0013-7227/83/1123-0788$02.00/0
Endocrinology

Vol. 112, No. 3
*Printed in U.S.A.*

# Diminished Luteinizing Hormone Pulse Frequency and Amplitude with Aging in the Male Rat*

A. E. KARPAS, W. J. BREMNER, D. K. CLIFTON, R. A. STEINER, AND D. M. DORSA

*Geriatric Research, Education and Clinical Center (D.M.D.), and Division of Endocrinology and Metabolism (A.E.K., W.J.B.), Veterans Administration Medical Center, and Departments of Medicine (A.E.K., W.J.B., D.M.D.), Pharmacology (D.M.D.), and Obstetrics and Gynecology (D.K.C., R.A.S.), and Physiology and Biophysics (R.A.S.), University of Washington, School of Medicine, Seattle, Washington 98195*

**ABSTRACT.** Aging in the male rat is associated with a reduction in circulating testosterone levels. One possible cause of this decline is an age-related alteration of central nervous system-mediated LH secretion. To examine the effects of age on the hypothalamo-hypophyseal system, in the absence of gonadal steroid feedback, we studied the pattern of pulsatile LH secretion in castrate male Sprague-Dawley rats, aged 3 months (young), 8 months (middle-aged), and 26 months (old). All animals were castrated, and after 3 weeks, they were implanted with indwelling atrial catheters. One day later, duplicate 25 μl blood samples were obtained at 4-min intervals for 4 h, while the animals were awake and unrestrained. Serum levels of LH, FSH, and testosterone were measured in animals before castration, and blood LH levels were measured in the postcastration, repeated sampling studies.

After castration, middle-aged and old animals exhibited significantly lower mean serum LH levels, associated with a diminished amplitude of LH secretory episodes compared to young rats. In the oldest group, LH pulse frequency was significantly lower compared to middle-aged and young animals. Since the control of LH secretory episodes resides in the central nervous system, we propose that alterations in frequency of LH pulses observed in the aged, castrate male rat are the result of a diminished functional capacity of LHRH-containing neurons or of neurotransmitters that modulate their activity in the aging brain. (*Endocrinology* **112:** 788, 1983)

AN AGE-RELATED decrease in plasma testosterone (T) levels has been described for the male of a variety of species including humans (1–4), mice (5), and rats (6–10). Whether this age-related decline of circulating T is due to primary testicular failure or to a decrease in the function of central nervous system (CNS)-pituitary mechanisms underlying gonadotropin secretion remains unresolved. Two observations suggest that the age-related decline in T production may not be the result of a simple primary testicular defect in the rat. First, Leydig cell number does not appear to change with age in the rat (11). Second, with repeated administration of human CG the aging rat testis can be stimulated to increase its T production, returning plasma levels to the normal range observed in young animals (9). These findings suggest that the age-related decline in testicular steroidogenic capacity may be the result of diminished gonadotropin secretory capacity in elderly rats. In support of the idea that the CNS-pituitary axis is hypofunctional in aged rats is the observation that they fail to show an appropriate compensatory rise in gonadotropin secretion which would be expected in response to the decline in plasma T levels associated with aging (11–17). In addition, aged rats show other CNS-gonadotropin related abnormalities such as diminished diurnal alterations of circulating LH levels (18). This observation suggests that as the animal ages, the CNS-pituitary axis undergoes changes which alter its capacity to respond to a diminishing T feedback signal and also make it less responsive to CNS-mediated environmental input.

We hypothesized that alteration of the neural substrates which determine the pattern of LH secretion may attend the process of aging and could, therefore, be responsible for the declining testicular function. It has been postulated that aging enhances the sensitivity of CNS-gonadotropin regulatory systems to testicular feedback signals (19). To eliminate this complication, we have characterized the functional capacity of the aged CNS-gonadotropin axis by examining minute-to-minute secretory profiles in aged, castrate rats and compared these profiles to those observed in younger animals. We report finding an age-related decline in LH pulse fre-

Received August 11, 1982.
Address requests for reprints to: Daniel M. Dorsa, Ph.D., Geriatric Research, Education, and Clinical Center (182B), Veterans Administration Medical Center, 4435 Beacon Avenue South, Seattle, Washington 98108.
* This work was supported by the Veterans Administration and by NIH Grants HD-12625 and HD-12629.

quency and amplitude and suggest that a CNS-dependent mechanism is responsible for altered gonadotropin secretion and may ultimately contribute to the testicular failure associated with aging.

## Materials and Methods

*Animals and accommodations*

We studied two groups of animals. In group I were intact male Sprague-Dawley rats (Tyler Labs, Bellevue, WA) aged 3 (n = 9) and 24 months (n = 9). This group was used to assess the effects of aging on baseline serum LH, FSH, and T levels in intact animals. Group II were male Sprague-Dawley rats (Charles River Labs, Wilmington, MA), aged 3 (n = 8), 18 (n = 7), and 26 (n = 5) months, obtained through the Aged Animal Program of the National Institute on Aging. Group II animals were used to study the effects of aging on the patterns of pulsatile LH secretion in castrate animals. The 18- and 26-month animals had been barrier-reared after 6 months of age. After arrival at the Seattle VA Medical Center, the animals were maintained in air-conditioned, constant-temperature rooms with artificial light at 12 h light-dark schedule (light on at 0600 h). Rat chow and water were supplied *ad libitum*.

*Bleeding and surgical procedures*

We obtained blood from animals in group I for the measurement of baseline serum LH, FSH, and T levels, using jugular venous puncture while maintaining the animals under ether anesthesia. We castrated all animals in group II, while maintaining them under ether anesthesia. Immediately before castration, blood was obtained by cardiac puncture for the determination of serum T levels. Animals were treated postoperatively with 30,000 U of procaine-penicillin (im). Repetitive blood sampling for blood LH measurement was performed 3 weeks after castration. Between 24–48 h before serial blood sampling, we implanted an indwelling right atrial catheter through the jugular vein, according to a modification of the method described by Terkel and Urbach (20). On the day of sampling, we attached a 2-foot extension of PE 50, 23-g tubing to the distal end of the catheter, which permitted remote blood sampling without disturbing the animal. During this time, the animals could move freely about their cages and could eat and drink normally. Blood was obtained every 4 min for 4 h beginning at 0900 h. We withdrew the deadspace volume of the connecting tubing, and then, with a Hamilton syringe, collected two 25-$\mu$l aliquots of whole blood, which we dispersed into tubes with 225 $\mu$l of Tris buffer. We flushed the catheters with saline after each sample and an additional volume of saline equal to that withdrawn was replaced at hourly intervals. Samples were frozen at −20 C until assayed. During the experiment, animals did not show any apparent disturbances of their gross behavior (grooming, sleeping, drinking, *etc.*). At autopsy, aged animals showed no incidence of brain or pituitary abnormalities or gross evidence of tumors on visual inspection.

*RIAs*

We used NIADDK kits to measure LH (S-4 antiserum, Reference Preparation-1 standard) and FSH (S-10 antiserum, Reference Preparation-1 standard) in whole blood and serum samples. The presence of red cells in the unknown whole blood samples was compensated by adding an equivalent volume of washed cells to all standard tubes. The minimum sensitivity of the LH assay was 1 ng/tube. The intra- and interassay coefficients of variation were 6% and 8%, respectively. Sensitivity of the FSH assay was 8 ng/tube with intra- and interassay coefficients of variation of 4% and 8%, respectively. T levels were measured in serum with reagents supplied by the World Health Organization Matched Reagent Programme. The antiserum was raised againt T linked at the three position by carboxymethyloxime to BSA. This antiserum exhibits a cross-reactivity of 15% with DHT, 6% with 5 $\alpha$-androstenediol, and 2% or less with all other steroids tested. We extracted serum samples with ether, and used dextran-coated charcoal to separate free from bound hormone. Minimum sensitivity was less than 10 pg/tube (0.1 ng/ml). Intra- and interassay variability were 5% and 10%, respectively.

*Data analysis*

To determine LH pulse frequency and amplitude we analyzed serial blood LH data from individual animals, using the two-part procedure. The first part consisted of a one-way analysis of variance to determine whether the data contained any significant hormone fluctuations over time. The analysis of variance was also used to estimate the signal-to-noise ratio (SNR). The SNR is an indicator of the relative contribution of real variations in hormone concentration (signal) and measurement variability (noise) to the overall pattern observed in the experimental data. The second part of the analysis involved the use of cycle detection to assess pulse frequency (or interpulse interval) and amplitude (21). Briefly, cycle detection is a computerized, iterative procedure that scans the data seeking local, sequential increases and decreases greater than a threshold value. One complete cycle is defined as two increases greater than the threshold. The threshold value is initially set at 2.7 times the SD of the noise, and on each subsequent iteration, it is readjusted as a function of both frequency and amplitude to equalize the probability of obtaining a false cycle with the probability of missing a true cycle. The formula used for the threshold adjustment is: $T = A\ (0.7 - 1.75\ Nc/Ns)$, where T = new threshold, Nc = number of cycles detected, and Ns = number of samples. The program continues either through 20 iterations or until the estimated parameters A and Nc stabilize. The cycle detection procedure has been validated by computer simulation of both sawtooth and sinusoidal wave forms; it works well for SNRs as low as 1.5.

We used Mann-Whitney *U* test to identify significant differences in LH pulse amplitude, frequency, and average blood LH levels between the three age groups. Statistical comparisons in intact groups were performed with the Student's *t* test. All variance measures reported here represent the SEM.

## Results

*Serum LH, FSH, and T levels in intact animals*

In intact rats (group I) we observed significantly lower serum T levels in the old compared to young animals

(Table 1). LH levels in old animals were approximately half those observed in young; FSH levels tended to be lower, but this difference was not statistically significant. Similarly, mean plasma T levels measured immediately before castration in group II were: young, 4.74 ± 0.63 ng/ml; middle aged, 2.4 ± 0.34 ng/ml; old, 0.7 ± 0.1 ng/ml. Plasma T levels in the middle-aged animals were significantly lower than in the young animals ($P < 0.01$); T levels in the old rats were significantly lower than in both middle-aged and young animals ($P < 0.001$).

*Castrate animals (group II)*

All animals exhibited a distinct pulsatile pattern of blood LH levels and this pattern varied with the age of the animals. Figure 1 depicts results from representative animals from the three different age groups. This illustrates the lower mean blood LH levels in the middle-aged and old animals compared to the individuals from the young group. LH interpulse interval can be observed to be similar in the young and middle-aged animal but was considerably longer in the old rat. Figure 2 depicts the results of grouping data from all animals of each age group. Mean LH level and LH pulse amplitude in the old and middle-aged groups were reduced to approximately 25% and 50%, respectively, of the levels observed in the young group. Mean interpulse interval was significantly longer in the old group than in the young (*i.e.* a 33% decrease in frequency of LH pulses).

TABLE 1. Plasma gonadotropin and testosterone levels in young and old male rats

| | LH (ng/ml) | FSH (ng/ml) | T (ng/ml) |
|---|---|---|---|
| Old (n = 9) | 20.1 ± 2.2 | 239 ± 11.2 | 1.51 ± 0.3 |
| Young (n = 9) | 46.3 ± 9.6 | 268 ± 11.4 | 5.8 ± 1.4 |
| Young *vs.* old | $P < 0.05$ | NS | $P < 0.001$ |

Values are mean ± SEM.

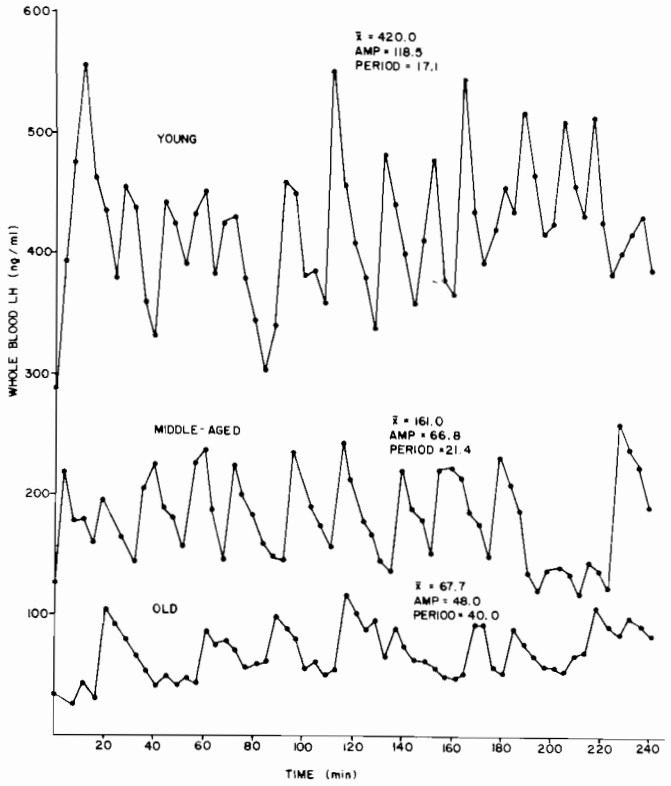


FIG. 1. Typical LH patterns in individual castrate male rats from each of the three age groups. x̄, Mean; AMP, amplitude; PERIOD, pulse period.

## Discussion

Our study has shown that old, castrate male rats exhibit a markedly reduced LH pulse frequency and amplitude compared to younger animals. These results suggest that the functional capacity of the CNS-LHRH pulse generator becomes compromised with the process of aging. Coquelin and Desjardins (5) have recently reported reduced pulsatile LH secretion in intact male mice. Because in our study this difference in LH secretory patterns between young and old rats can be revealed in the absence of gonadal steroid negative feedback, we have provided evidence that the age-dependent change is not simply the result of a differential T negative feedback sensitivity between the groups. We deduce that, with aging in the rat, there is a progressive degeneration of the mechanisms governing pulsatile LH secretion, and we suggest that this may account for the reported degenerative changes in testicular steroidogenesis (1–10). Finding a lower LH pulse frequency in old compared to young animals implies the development of a primary defect in the generating capacity of hypothalamic LHRH neurons. Finding a reduced LH pulse amplitude in old

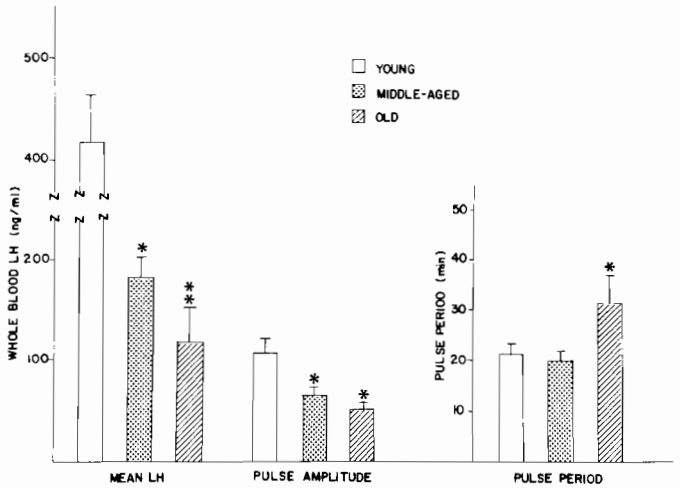


FIG. 2. Mean blood LH, LH pulse amplitude and interpulse pulse interval in castrate male rats (group II) from the three age groups. *Bars* represent mean ± SEM for young (n = 8), middle-aged (n = 7), and old (n = 5) age groups. *, $P < 0.05$ compared to young group; ⁑, $P < 0.01$ compared to young group.

animals could be due either to a diminution of LHRH secretion or to a reduction in pituitary responsiveness to LHRH (or both).

The idea that a defect in hypothalamic LHRH secretion is responsible for the degenerative changes in the patterns of LH secretion we have observed in old rats is reinforced by direct evidence provided by Miller and Reigle (22), who demonstrated a decrease in hypothalamic LHRH secretion in aged rats. Immunohistochemical evidence of LHRH neuronal deterioration with aging has also been reported (23), again bolstering the concept that LHRH secretion becomes compromised with aging.

The degeneration of the LHRH pulse generating system with aging may be related to a declining number of LHRH secreting neurons themselves. A recent report by Enright (24) suggests, on theoretical grounds, that the inherent stability and performance of a neural oscillator may depend on the number of pacemaker cells within the network. Therefore, the degenerative changes we observed (markedly reduced frequency and amplitude) in the operating characteristics of the LHRH pulse generator of aged rats may be the result of a loss of pacemaker cells within the LHRH oscillator.

The secretory activity of LHRH neurons is influenced by a variety of other transmitter and modulator substances. Aging has been reported to influence the activity of important neural regulators of LHRH secretion (see Ref. 25 for review), such as catecholamines (26, 27) and endogenous opiate peptides (28–30), and it may be that these changes are responsible for altered LHRH secretory dynamics.

The pituitary of aged animals may be less capable of responding to the LHRH stimulation. That the pituitary of old animals may be hyporesponsive to LHRH stimulation is supported by reports of both *in vivo* and *in vitro* studies, which demonstrate decreased LH production in response to LHRH exposure (14, 16, 18); changing pituitary responsiveness to LHRH may occur either as a direct result of pulsatile LHRH secretion or may be intrinsic to the aging pituitary itself. Our studies cannot differentiate between these possibilities. The LH molecule itself manifests degenerative changes with aging. There is a reduced biological potency of the LH molecule with aging, and this change in bioactivity is associated with alterations in its physiochemical properties (31).

In summary, we have provided evidence for degenerative changes in the neural mechanisms governing gonadotropin secretion in the castrate, aged male rat. These results are consistent with the hypothesis that aging is associated with alterations of the neural substrates which determine the frequency of LHRH secretory episodes. The diminution of magnitude of LH release observed may be due to the amount of LHRH secreted per episode or to a diminished pituitary responsiveness to LHRH. We also suggest that the degeneration of testicular steroidogenesis characteristic of elderly rats may be the result of chronic understimulation by pituitary gonadotropin.

## Acknowledgments

We are very grateful for the excellent technical assistance of Vasumathi Sundarraj, Patricia Payne, and Judy Tsoi and the secretarial work of Patricia Jenkins, Maxine Cormer, and Cindy Schuler. We appreciate the gifts of assay reagents from the NIADDK and the World Health Organization.

## References

1. Kinsey AC, Pomeroy WB, Martin CE 1948 Sexual Behavior in the Human Male. WB Saunders, Philadelphia
2. Stearns EL, MacDonell JA, Kaufman BJ, Padua R, Lucman TS, Winter JSD, Faiman C 1974 Declining testicular function with age. Am J Med 57:761
3. Baker HWB, Burger HG, DeKretzer DM, Hudson B, O'Conner C, Wang C, Mirovics A, Court J, Dunlop M, Rennie GC 1976 Changes in the pituitary testicular system with aging. Clin Endocrinol (Oxf) 5:349
4. Vermeulen A, Rubens R, Verdonk L 1972 Testosterone secretion and metabolism in male senescence. J Clin Endocrinol Metab 34:730
5. Coquelin A, Desjardins C, Luteinizing hormone and testosterone secretion in young and old male mice. Am J Physiol, in press
6. Ghanadian R, Lewis JG, Chisholm GD 1975 Serum testosterone and dihydrotestosterone changes with age in the rat. Steroids 25:753
7. Miller AE, Reigle GD 1975 Aging effects on hypothalamic catecholamine and testosterone secretion in the male rat. Fed Proc 34:303
8. Chan SW, Leathem JH, Esashi T 1977 Testicular metabolism and serum testosterone in aging male rats. J Endocrinol 76:551
9. Harman SM, Danner RL, Roth GS 1978 Testosterone secretion in the rat, response to chorionic gonadotropin with age. Endocrinology 102:540
10. Miller AE, Reigle GD 1980 Serum testosterone and response to HCG in young and old male rats. J Gerontol 33:197
11. Kaler LW, Neaves WB 1982 The androgen status of aging male rats. Endocrinology 108:712
12. Gray DG 1978 Changes in the levels of LH and testosterone in aging male rats. J Endocrinol 76:551
13. Bethea CL, Walker RF 1977 Age related changes in reproductive hormones in Leydig cell responsiveness to LHRH in the male laboratory rat. J Gerontol 34:21
14. Reigle GD, Meites J 1976 Effect of aging on LH and prolactin after LH L-dopa, methyl dopa, and stress in the male rat. Proc Soc Exp Biol Med 151:507
15. Reigle GD, Meites J, Miller AE, Woods SM 1977 Effect of aging on hypothalamo releasing and prolactin inhibiting activities and responsiveness to LHRH in the male laboratory rat. J Gerontol 32:13
16. Bruni JF, Hive HOH, Marshall S, Meites J 1980 Effects of single and multiple injections of synthetic GNRH on LH, FSH, and testosterone in young and old male rats. Biol Reprod 17:123
17. Saksena SK, Lau IF 1979 Variations in serum androgens, estrogens, progestins, gonadotropin and prolactin levels in male rats from prepubertal to advanced age. Exp Aging Res 5:179
18. Miller AE, Reigle GD 1982 Temporal patterns of serum LH and testosterone and endocrine response to LHRH in aging male rats. J Gerontol 37:522
19. Gray GD, Smith ER, Davidson JM 1980 Gonadotropin regulation in middle-aged male rats. Endocrinology 107:2021
20. Terkel J, Urbach L 1974 Chronic intravenous cannulation technique adapted for behavioral studies. Horm Behav 5:141

21. Steiner RA, Bremner WJ, Clifton DK 1982 Regulation of luteinizing hormone pulse frequency and amplitude by testosterone in the male rat. Endocrinology 111: 2055
22. Miller AE, Reigle GD 1978 Hypothalamic LH releasing activity in young and aged intact and gonadectomized rats. Exp Aging Res 4:145
23. Hoffman GE, Sladek JR 1980 Age-related changes in dopamine, LHRH and somatostatin in the rat hypothalamus. Neurobiol Aging 1:27
24. Enright JJ 1980 Temporal precision in circadian systems: a reliable plot from unreliable components. Science 209:1542
25. Timiras PS, Choy VJ, Judson DB 1982 Neuroendocrine pacemaker for growth, development, and aging. Age Ageing 11:73
26. Ponzio F, Calderini G, Lanusao G, Vantim G, Toffano G, Algeri S 1982 Changes in monoamines and their metabolite levels in some brain regions of aged rats. Neurobiol Aging 3:23
27. Ponzio F, Brunello N, Algeri S 1978 Catecholamine synthesis in brain of aging rats. J Neurochem 30:1617
28. Barden N, Dupont A, Labrie F, Merand Y, Rouleau D, Vaudry H, Boissier JR 1981 Age-dependent changes in the $\beta$-endorphin content of discrete rat brain nuclei. Brain Res 208:209
29. Steger RW, Sontag WE, VanVugt DA, Forman LJ, Meites J 1980 Reduced ability of naloxone to stimulate LH and testosterone release in aging male rats: possible relation to increase in hypothalamic met-enkephalin. Life Sci 27:747
30. Forman LJ, Sontag WE, VanVugt DA, Meites J 1981 Immunoreactive $\beta$-endorphin in the plasma, pituitary and hypothalamus of young and old male rats. Neurobiol Aging 2:281
31. Conn PM, Cooper R, McNamara C, Rogers DC, Schoenhardt L 1980 Qualitative change in gonadotropin during normal aging in the male rat. Endocrinology 106:2549