < 0.01$ second challenge) (Fig. 1).

Of the six animals in the 24-month-old group, two had an identifiable pituitary tumor at postmortem examination. The baseline levels of LH in the animals with a pituitary tumor (0.12 ± 0.01 ng/ml) were not significantly different from those in healthy animals (0.1 ± 0.03 ng/ml). Similarly, the response to both challenges of GnRH in the animals with pituitary tumors did not differ significantly either from that in healthy animals. Therefore, data from these two animals were included for statistical comparisons.

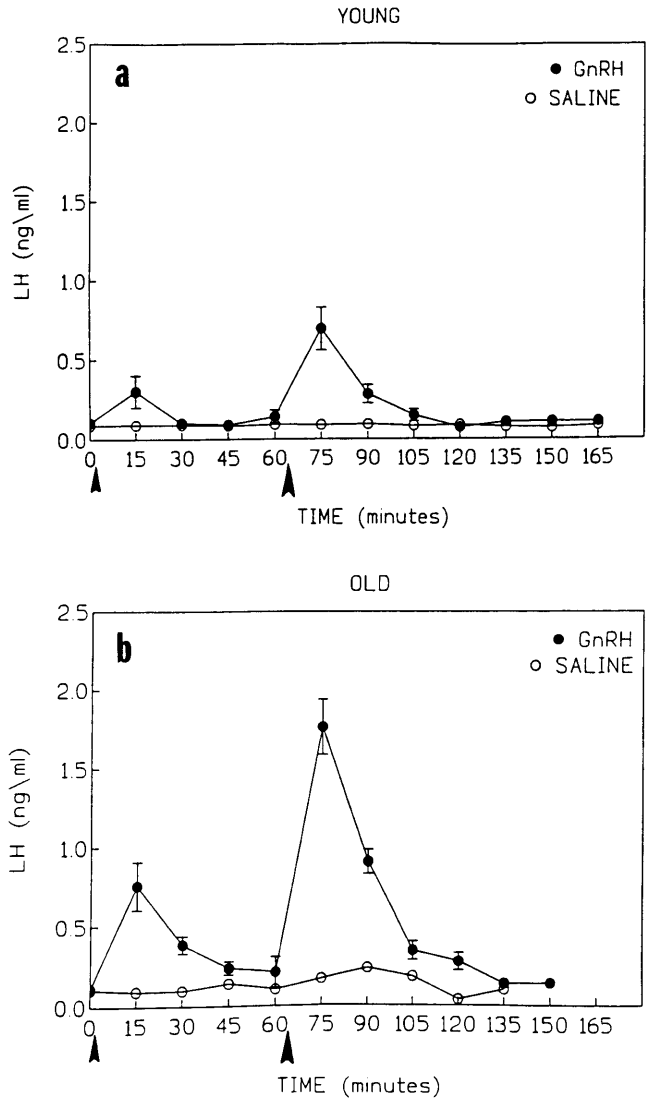

Figure 1. Effect of GnRH and saline administration on LH secretion in (a) young (5–6 months), cycling and (b) old (24 months), acyclic mice. Animals were OVX and E_2-treated for 6 days, and serial blood samples were obtained as described in the text. Animals were challenged with GnRH 5 μg/5 μl saline/kg body wt immediately after the 0-min sample followed by 15 μg/15 μl saline/kg body wt 1 hr after the 0-min sample or saline 5 μl/kg body wt followed by 15 μl/kg body wt. Each data point is mean ± SE of $n = 6$ for both young and old animals receiving GnRH and $n = 3$ for the saline controls.

The average serum E_2 levels on Day 7 in young (11.6 ± 3.3 pg/ml) and old (8.0 ± 0.9 pg/ml) mice were not significantly different. The average uterine weights also did not vary significantly between young (0.06 ± 0.04 g) and old (0.14 ± 0.03 g) mice.

Discussion

These results show that the capacity of the pituitary to respond to exogenous GnRH does not decline as a function of age in old C57BL/6J female mice since the serum LH response in old animals was of an equal or higher magnitude than that in young animals. These data also demonstrate that there is no apparent age-related difference in the time required by the pituitary to produce peak levels of LH in response to exogenous GnRH.

Previous studies have reported either no change or a decrease in the capacity of the pituitary to respond to GnRH with age. Cooper *et al.* (14) found no difference in the serum LH response to a single intracardiac injection of GnRH in anesthetized young (3–4 month), middle aged (10–11 month), and old (24–28 month) virgin female rats matched according to their endocrine status. LH response to multiple injections of GnRH through the jugular vein in anesthetized intact young rats (4–5 month) in estrus or diestrus was found to be similar to that of old retired breeder (24–28 month) constant estrus, and persistent diestrus rats by Miller and Reigle (15). Other studies have reported a diminished LH response to GnRH in older rodents. Watkins *et al.* (16) studied intact rats and observed that the LH response to GnRH was decreased in old (23–30 month) constant diestrus or constant estrus rats compared with young (4–6 month) proestrus, diestrus, or estrus rats. In the study of Watkins *et al.* (16), the blood samples were obtained by orbital sinus puncture under light ether anesthesia. Hwang *et al.* (17) have found that the response to a single injection of GnRH was reduced in OVX (3–4 weeks) or OVX (3–4 weeks) and E_2-treated old (21–24 month) as compared with young (3–4 month) similarly treated rats. In another study (18), pituitary responsiveness to GnRH was reduced in 8- to 12-month-old retired breeders in persistent diestrus compared with 3- to 4-month-old cycling proestrus females. They concluded that the variation in the response from the pituitary depends upon the antecedent steroidal milieu during the treatment period, which differed in the persistent diestrus compared with the proestrus rats. Thus, in previous studies the GnRH response of the pituitary gland of old rodents was either similar to that of younger animals or the response was diminished. In the present study, both the young and old group of mice were OVX and E_2 treated and serial blood samples were obtained from the indwelling jugular catheter in conscious, freely mobile animals. In contrast to other findings, the response to either dose of GnRH was greater in the old mice compared with the young ones.

There is no reduction in the number of pituitary cells labeled immunocytochemically for LH at the light microscopic level in old constant diestrus rats (19), suggesting that the anatomical substrate for production of LH is unimpaired in the aging rat. Furthermore, pituitary content of LH remains unaltered with age in female rats (19, 20) or mice (21). Hence, pituitary reserves of LH of old rats appear to be adequate for mounting a surge. The LH-releasing ability of pituitary cells from old rats measured *in vitro* is identical to that of pituitary cells from young females (22). It is possible

that a change in the molecular form of LH occurs with age, with consequent alteration in its clearance and half-life (23). Higher molecular weight due to increased sialation associated with decreased clearance of LH has been reported in old male rats (24). Therefore, qualitative rather than quantitative changes in LH production may be responsible for some of the age-related alterations in the LH response observed in the present study. Pituitary cells have been reported to have a reduction in the density of GnRH receptors and an impairment of the intracellular postreceptor mechanisms in old male rats (7, 25, 26). Similar alterations in aged female mice either are not present or are not sufficient to diminish the GnRH response, since the LH response was greater in old mice.

The present studies suggest that an alteration in the hypothalamic interactions between the GnRH neurons and the pathways regulating them, rather than the capacity of the pituitary to respond to GnRH, is more likely to play a role in age-related dysfunction in LH secretion associated with reproductive decline in the 24-month-old C57BL/6J female mice in constant diestrus. It remains to be established whether this alteration in hypothalamic function is intrinsic to aging or is subsequent to age-related changes in ovarian function.

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