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# Serum LH Levels Following Multiple LHRH Injections in Aging Rats[1, 2] (40084)

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Reduced reproductive function is characteristic of aging in mammalian species. Although the precise neuroendocrine mechanisms involved with age-related alterations in reproduction remain unresolved, current information suggests that age may affect several components of the hypothalamic–hypophyseal–gonadal control system.

Recent studies have shown reduced serum testosterone levels in aged male rats and humans (1, 2). Ovarian steroid production also changes in aged rats. Normal ovarian cyclicity is replaced by periods of constant estrous and repetitive pseudopregnancies (3). The effects of age on gonadal function are related to the effects of aging on gonadotropin secretion. Although the reduction in gonadal steroid production with age in humans is accompanied by increased blood gonadotropin concentrations (4, 5), there is no evidence for similar increases in gonadotropin release accompanying the reduced gonadal steroid secretion in aged rats. We recently reported decreased serum LH levels in aged male rats (6). In female rats the effect of age on serum LH levels is variable and related to the variable ovarian states of the aged rat. Although aged constant estrous rats have higher serum LH concentrations than do young cycling female rats in estrous or diestrous stages of their ovarian cycles, the LH level in the aged rats is noncyclic and is much less than that found in young rats at proestrus (7). This study also showed that aged rats with repetitive pseudopregnancies or aged anestrous rats have serum LH levels which are lower than LH levels in young rats at diestrus. Additional studies have shown that ovaries from young rats grafted into aged female rats resume the endocrine state of the recipient before the transplant (3).

These studies suggest significant age effects on pituitary gonadotropin secretion. In most reproductive states old rats have decreased serum LH levels. These low LH concentrations suggest chronically reduced pituitary LH secretion. In addition we have reported smaller increases in serum LH following intravenous LH releasing hormone (LHRH) injections in aged compared to young rats of both sexes (6, 7). The increase in serum LH following acute LHRH injection in aged rats indicates that their pituitaries are capable of secreting greater amounts of LH than they normally maintain. The long-term reduced LH secretory activity which is characteristic of the aged rat could at least partially account for our reported decrease in pituitary responsiveness to actue LHRH in these animals. The current study was designed to consider the effects of more sustained LHRH stimulation on serum LH concentration in aging laboratory rats.

*Materials and methods.* The rats used in these studies were on the Long-Evans strain. Young adult animals were 4 and 5 mo of age, the aged groups ranged from 24 to 28 mo of age. The aged females were multiparous rats obtained as retired breeders at 9 mo of age (Blue Spruce Farms, Altamont, N.Y.). The young females and both groups of males were from the same lineage and had been reared in our colony. The rats were housed in a temperature controlled (22° ± 1°) and artificially lighted room (12 hr light cycle) and maintained on Wayne Lab-Blox and water supplied *ad libitum*. Female rat experimental groups included aged females with either constant estrous or persistent diestrous (repetitive pseudopregnant) vaginal smears and young females at estrus or diestrus day 2 of their ovarian cycles. Old rats were considered to be constant estrus or persistent diestrus if they had at least 10 consec-

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utive days of cornified or leucocytic vaginal smears, respectively. All rats were under health supervision of the Michigan State University Laboratory Animal Care veterinarians. The rats were maintaining stable body weights and were free of any obvious acute or chronic disease.

Experiments were begun at 10:00 AM to minimize variation in pituitary response which could be caused by diurnal patterns of endocrine secretion. A pretreatment blood sample was taken by orbital sinus puncture under light ether anesthesia. All experimental groups (12 rats/group) received three serial intravenous injections of LHRH. Initially, 500 ng of LHRH (Eli Lily, Inc., Indianapolis, IN) was injected into the exposed jugular vein. Serial blood samples averaging about 1 ml were taken from each rat 15, 75, 90, 150 and 165 min after the first LHRH injection. Similar intravenous LHRH treatments (500 ng) were also administered at the 75 and 150 min blood sampling intervals. Serum LH was measured by double antibody radioimmunoassays (8). The antibody used in these assays was the NIAMD anti rat LH. The reference hormone was NIAMD rat LH-RP 1.

The statistical analysis of the data (Michigan State University CDC 6500 computer) included analysis of variance of the pretreatment LH concentrations and multivariant analysis of variance to determine interaction between age groups, treatments, and treatment responses.

*Results.* Figure 1 compares serum LH levels in young, day 2 diestrous and aged pseudopregnant (persistant diestrous) female rats. Serum LH concentration in the samples taken prior to LHRH administration (0, 75, and 150 min) were different in the aged compared to the young groups ($P < 0.05$). LH was higher in the young group than the aged group at 0 time (16.3 vs 8.6 ng/ml). However, in the 150 min sample, LH levels in the aged group far exceeded that of the young rats (141.0 vs 35.7 ng/ml). Serum LH concentration was increased after each LHRH injection in both age groups ($P < 0.0001$). The increase in serum LH 15 min following each injection was greater in both the young and aged groups with each successive LHRH injection ($P < 0.01$). Although the increase in serum LH following the first LHRH injection was greater ($P < 0.05$) in the young than the aged group (112 vs 64 ng/ml), the differences between pretreatment and the 15 min postinjection LH values in the young and aged groups following the second and third LHRH injection were not different.

The effects of multiple LHRH injections

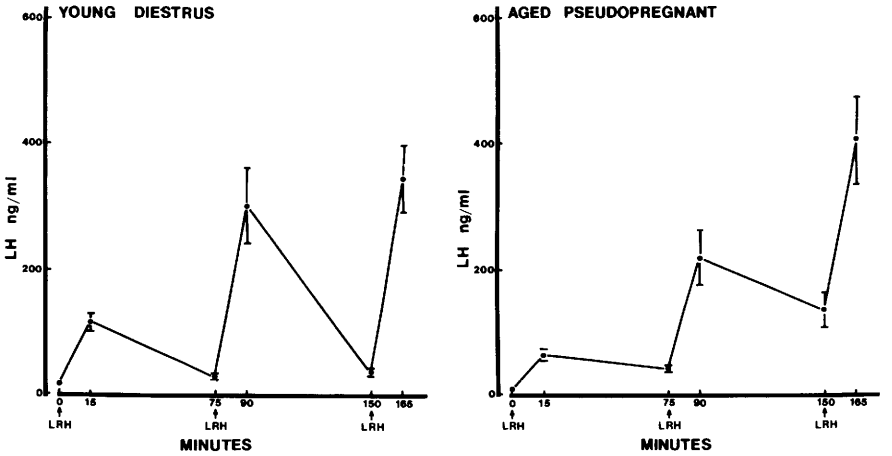


FIG. 1. The effect of serial intravenous LHRH injections on serum LH concentration in young diestrous and aged persistent diestrous female rats. ($n = 12$/group). LH is expressed as ng/ml of serum with indicated standard error of the means. Serial samples were taken under light ether anesthesia before LHRH injection (0, 75, and 150 min) and 15 min following LHRH stimulation (15, 90, and 165 min). Serum LH was higher in young than the aged group at the 15 min sample ($P < 0.05$). The increase in LH was greater after the second and third LHRH injection in both groups ($P < 0.01$). The pretreatment LH concentrations were increased prior to the second and third LHRH injections in the aged group ($P < 0.05$).

on serum LH concentrations in young estrous and aged constant estrous female rats are illustrated in Fig. 2. LH levels in the blood samples taken from the aged group before each LHRH injection (0, 75 and 150 min), increased with each sampling interval ($P < 0.01$). Mean LH concentrations increased from 13.5 to 79.1 and 207.9 ng/ml from the 0 min to the 75 min to the 150 min sampling interval. Serum LH was increased after each LHRH injection in both age groups ($P < 0.0001$). The increase in LH in the 15-min sample was greater for the young than the aged groups ($P < 0.01$). Although the increases in serum LH concentrations in the young group were similar after the second and third LHRH injection to the response after the first injection, the aged group had greater increases in LH after the second and third LHRH injection (the increase in serum LH after the first injection was 163 ng/ml; after the second, 474 ng/ml; and after the third, 479 ng/ml). In addition, the increases in LH were similar after the second and third LHRH injections in the young and aged groups.

The response of young and aged male rats to the three injections of LHRH is plotted in Fig. 3. Although the mean preinjection LH levels of the young male group were consistently higher than LH in the aged males (49.1 vs 27.0 ng/ml at 0 min; 40.1 vs 21.8 ng/ml at 75 min; and 32.6 vs 27.8 ng/ml at 150 min), these differences were not significant. Both age groups had increased serum LH after each LHRH injection ($P < 0.0001$). Although the increase in LH after the first LHRH injection was greater ($P < 0.05$) in the young than in the aged group (182.0 vs 117.1 ng/ml in the 15-min samples), the increase in LH over the preinjection hormone level was similar after the second and third LHRH injections for both age groups.

*Discussion.* These data suggest substantial differences in young and aged rat pituitary response to multiple LHRH stimulations. Levels of serum LH measured after the initial LHRH injection were uniformly lower in aged compared to young experimental groups. This finding is in agreement with our recent reports in aged rats of both sexes (6, 7). However, the results of this study show that age-related differences in serum LH are much less prominent following multiple LHRH stimulations. The marked increase in serum LH following the second and third

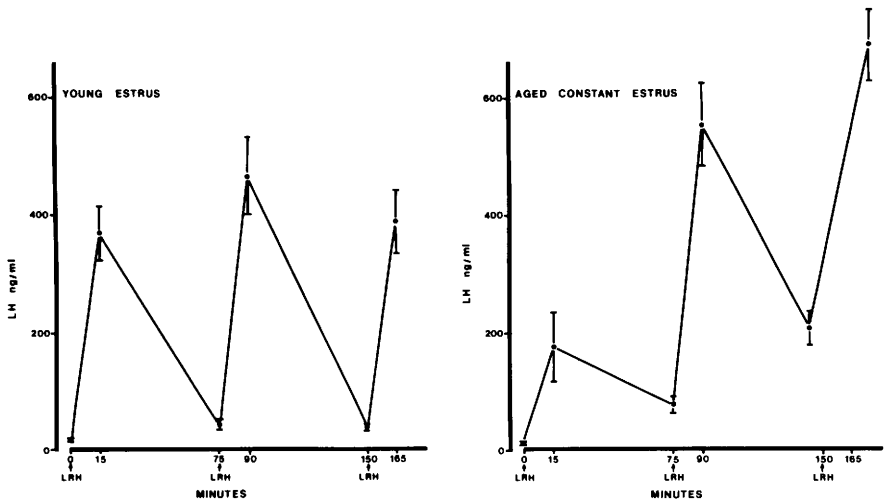


FIG. 2. The effect of serial intravenous LHRH injections on serum LH concentration in young estrous and aged constant estrous female rats ($n = 12$/group). LH is expressed as ng/ml of serum with indicated standard error of the means. Serial blood samples were taken under light ether anesthesia before LHRH injection (0, 75, and 150 min) and 15 min following LHRH stimulation (15, 90, and 165 min). Serum LH was higher in young than the aged group at the 15 min sample ($P < 0.01$). The increase in LH was greater after the second and third LHRH injections in the aged group ($P < 0.01$). The pretreatment LH concentrations were increased prior to the second and third LHRH injections in the aged group ($P < 0.01$).

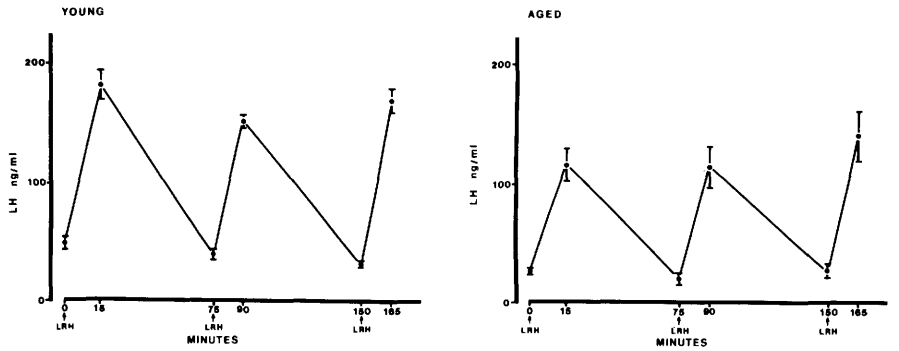


FIG. 3. The effect of serial intravenous LHRH injections on serum LH concentration in young and aged male rats ($n$ = 12/group). LH is expressed as ng/ml of serum with indicated standard error of the means. Serial blood samples were taken under light ether anesthesia before LHRH injection (0, 75 and 150 min) and 15 min following LHRH stimulation (15, 90, and 165 min). Serum LH was higher in the young than the aged group at the 15 min sample ($P < 0.05$).

LHRH treatments in the aged female rats and the young diestrus female group is similar to the accepted self-priming effects of LHRH previously shown to be involved in the proestrus LH surge. It is presumed that the increased responsiveness to LHRH involves changes in gonadal steroids which affect pituitary responsiveness to LHRH (9), to the self-priming effects of LHRH on pituitary LH secretion (10) which results in increased LH synthesis or secretory processes within pituitary gonadotrophs. The marked increase in serum LH levels in the preinjection blood samples of the aged female groups suggest either a more sustained LH release following LHRH or more likely, a slower rate of removal of the hormone from the circulation in these animals.

Although the current study indicates that the aged rat can sustain substantial serum LH levels under experimental conditions of high LHRH stimulation for limited time periods, there is ample evidence that the responsiveness of the hypothalamic–hypophyseal unit to LH controlling input is impaired in the aged rat. We and others have reported low basal serum LH levels in aged male rats (6, 9) and in aged female rats in various reproductive states (7, 11). A recent report suggests that serum LH levels during the proestrous surge are reduced in 12 mo cycling rats compared to 3–5 mo old cycling rats (12). In addition, it has been reported that pituitary concentrations of LH are smaller in aged male and female rats compared to young adult controls (13, 14). These low blood and pituitary gonadotropin concentrations have been implicated in the decrease in reproductive performance of aged rats of both sexes (1, 3, 15). We have shown decreased serum testosterone levels in aged male rats (1). If the reproductive control systems of these aged males were functionally normal, this decrease in testosterone would be detected in the hypothalamic–hypophyseal feedback system and result in increased LH secretion. Since aged male rats have increased serum testosterone after HCG injection, indicating that they are capable of responding to gonadotropin, we conclude that the reduced serum testosterone in aged male rats reflects reduced gonadotropin stimulation of the testis.

We and others have hypothesized that the most fundamental age-related alteration in the reproductive control system occurs in the neural regulation of hypothalamic hormone release (3, 5, 17). Hypothalami of aged male rats contain sufficient gonadotropin releasing activity to stimulate LH release from incubated rat pituitaries (13). Aged male and female rats have smaller increases in serum LH and FSH following orchidectomy and ovariectomy (9, 18). These findings indicate that although the reproductive control system in aged rats respond to decreased negative feedback following gonadectomy, the response is less than that which occurs in the young adult rat. The constant estrous state in aged female rats seems involved with failure of hypothalamic mechanisms responsible for the proestrous LH surge. The preoptic

area of the rat hypothalamus contains gonadotropin releasing hormone (19). Electrical stimulation of this region can cause ovulation in rats (20) and lesions of the preoptic area have been shown to reduce gonadotropin releasing hormone content in this region (21) and cause persistent vaginal cornification in young female rats (19). In addition, the preoptic area has been reported to be the site of the stimulatory effect of estrogen on gonadotropin release (22). Aged constant estrous rats have sufficient serum LH levels to stimulate minimal follicular development and estrogen secretion (23). Although electrical stimulation of the preoptic area under these conditions will stimulate ovulation (24), the hypothalamic mechanisms have apparently become less responsive to endogenous estrogen stimulation of gonadotropin release. The results of the present study show that aged rats can sustain high levels of serum LH if the hypothalamic regulation of LH release is circumvented by the injection of LHRH, suggesting that the ability of the hypothalamus to secrete gonadotropin releasing factors is impaired in the aged rat.

Although the precise mechanisms involved in the deterioration of hypothalamic function in the aged rat are not understood, a substantial amount of experimental data suggests that hypothalamic catecholamines may be involved. Hypothalamic catecholamines have been shown to influence anterior pituitary secretions presumably either by affecting the release of hypothalamic pituitary regulating hormones or by the catecholamines acting directly on the pituitary. Increased hypothalamic catecholamines have been strongly implicated in the release of pituitary LH and the inhibition of pituitary prolactin release (25). The decrease in serum LH and increase in serum prolactin we have found in the aged rat is consistent with a hypothesis of decreased hypothalamic catecholamine function. This hypothesis is supported by reports of decreased hypothalamic catecholamine content (26, 27) and reports of decreased neuronal catecholamine turnover rates in aged rats and mice (26, 28). However, a great deal more experimentation is required to understand the molecular basis for changes in hypothalamic catecholamine function in aging rats and to understand how changes in hypothalamic catecholamines may be related to alterations in hypothalamic responsiveness to multiple stimulatory and inhibitory inputs it receives.

*Summary.* The effect of three serial injections of 500 ng of LHRH on serum LH was tested in young (4–5 mo) and aged (24–28 mo) male and female Long–Evans rats. Aged female groups were rats showing constant estrous or pseudopregnant vaginal cytology (at least 10 days of cornified or leucocytic vaginal smears, respectively) which were compared to young female rats at estrous and diestrous stages of their ovarian cycle. The increase in serum LH following the first LHRH stimulation was less in the aged than in the young groups. Serum LH concentrations in the aged female group were progressively increased following the second and third LHRH injections. The increase in serum LH following the second and third LHRH injections was similar in young and aged female groups. LH concentration in blood samples collected before LHRH injection was progressively increased in both aged female groups. Although LH was greater in young than aged males after the first LHRH injection, LH concentrations after the second and third injections were similar in both ages. These results indicate that aged rat pituitaries can sustain higher serum LH concentrations than are normally found in their circulation and suggest that failure of hypothalamic hormonal stimulation of LH release contributes to the loss of reproductive function in aging. The increase in LH following multiple LHRH injections in aged female rats reflects increased pituitary responsiveness following the priming effect of the first stimulation and reduced LH clearance.

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