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Relation of Aging to Hypothalamic LHRH Content and Serum Gonadal Steroids in Female Rats (40530)[1]

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Aging female rats progress from regular to irregular estrous cycles, then to a constant estrous (CE) or a pseudopregnant-like (PP) condition, and finally to an anestrous (AN) state (1). Old CE, PP, and AN rats show no cyclic variation in serum LH and FSH levels, although basal LH levels in old CE and PP rats are similar to basal values in young cycling rats (2, 3). Old AN rats have undetectable serum levels of LH although FSH levels are not different than in young diestrous rats (2, 3). All three categories of old rats also exhibit no cyclic variations in serum estradiol or progesterone (3).

Old CE and PP rats respond normally or show only a slight reduction in LH release in response to exogenous LHRH administration (4, 5), but a greatly reduced gonadotropic response to the positive feedback action of ovarian steroids when compared to young female rats (4, 6). Since a reduction in hypothalamic LHRH synthesis and/or release could explain the decrease in the positive feedback response and the lack of cyclic LH surges, the present study was undertaken to measure LHRH in the anterior, medial basal, and posterior hypothalamus of old CE, PP, and AN rats and in young cycling female rats. Serum estradiol and progesterone also were assayed because of their possible influence on hypothalamic LHRH content (7).

Materials and methods. Multiparous female Long–Evans rats, 10–12 months old, were purchased from Blue Spruce Farms (Altamont, N.Y.) and housed in stainless-steel cages in an air-conditioned, temperature-controlled (24° ± 2°) room. Light was provided daily from 0600–2000 hr by fluorescent lamps, and the animals were fed a diet of Purina Lab. Chow (Ralston Purina Co., St. Louis, Mo.) and tap water *ad libitum.* When the animals reached 20–24 months of age, daily vaginal smears were collected. Animals that exhibited vaginal cornification continuously for 20 days or more were considered to be in CE, those that showed diestrous periods (predominantly leukocytic smears) for 10–30 days interspersed with 1–2 days of estrus or proestrus were judged to be PP, and rats that showed no cyclic activity and only vaginal leukocytes were considered to be AN. Four-to-five-month-old 4-day cycling rats were used as controls (C). Animals that became diseased were not included in this study.

Young and old rats were decapitated between 1000 and 1100 hr and trunk blood was collected for steroid assays. An additional group of young proestrous (PE) rats was killed between 1600 and 1700 hr. The brains were quickly removed and frozen on dry ice. The frozen brains were then sectioned into the regions described by Araki *et al.* (8). The anterior hypothalamus (AH) consisted of a tissue section 5-mm deep, extending from the rostral border to the caudal border of the optic chiasm and laterally to the lateral hypothalamic sulci. The medial basal hypothalamus (MBH) consisted of a tissue block extending from the caudal border of the optic chiasm to the caudal end of the pituitary stalk and laterally to the hypothalamic sulci. The posterior hypothalamus (PH) consisted of the block of tissue extending from the caudal limit of the pituitary stalk to the rostral margin of the mammillary body and laterally to the lateral hypothalamic sulci. These hypothalamic sections were sonified in 1 ml of 0.1 *N* HCl, the homogenates were neutralized with 1 ml of 0.1 *N* NaOH, and after centrifugation at 2000*g* for 30 min, the supernatants were diluted with pH 7 phosphate-buffered saline to concentrations suitable for LHRH

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radioimmunoassay (RIA).

Serum estradiol and progesterone were assayed in duplicate at two dilutions as previously described (3). Antiestradiol-6-BSA, GND No. 244, and anti-progesterone-11-BSA, GDN No. 337, were kindly provided by Dr. G. D. Niswender (Colorado State Univ., Ft. Collins, Co.). Hypothalamic LHRH was measured by the double-antibody RIA described by Nett *et al.* (9). Anti-GnRH-serum, R-42 pool, also provided by Dr. G. D. Niswender, was used at a dilution of 1: 280,000. Synthetic LHRH (lot CN-79, 479-11K, TM 10455x151-2, Parke Davis Co., Detroit, Mich.) was used as a reference standard and for radioiodination by the chloramine-T method. Samples were assayed in duplicate at two dilutions. Analysis of variance and Duncan's multiple range test were used to analyze the data. The results were considered to be significant if $P < 0.05$.

Results. Hypothalamic LHRH content. LHRH content in the medial basal hypothalamus (MBH) of young cycling female rats was maximal on the morning of PE, but decreased significantly by PE afternoon ($P < 0.01$) (Fig. 1). LHRH content was significantly elevated above PE afternoon levels on the morning of estrus ($P < 0.05$), but dropped to low levels by the morning of DE-2. LHRH levels on DE-2 were not significantly different than levels on the afternoon of PE. The difference between DE-2 and E only bordered on significance ($0.05 < P < 0.1$). MBH-LHRH levels in the old CE rats were similar to young DE-2 rats, but significantly less than seen in young estrous rats ($P < 0.05$). Old PP rats had MBH-LHRH levels intermediate to, but not significantly different than levels seen in young E or DE-2 rats. LHRH content in the MBH of old AN rats was significantly lower than in any other group ($P < 0.05$).

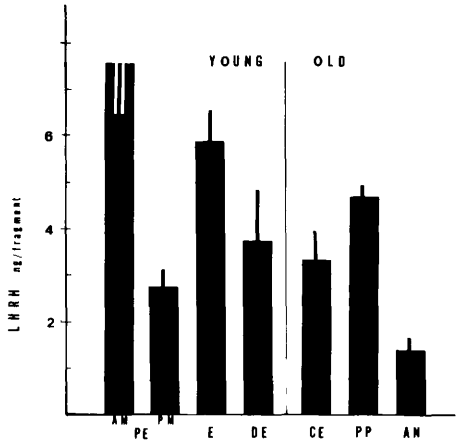

FIG. 1. LHRH content of the medial basal hypothalamus (MBH) of young proestrous (PE), estrous (E), diestrous Day 2 (DE-2) old constant estrous (CE), pseudopregnant-like (PP), and anestrous (AN) female rats. Rats were decapitated at 1000–1100 hr with the exception of PE rats which also were killed at 1600–1700 hr. Values are expressed as the mean ± SE of six to eight rats per group.

LHRH content in the anterior hypothalamus (AH) of young rats showed a small nonsignificant decrease only on the afternoon of PE (Fig. 2). Values in the old CE, PP, and AN rats were not significantly different than those in young rats on the day of E or DE-2. LHRH content in the posterior hypothalamus (PH) was less than 160 pg per hypothalamic fragment, and showed no significant variation due to either age or stage of cycle (not shown).

Serum gonadal steroids. In young rats, serum estradiol concentrations were high on the morning and afternoon of PE and fell to low levels by E (Table 1). DE-2 levels were significantly higher than E values ($P < 0.05$), but significantly lower than PE levels ($P < 0.01$). Old CE and PP rats had serum estradiol concentrations similar to those in young DE-2 rats. Old AN rats had estradiol levels similar to those in young E rats.

Serum progesterone concentrations in

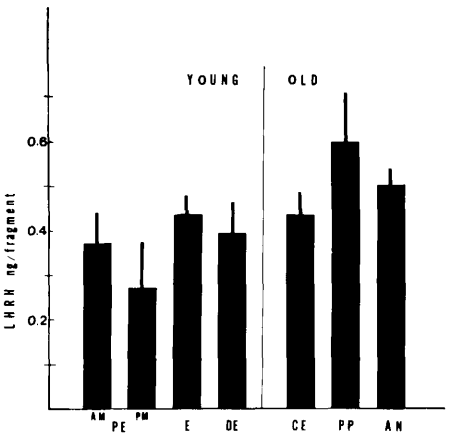

FIG. 2. LHRH content of the anterior hypothalamus (AH) of the rats described in Fig. 1.

young rats were low on the morning of PE, rose to surge values on the afternoon of PE, and dropped to basal levels on E and DE-2 ($P < 0.05$). (Table 1). Progesterone levels in old CE rats were similar to basal levels in young rats. Old PP rats had significantly elevated serum progesterone levels when compared to old CE rats or young E rats ($P < 0.05$). Progesterone levels in old AN rats were significantly lower than in any other group ($P < 0.05$).

Discussion. Our present results suggest that cyclic fluctuations of hypothalamic LHRH occur in young female rats, in agreement with the results published by several other laboratories (8, 10, 11). Although differences in hypothalamic content of LHRH are not necessarily indicative of release rate, Barr and Barraclough (10) demonstrated a precipitous decline in MBH-LHRH prior to the beginning of the proestrous LH surge followed by rhythmic rises and falls of LHRH in association with increasing levels of plasma LH. In both the present and the previously cited study (10), LHRH levels were significantly lower at 1700 hr than at 1000 or 1200 hr on the day of PE. Kalra and Kalra (11) described a fall in MBH-LHRH between 1407 and 1445 hr, followed by a rise between 1455 and 1555 hr; the preovulatory LH surge began at 1605 hr.

The observations that MBH-LHRH and serum LH levels (3) are similar in old CE and PP rats, and that MBH-LHRH and serum LH levels are reduced in old AN rats, suggest a possible causal relationship between MBH-LHRH and serum LH values. Although cyclic fluctuations in MBH-LHRH during diestrus and estrus in young rats are not reflected by serum LH changes (10, 11), MBH-LHRH in the old AN rats is significantly lower than in young DE or E rats, and may not be compatible with the maintenance of basal LH secretion. Serum LH was reported to be undetectable in old AN rats (2).

TABLE 1. SERUM ESTRADIOL AND PROGESTERONE LEVELS IN YOUNG AND OLD RATS

	Estradiol (pg/ml)	Progesterone (ng/ml)
Young		
Proestrus (AM)	53.7 ± 5.4[a]	11.7 ± 2.4
Proestrus (PM)	72.1 ± 6.4[a]	31.0 ± 3.9
Estrus	19.2 ± 1.9	14.0 ± 2.2
Diestrus-2	29.0 ± 2.0	16.7 ± 2.6
Old		
CE	33.6 ± 3.3	15.5 ± 2.8
PP	28.7 ± 4.8	25.2 ± 6.5[b]
AN	19.8 ± 2.5[c]	6.5 ± 0.88[c]

[a] Mean ± SEM. $P < 0.05$ compared with estrus, diestrus-2, CE, PP, and AN.
[b] $P < 0.05$ compared to E and CE.
[c] $P < 0.05$ compared to CE and PP.

Kalra (12) suggested that LHRH may be synthesized in the AH and then transported to the MBH for storage and release. AH-LHRH levels in the present study were not affected by either age or stage of the cycle. The reduced MBH-LHRH levels in the old AN rats, despite normal AH-LHRH levels may represent a deficiency in transport of LHRH from a region of synthesis to a region of storage and release.

Although hypothalamic LHRH and serum steroid levels fluctuate independently in cyclic rats (11), total hypothalamic LHRH content decreased significantly after castration of male or female rats (7, 8). Castration lowered MBH-LHRH to a greater degree than AH-LHRH (8). Steroid replacement restored LHRH levels to normal or higher than normal levels in castrated rats (7, 8). In the present study, a relationship between serum steroid concentrations and hypothalamic LHRH content was seen in the old rats. Old AN rats had low MBH-LHRH levels and low estradiol and progesterone values. Old PP and CE rats both had significantly higher MBH-LHRH levels and significantly higher steroid levels than old AN rats. The higher LHRH content in the old PP than in the old CE rats, although only approaching significance, may reflect the combination of similar estradiol values together with significantly higher serum progesterone in the PP rats. The combination of estrogen and progesterone was reported to be more effective in increasing hypothalamic LHRH levels than estrogen alone (7).

In conclusion, it appears that sufficient hypothalamic LHRH is present and released in old CE and PP rats to maintain basal LH secretion. Similar conclusions were reached by Miller and Riegle (13) in a recent report, using bioassay methods to measure total hypothalamic LHRH in young and old male and female rats. An LH surge does not occur

in old female rats, perhaps because the stimulus for cyclic release of LHRH is lacking. This may be due to a deficiency of catecholamines and an excess of serotonin turnover in the hypothalamus of old rats (14, 15). The low MBH–LHRH content in old AN rats may be responsible for their undetectable serum LH levels.

Summary. The relationships of age and reproductive state to hypothalamic LHRH content and gonadal steroids was studied in young and old female rats. LHRH content in the medial basal hypothalamus (MBH) of young female rats was maximal on the morning of proestrus (PE), but fell to a nadir on PE afternoon. MBH–LHRH rose again on the morning of estrus (E) and then dropped by 1000 hr on diestrous Day 2 (DE-2). Old constant estrous (CE) rats had MBH-LHRH levels that were significantly lower than in young E rats, while old pseudopregnant-like (PP) rats had intermediate MBH–LHRH levels, not significantly different from those in young E or DE-2 rats. LHRH content in the MBH of old anestrous (AN) rats was lower than in any other group. Anterior hypothalamic (AH)–LHRH in old CE, PP, and AN rats were similar and did not differ from those in young E or DE-2 rats.

Serum estradiol and progesterone peaked at PE in young cycling rats, but showed no cyclic variations in old rats. Old CE and PP rats had moderate estradiol levels comparable to those in young DE-2 rats, and AN rats had low estradiol levels comparable to those in young E rats. Old PP rats had higher progesterone levels than old CE or young E rats. Old AN rats had progesterone levels lower than in any other group. In general, these observations suggest that sufficient LHRH is present in old CE or PP rats to maintain basal LH secretion, but that the stimulus for cyclic LHRH release is lacking. Low to undectable LH levels seen in old AN rats may be the result of the low LHRH levels seen in these rats.

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