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Positive Feedback by Estrogen and Progesterone on LH Release in Old and Young Rats[1] (39609)

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A number of studies has indicated that the hypothalamopituitary system is less responsive to stimuli that normally release pituitary LH in old female rats that have ceased to cycle than in young cycling rats (1-3). This includes the response to electrical stimulation of the hypothalamus (4), to castration (5), to ether stress (6), and to LRH administration (7). We have reported that injections of progesterone can induce ovulation and reinitiate regular estrous cycles in old constant-estrous rats (8). Progesterone also was shown to induce release of LH within 4-7 hr in ovariectomized, estrogen-primed mature rats (8, 10), and is believed to participate with estrogen in producing the LH surge on the afternoon of proestrus in the cycling rat (11, 12). It was of interest, therefore, to compare the ability of the hypothalamopituitary system of young cycling rats and old rats showing irregular cycling or constant estrus (no ovulation) to respond to the positive feedback of estrogen followed by progesterone on LH release.

Materials and methods. Animals. One-hundred-and-fifty 8- to 9-month-old female Long-Evans rats were purchased from Blue Spruce Farms, Altamont, N.Y. The rats were housed eight per cage in a temperature-controlled (25 ± 1°) and artificially illuminated (14 hr daily) animal room, and were fed a diet of Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.) and tap water *ad libitum*. Daily vaginal smears were taken from each of the rats for 1.5 months before they were selected for experimentation. At 9 to 10 months of age, many of the rats began to show irregular estrous cycles (IRC) or constant estrus (CE). A group of IRC rats and a group of CE rats were unilaterally ovariectomized (OVX) at 10 to 11 months of age, in order to first determine the effect on compensatory hypertrophy of the remaining ovary. Ten days later, the other ovary was removed. For comparison, a group of normal-cycling female rats of the same strain were similarly OVX at 60 to 70 days of age. All OVX rats were given a single im injection of 0.2 ml of Bicillin (Wyeth Labs., Inc., Philadelphia, Penn.) postoperatively to prevent infection.

Experiment 1. At 25 days after both ovaries were removed, each rat was given a single sc injection of 8 μg of estradiol benzoate (EB) in 0.1 ml of corn oil per 100 g of body weight. Seventy-two hours later, each rat was given a single sc injection of 0.8 mg of progesterone in 0.1 ml of corn oil per 100 g of body weight. A blood sample of 1.0 ml was collected by ocular sinus puncture under light ether anesthesia immediately prior to administration of progesterone at 1100, 1500, 1700, 1900, and 2100 hr.

Experiment 2. At 52 days after removal of both ovaries, the young IRC and CE rats again were given EB, followed 72 hr later by progesterone. Sequential blood samples of 0.5-ml each were taken at 1100, 1500, 1700, and 1900 hr by cardiac puncture under light ether anesthesia. The injection of progesterone was given at 100 hr.

LH radioimmunoassay (RIA). Blood

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samples were stored in a refrigerator at 4° overnight before centrifuging at 4000*g* for 14 min in a Sorvall refrigerated centrifuge. The serum was stored in a 2-ml serum vial, capped with parafilm, and kept frozen at −20° until assayed. LH concentration in individual serum samples were determined by a double-antibody RIA using an ovine–ovine system (13). Ovine LH antiserum (#15), and purified ovine LH for radioiodination were generously provided by Dr. G. D. Niswender of Colorado State University, Fort Collins, Colo., and by Dr. L. E. Reichert, Jr., of Emory University, Atlanta, Ga., respectively. Concentrations of LH in the serum were expressed in terms of the reference standard, NIAMDD rat LH-RP-1. Only differences between groups of $P < 0.05$ were considered to be significant.

Results. Pituitary LH response to estrogen and progesterone 28 days after OVX. At 72 hr after a single injection of EB, serum concentrations of LH were 155 ± 25, 111 ± 22, and 123 ± 22 ng/ml in the OVX young, and former IRC and CE rats, respectively (Fig. 1, left panel). After administration of progesterone at 1100 hr, serum concentrations of LH in the young rats rose to 428 ± 93 ng/ml at 1500 hr and reached a peak of 1472 ± 234 ng/ml at 1700 hr. Thereafter, serum LH declined to 489 ± 94 and to 139 ± 16 ng/ml at 1900 and 2100 hr, respectively. In contrast, the former IRC rats showed only a small rise in serum LH in response to administration of progesterone, with a LH peak of 852 ± 190 ng/ml at 1700 hr. The previous CE rats showed a rise of serum LH similar to that of the young rats, reaching 1618 ± 320 ng/ml by 1700 hr.

The average body weights of the OVX young, and former IRC and CE rats were 307 ± 5, 355 ± 9, and 365 ± 11 g, respectively. Ten days after unilateral OVX, the old CE and IRC rats showed significantly less compensatory hypertrophy of the remaining ovary than in the young rats ($P < 0.05$). Since the doses of EB and progesterone were given on a per 100-g body-weight basis, the young rats received somewhat less EB and progesterone than the old rats.

Pituitary LH response to estrogen and progesterone 55 days after OVX. At the time when EB and progesterone were administered, the average body weights of the OVX young, and former IRC and CE rats were 341 ± 9, 379 ± 11, and 369 ± 9 g, respectively. The results in Fig. 1, right panel, show the patterns of serum LH responses. It can be seen that 72 hr after a single injection of EB, serum concentrations of LH were 132 ± 15 and 107 ± 24 ng/ml in the OVX young and former CE rats, respectively, whereas serum LH was only 68 ± 7 ng/ml in the former IRC rats. After injection of progesterone at 1100 hr, serum concentrations of LH in the young rats increased to 493 ± 72 ng/ml at 1500 hr, reached a peak of 2870 ± 750 ng/ml at 1700 hr, and declined thereafter to 1334 ± 598 ng/ml at 1900 hr. The previous IRG rats showed only a small LH response to progesterone increasing from 132 ± 27 to 521 ± 162 ng/ml by 1700 hr and to 647 ± 140 ng/ml by 1900 hr. Administration of progesterone induced an intermediate surge in serum LH in the previously CE rats, which increased from 221 ± 47 to 1141 ± 242 ng/ml at 1700 hr and to 1335 ± 283 ng/ml at 1900 hr. Serum LH in the old female rats rose continuously whereas in the young rats it reached a peak by 1700 hr.

Discussion. The present study demonstrates that after OVX, estrogen-primed young and old rats exhibited distinctly different patterns in release of LH 4 to 8 hr after progesterone administration. The surge in serum LH was markedly decreased in old IRC rats 28 days after OVX, and in both IRC and CE rats 55 days after OVX, as compared to that of young rats. Since we previously observed that old female rats showed a significantly smaller rise in pituitary LH release after ovariectomy than young rats (5), this together with the present results indicates that the ability to secrete LH in aging female rats is diminished both after removal of negative, or by positive feedback of ovarian hormones.

The results from the second experiment demonstrate that in the old OVX former IRC and CE rats, the LH rise after steroid treatment not only was smaller than in young rats, but also was delayed and/or prolonged. This is believed to constitute additional evidence that the LH release mechanism in old female rats is more sluggish than in young rats. This also is indicated in the

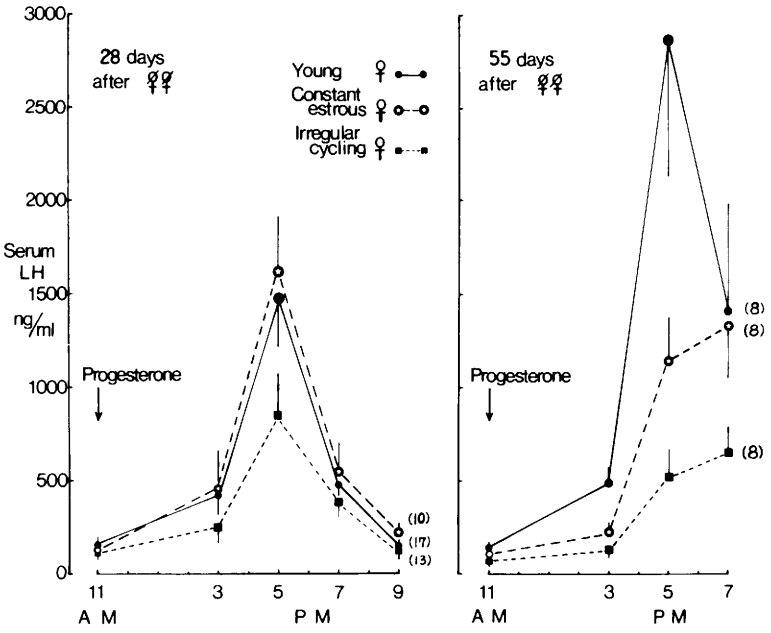

FIG. 1. Serum concentration of LH 25 or 55 days after ovariectomy (♀♀) and treatment with a single dose of estrogen (EB) followed 3 days later with a single dose of progesterone in young and old female rats. Prior to ovariectomy, the young rats showed regular estrous cycles and the old rats exhibited constant-estrus (CE) or irregular cycles (IRC). (*n*) = Number of rats per group.

significantly smaller compensatory hypertrophy of the remaining ovary after unilateral OVX in the old rats, in agreement with a previous report by Aschheim (14).

It is of interest that the OVX former CE rats showed a greater LH response to the steroid treatment than the former IRC rats, both at 28 and 55 days after OVX. It is possible that estrogen secreted by the old CE rats sensitized the hypothalamopituitary system to subsequent estrogen-progesterone stimulation. Aging CE rats have well-developed follicles in the ovaries and show persistent vaginal cornification, suggesting unhindered estrogen action, whereas IRC rats presumably are secreting progesterone as well as estrogen, perhaps thereby rendering the hypothalamopituitary system less sensitive to positive feedback by steroid hormones. It is remarkable that these differences between the two groups of old rats persisted so long after OVX.

The present results suggest that the reduced LH response to the gonadal steroids in the old as compared to the young OVX rats does not depend entirely on the previous ovarian influence. We have observed similar differences in the LH response to castration alone in young and old male and female rats (5). We have suggested that a fundamental change in the hypothalamopituitary system occurs in aging rats, and believe this is associated at least in part with alterations in hypothalamic monoaminergic activity (1, 15). Preliminary evidence indicates that in old rats, hypothalamic catecholamines are reduced and serotonin is increased (16). Catecholamines, particularly norepinephrine, are believed to stimulate and serotonin to inhibit gonadotropin release in rats (16). In addition, pituitary LH release in response to LRH stimulation was shown to be reduced in old as compared to young rats (7). Thus, several mechanisms operate to reduce gonadotropin release in response to many stimuli in old as compared to young rats.

Summary. Young cycling rats 60 to 70 days of age, and old irregular-cycling or constant-estrous rats 10 to 11 months of age were ovariectomized. At 25 or 52 days after ovariectomy, they were each given a single

injection of 8 μg of estradiol benzoate/100 g of body weight, followed 72 hr later by a single injection of 0.8 mg of progesterone/100 g of body weight. Radioimmunoassay of serum LH from blood samples removed 4, 6, 8, and 10 hr after progesterone administration showed that LH rose significantly more in the young than in the old, former constant-estrous rats 25 days after ovariectomy, and more than in either group of old rats at 55 days after ovariectomy. These results indicate that the positive feedback of estrogen and progesterone on LH release is reduced in old as compared to young cycling female rats, and is believed to account at least in part for the irregular cycling, and failure of ovulation in the old constant-estrous rats.

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