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Effects of Aging on LH and Prolactin after LHRH L-Dopa, Methyl-Dopa, and Stress in Male Rat[1, 2] (39246)

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Aging is associated with a progressive reduction in certain endocrine functions. Our studies indicate that age-related changes occur in hypothalamic-pituitary-adrenocortical and gonadal control systems. Aged constant estrous rats can regain cyclic ovarian activity after hypothalamic stimulation (1, 2), but L-dopa induced inhibition of prolactin release and stimulation of LH release via the hypothalamus are reduced in aged female rats (3). We have recently reported that synthetic LHRH shows less ability to increase LH release in aged female rats (4), and these and other aged mammals have shown reduced responsiveness of the ovary (5), adrenal cortex (6, 7), and testis (8) to different stimuli. The present study determined the effect of aging in the male rat on hypothalamic responsiveness to changes in catecholamine activity and to stress and anterior pituitary responsiveness to LHRH.

Materials and methods. Young adult (4-6 months) and aged (22-30 months) male Long-Evans (Blue-Spruce Farms, Altamont, N. Y.) were used in these studies. The rats were housed in a temperature controlled (72 ± 2° F) and artificially lighted (12-hr light cycle) room and maintained on Wayne Lab-Blox and water supplied *ad libitum*. Mean body weights for the young and aged rats were 432 and 468 g, respectively.

Rats included in the catecholamine and LHRH portions of the study were removed from their cages and transported to a surgery room before experimentation. A pretreatment blood sample was taken via the orbital sinus under light ether anesthesia. Separate groups of 10 rats were injected with 0.5 ml of 0.85% NaCl or 0.5 ml of 0.85% NaCl containing 15 or 150 mg of methyl dopa (Merck, Sharp and Dohme, Rahway, N. J.), or 5 or 50 mg of L-dopa (Hoffman-LaRoche, Inc., Nutley, N. J.). Rats in the LHRH experiment receiving iv injection of 0.5 ml of 0.85% NaCl or 0.5 ml of 0.85% NaCl containing 500 ng of LHRH (Eli Lily Co., Indianapolis, Indiana). Serial blood samples were taken at 15 and 45 min after LHRH injection, and 15, 30, 60, and 120 min after L-dopa or methyl dopa treatment.

The effect of acute stress of handling and serial bleeding on serum LH was assessed in separate groups of young and aged male rats. Individual rats were rapidly transferred from their cages directly into ether chambers and an orbital sinus blood sample was taken between 40 and 60 sec after initial animal disturbance (designated as the 1-min sample). The rats were re-anesthetized and additional blood samples were taken 4, 8, and 15 min after the initial animal disturbance.

Serum LH and prolactin were measured by standard double antibody radioimmunoassay procedures (9, 10). The reference hormones in these assays were NIAMD rat LH-RP-1 and NIAMD rat prolactin-RP-1. Differences between groups were analyzed by Student's *t* test, and a probability of 0.05 was considered as significant.

Results. The response of young and aged male rats to 500 ng of LHRH is plotted in Fig. 1. Serum LH in the aged control group was again lower than that of the young control group throughout the 45-min period. Serum LH levels rose sharply in both groups given 500 ng of LHRH, but the increase was

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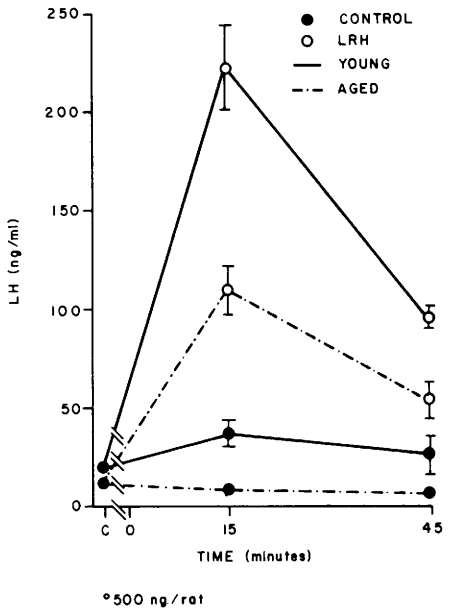

FIG. 1. Serum LH in young and aged male rats is plotted as a function of experimental interval after iv injection of saline (Control) or 500 ng of LHRH. Serial blood samples were taken before drug injection (C) and at 5, 15, and 45 min after LHRH treatment.

smaller in the aged than in the young group. Although LH levels were reduced by the 45-min sampling interval, LH levels in the young group remained greater than those in the aged group, and both of the LHRH treated groups had higher LH levels than their respective control groups.

The effects of 5 and 50 mg of L-dopa on serum LH and prolactin are shown in Fig. 2. Pretreatment serum LH levels were higher in young as compared to aged groups. Although mean concentrations of serum LH were increased in the young group, which received the 50 mg treatment, the differences were not significant. L-Dopa treatment dramatically reduced serum prolactin levels in both age groups. In the young rats, the 5-mg L-dopa dose produced a sharp reduction in serum prolactin within 15 min after drug injection. Prolactin levels in the young group given the 5-mg injection remained low at 30 and 60 min, and although prolactin was increased at 120 min, it remained significantly lower than that of the control group. Prolactin levels in the young group given 50 mg of L-dopa were similarly reduced at 15 min after drug injection and remained low throughout the 120-min sampling interval.

Serum prolactin in the aged group given the 5-mg L-dopa treatment was not decreased 15 min after drug administration. Although prolactin in the aged group given the 5-mg dose was decreased at 30 and 60 min after injection, these prolactin concentrations remained higher than those of the young group receiving similar L-dopa treatment. Prolactin levels in the aged group given the low dose of L-dopa were similar to those of the control group at 120 min after treatment. The 50-mg L-dopa treatment caused a marked reduction in serum prolactin in the aged group. Although the total magnitude of the reduction in serum prolactin was similar in young and aged groups given 50 mg of L-dopa, the effect occurred more rapidly in the young rats. The young group had a maximal reduction of serum prolactin by 15 min, but maximum prolactin suppression in the aged group was not reached until 60 min.

Figure 3 illustrates the effects of methyl dopa on serum prolactin and LH. Again the pretreatment LH levels were higher in young than in aged rats. Although the average LH levels of the young groups were decreased with increasing experimental interval time, serum LH levels did not change during the experimental interval in the aged groups. Methyl dopa resulted in increased serum prolactin by 15 min after drug injection, which was sustained throughout the treatment period. The response to 15 and 150 mg of methyl dopa was not significantly different in either age group throughout the experimental period.

Figure 4 shows the effect of acute stress on serum LH levels in groups of young and aged male rats. Groups of rats were serially exposed to ether anesthesia and blood sampling at 1, 4, 8, and 15 min after first cage disturbance. Serum LH was sharply increased by 4 min after initial animal disturbance in the young group. This increase in serum LH was sustained throughout the 15-min sampling period. On the other hand, the low initial serum LH levels were not affected by this acute stress treatment in the aged male group.

Discussion. These experiments show that there are substantial differences in the re-

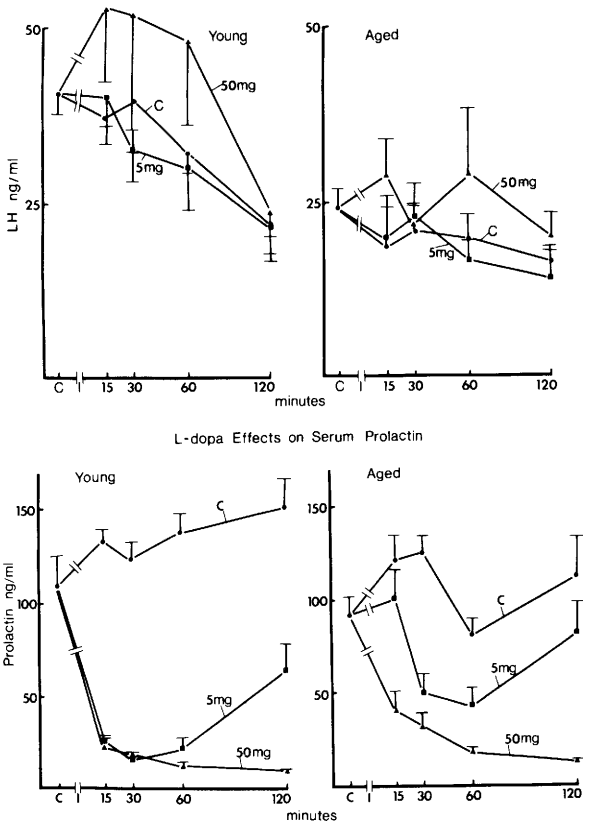

FIG. 2. Serum LH and prolactin concentration in young and aged male rats are plotted as a function of experimental interval after ip injection of saline (C), or 5 or 50 mg of L-dopa. Serial blood samples were taken before drug injection (C) and at 15, 30, 60, and 120 min after L-dopa treatment.

sponsiveness of the hypothalamic-pituitary mechanisms controlling LH and prolactin secretion in the aged as compared to the young mature male rat. Pretreatment LH levels were consistently lower in the old than in the young rats. This reduction in serum LH suggests that the reduced reproductive capability of aged male rats is at least in part related to reduced gonadal stimulation. The reduced responsiveness of the aged male rats to LHRH is in agreement with our recent report in aged female rats (4) and may at least partially explain the reduction in serum LH in these rats. This decrease in LHRH responsiveness is also supported by recent unpublished data from our laboratory indicating that LH secretion following LHRH addition to aged male rat pituitary incubates was only about half the LH secretion stimulated by similar amounts of LHRH in young male pituitary incubates. These data do not indicate whether decreased pituitary responsiveness to LHRH is due to the decrease in pituitary LH content (11), which may restrict the LH pool available for release or to other factors which reduce the effectiveness of LHRH stimulation of pituitary LH secretion, such as reduced LHRH binding or decreased activation of adenyl cyclase within aged pituitary gonadotrophs.

The inability of acute L-dopa injections to increase serum LH in the young or aged groups differs from our findings in female rats (3). This suggests the existence of a sex difference in responsiveness of the hypothalamus to L-dopa-induced stimulation of LH secretion. Recent reports indicate that hypothalamic norepinephrine rather than dopamine is the primary amine stimulating LH secretion (12, 13). The current data suggest that sex differences may alter the ability of systemic L-dopa injections to sufficiently increase hypothalamic norepineph-

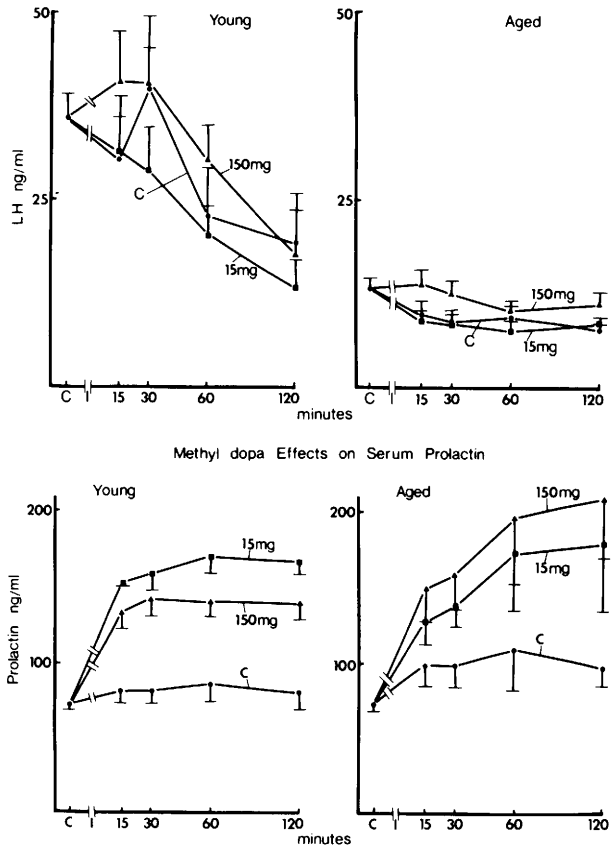

FIG. 3. Serum LH and prolactin concentrations in young and aged male rats are plotted as a function of experimental interval after ip injection of saline (C), or 15 or 150 mg of methyl dopa. Serial blood samples were taken before drug injection (C), and at 15, 30, 60, and 120 min after methyl dopa treatment.

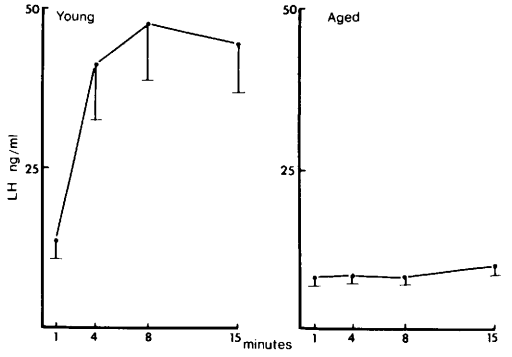

FIG. 4. Serum LH in young and aged male rats is plotted as a function of acute stress. Serial blood samples were taken from each rat at 1, 4, 8, and 15 min after initial animal disturbance.

rine levels in the time interval used to stimulate pituitary LH release.

The rapid increase in serum LH in the young rats subjected to the acute stress of handling, anesthesia, and serial blood sampling is in agreement with our recent report (14) showing that ether stress can induce LH release in male rats. The complete lack of stimulation of serum LH in the aged male rats by acute stress is consistent with the hypothesis of decreased hypothalamic-pituitary responsiveness in the aged rat. Previously, cervical stimulation of old constant estrous rats was shown not to induce LH release as in young rats (1). The decreased responsiveness of the old rats may be due in part to a deficiency in hypothalamic catecholamine activity.

The effects of systemic L-dopa and methyl dopa injections on serum prolactin are in agreement with previous reports (15, 16). Aged mice showed no differences in blood L-dopa turnover compared to young controls (17). The decreased ability of the 5-mg L-dopa treatment to lower serum prolactin

in the aged rats indicates substantial differences in the sensitivity of the hypothalamic-pituitary mechanisms controlling prolactin secretion to increased availability of this catecholamine precursor. This difference reflects either an increased capacity to secrete prolactin in aged as compared to young rats or a greater capacity to resist stimuli that would normally decrease prolactin release in the aged rats or both.

The indication that aged male rats have greater capacity to secrete prolactin is supported by the observation that serum prolactin levels in nonstressed aged male rats are greater than in young male controls. In a separate experiment serum prolactin was measured in groups of aged (n = 19) and young (n = 30) male rats within 40–60 sec after initial animal disturbance in their cages, a procedure we have previously shown to be comparable to measuring nonstressed serum hormone levels after rapid decapitation (14). Nonstressed serum prolactin levels averaged 14.4 ± 1.5 ng/ml in the young rats, and 44.0 ± 6.7 ng/ml in the aged group. For the present study both young and aged groups were removed from their cages, transported to a surgery room, and held for several minutes before beginning the experiment. The pretreatment serum prolactin levels averaged between 66 and 110 ng/ml and reflected this stress stimulation. The response to this pretreatment stress was not consistently different between young and aged male rats.

Summary. Hypothalamic-pituitary control of prolactin and LH secretion was tested in young (4–6 months) and aged (22–30 months) male Long-Evans rats given L-dopa, methyl dopa, LHRH, or stress treatments. Pretreatment serum LH levels were consistently higher in young than in the aged groups. The increase in serum LH after LHRH injection was only about half as much in aged as compared to young control males. Although acute stress caused a prompt increase in serum LH in young male rats, this treatment was without effect in the aged group. Methyl dopa treatment stimulated serum prolactin secretion in both young and old rats. Although L-dopa treatment caused a reduction in serum prolactin in both age groups, the sensitivity, magnitude, and duration of the reduction was smaller in the aged rats.

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