Testicular LH Receptors During Aging in Fisher 344 Rats

ARMANDO AMADOR, RICHARD W. STEGER, ANDRZEJ BARTKE, ANTHONY JOHNS, THERESA M. SILER-KHODR, C. RICHARD PARKER, Jr.,* AND ALEXANDER M. SHEPHERD†

*From the Department of Obstetrics and Gynecology, †Department of Pharmacology, the University of Texas Health Science Center at San Antonio San Antonio, Texas, and the *Department of Obstetrics and Gynecology, the University of Texas Health Science Center at Dallas, Dallas, Texas*

Levels of serum LH, prolactin, testosterone, progesterone and 17-OH progesterone and the testicular concentration and total content of LH receptors were measured in 4-, 11-, 18-, and 27-month-old Fisher 344 rats. All 27-month-old rats had Leydig cell tumors. At first, testicular LH receptor levels decreased with age, but with the appearance of the testicular tumors, these levels increased dramatically. Serum prolactin levels fluctuated with age, but were significantly decreased in 27-month-old rats, as were serum LH levels. Serum testosterone levels decreased steadily with age, while the testosterone-LH receptor ratio remained constant until the appearance of the testicular tumors, after which the ratio decreased precipitously. Serum progesterone levels remained constant throughout the life of Fisher 344 rats until the appearance of testicular tumors, when they increased dramatically. Serum 17-OH progesterone levels were increased significantly at 11 and 27 months as compared to four months of age, but levels at 18 months were similar to those seen in the 4-month-old animals. Therefore, in aged Fisher 344 rats with spontaneous Leydig cell tumors, there is an alteration in the testicular testosterone synthesizing pathway at a step after progesterone.

Key words: LH receptors, testicular tumors, Fisher 344 rats, aging, LH, prolactin, testosterone, progesterone.

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Rats of the Fisher 344 strain are characterized by a high incidence of spontaneous tumors, including neoplasias, during aging. Thus, while spontaneous Leydig cell tumors are rare in rats, as they are in humans, and malignancy in these tumors is even less frequent, most old male Fisher 344 rats have Leydig cell tumors, and many of these tumors are malignant (Thompson et al, 1961; Jacobs and Huseby, 1967; Walsh, 1979). Serum and pituitary prolactin levels and serum estradiol levels have been reported to increase with age in Fisher 344 rats, whereas serum and pituitary FSH levels and pituitary LH levels decrease (Turek and Desjardins, 1979). In old Fisher 344-derived, inbred CDF rats with tumors consisting of hyperplastic Leydig cells, plasma testosterone and androstenedione levels were similar to those in non-tumor-bearing animals of the same age (Sweeney et al, 1983). In these animals, basal testosterone production *in vitro* was not different in testes with or without tumors, while testosterone response to hCG *in vitro* was lower in testes with tumors. Isolated tumor cells (hyperplastic Leydig cells) produced less testosterone *in vitro*, under both basal and hCG-stimulated conditions, than did normal Leydig cells. Furthermore, both basal and hCG-stimulated progesterone secretion *in vitro* was markedly increased in tumor-bearing testes.

The present study was undertaken to analyze the changes in testicular LH receptors during aging in Fisher 344 rats, to relate these changes to serum steroid levels and to examine the influence of spontaneously occurring Leydig cell tumors on these parameters.

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Reprint requests: Dr. R. W. Steger, Department of Obstetrics and Gynecology, the University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284.

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Materials and Methods

Animals

Fisher 344 rats of different ages (four, 11, 18, and 27 months) were obtained from Charles River Breeding Laboratories (Wilmington, MA). The animals were maintained in a room with controlled illumination (14 hours light:10 hours darkness) and temperature (22 ± 2 C), with free access to commercial food (Wayne Breeder Blox) and tap water. The animals were sacrificed by decapitation, without the use of anesthetics, immediately after removal from the cage. Trunk blood was collected for hormone measurements and the testes were removed for binding studies.

Measurement of hCG Binding and Plasma Hormone Levels

Testicular ^{125}I-hCG binding was measured by radioreceptor assay using procedures described previously (Amador et al, 1983). The ^{125}I-hCG (CR-121, NIH) used had an average specific activity of 51 μCi/μg and an average maximum binding of 47%. The protein content of the testicular membrane preparations used for measuring hCG binding was determined by a modification of Lowry's method (Markwell et al, 1978), using bovine albumin as the standard.

Testosterone levels were determined by radioimmunoassay (RIA) using procedures previously described (Wolfe et al, 1981). The levels of prolactin and LH were measured by RIAs which have been described previously (Amador et al, 1983) and had intra-assay coefficients of variation of 10.1% and 2.1%, respectively. Progesterone levels were measured by RIA after extraction of serum samples with hexane: ethylacetate (9:1, v:v), using ^{3}H-progesterone as an internal trace to monitor losses during the procedure. The antiserum used (Endocrine Sciences, Tarzana, CA) showed 21%, 15%, and 6% crossreactivity with 5-pregnan-3,20-dione, 17-OH pregnenolone, and 21-OH pregnenolone, respectively. Saturated ammonium sulfate was used as a gamma-globulin precipitating agent. The intra-assay coefficient of variation was 3.7%. The average sensitivity of this RIA was 0.57 ng/ml serum. Selected serum samples were also subjected to column chromatography and progesterone measurement by another RIA procedure which has been described by Parker et al (1975). The results of this assay were in very close agreement with those of the first assay, and are not reported here. Serum 17-OH progesterone was assayed after extraction and purification on celite columns as previously described (Parker et al, 1975).

Statistics

The data were evaluated using two-way ANOVA and the Student-Newman-Keuls test. The data were tested for normality of distribution by the Kolmogorov-Smirnov test, and for homogeneity of variance by Barlett's test, and was log or square-root transformed as needed (Sokal and Rohlf, 1973; 1981).

Results

Testicular LH receptor concentration and total content (or receptor levels) changed with age in Fisher 344 rats. Compared to the level measured in 4-month-old animals, they declined in 11- and in 18-month-old rats (Fig. 1). Upon gross inspection little, if any, normal testicular tissue was found in 27-month-old rats, and the gonads consisted essentially of tumor tissue. The LH receptor levels in these tumors were much greater than those measured in the testes of 4-, 11-, or 18-month-old rats. In these tumorous testes, the LH receptor concentration was five times higher, and the LH receptor content was 14 times higher, than the corresponding values in 4-month-old animals (Fig. 1).

Serum testosterone levels declined with age, with levels in 18-month-old rats being significantly lower than in 4-month-old ones. Moreover, testosterone levels in 27-month-old animals were significantly lower than in all other age groups (Fig. 2). Serum prolactin levels varied among 4-, 11-, and 18-month-old rats, but these apparent differences

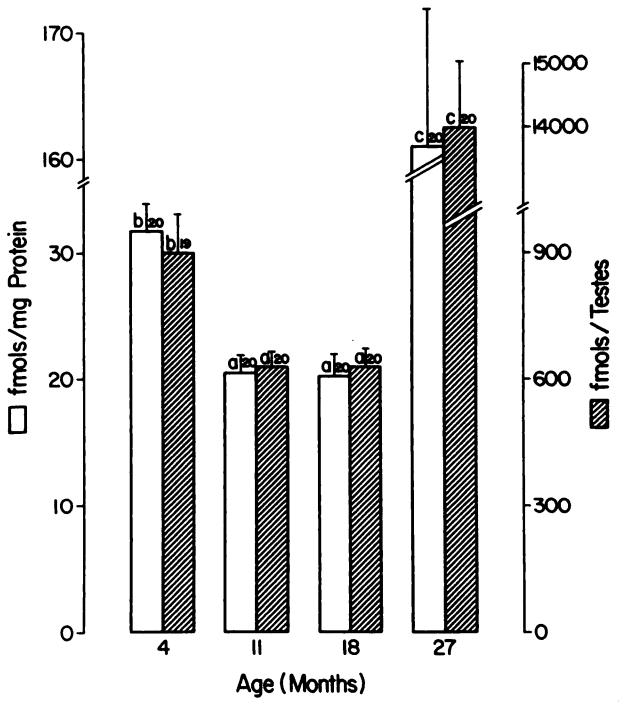

Fig. 1. Binding of ^{125}I-labeled hCG to testis membranes as a function of the age of F344 rats. Values are expressed as means ± SEM. Numbers indicate number of rats per point. Where letters are different, $P < 0.05$ (Student-Newman-Keuls procedure of the multiple range test).

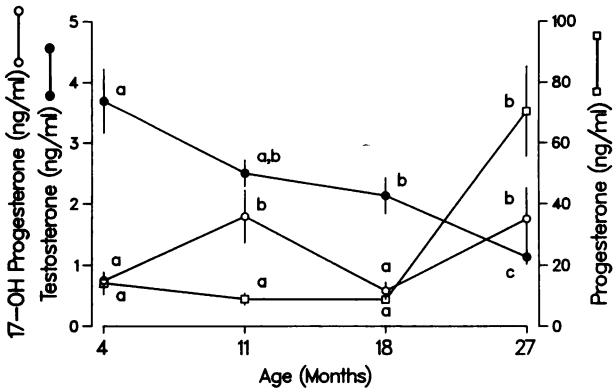

Fig. 2. Serum testosterone, progesterone and 17-OH progesterone levels as a function of the age of F344 rats. Values are expressed as means ± SEM. Where letters are different, $P < 0.05$ (see Fig. 1).

were not significant (Fig. 3). However, in 27-month-old rats, prolactin levels were significantly decreased. No significant differences in serum LH values were measured between 4-, 11-, and 18-month-old rats, although LH levels were numerically lowest in 18-month-old rats. In 27-month-old rats, serum LH levels were significantly decreased (Fig. 3). Serum progesterone values measured in 4-, 11-, and 18-month-old rats did not vary significantly among age groups (Fig. 2). However, in 27-month-old rats, serum progesterone was dramatically elevated (Fig. 3). Serum 17-OH progesterone levels increased significantly between four and 11 months of age, but then dropped by 18 months to levels not different from those of 4-month-old rats (Fig. 2). At 27 months, serum 17-OH progesterone concentrations were again elevated to levels seen in 11-month-old animals.

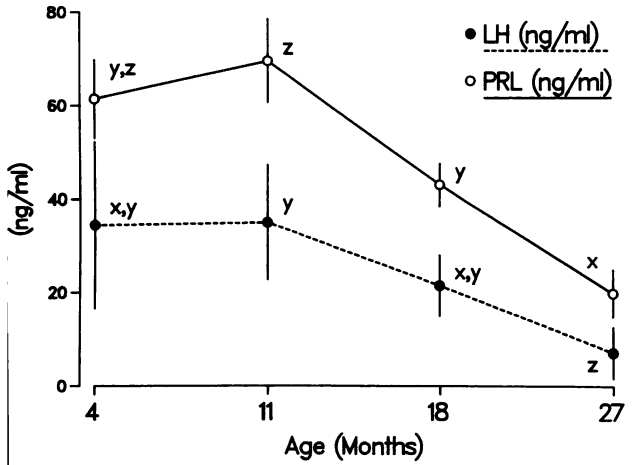

Fig. 3. Serum LH and prolactin levels as a function of the age of F344 rats. Values are expressed as means ± SEM. Where letters are different, $P < 0.05$ (see Fig. 1).

Discussion

The present study demonstrates a significant age-related decrease in testicular LH receptors, serum testosterone and serum LH between four and 18 months of age in male F-344 rats. In these age groups, the changes in testicular LH receptors and in serum testosterone levels were correlated, but this correlation disappeared with the development of Leydig cell tumors. In 27-month-old rats, all of which had testicular tumors, testicular LH receptor levels were very high, while serum testosterone levels were lower than in younger animals. The relationship of the LH receptor levels and testicular steroidogenesis can be assessed from the testosterone/receptor ratio as shown in the following formula:

$$\text{T/R ratio} = \frac{\text{pg testosterone/ml}}{\text{fmols LH receptor/mg protein}}$$

Comparison of these ratios in the different age groups indicates that the LH receptor-steroidogenesis interaction remained practically constant in 4- to 18-month-old rats without tumors, and that this measure of testicular steroidogenic "efficiency" became extremely low in 27-month-old tumor-bearing rats (T/R ratios: four months, 116; 11 months, 122; 18 months, 105; 27 months, 7). The dramatic decrease in T/R ratio in 27-month-old rats was concomitant with an equally dramatic increase in serum progesterone levels.

The large increase in serum progesterone levels, despite only slightly elevated 17-OH progesterone levels (present study), and normal or decreased androstenedione and testosterone levels (Sweeney et al, 1983, and present study) suggests that the tumorous Leydig cells are unable to process progesterone to testosterone efficiently. It is also possible that the tumors do not metabolize progesterone, and a small population of normal Leydig cells continues to exist and can account for the limited testosterone production seen in old F-344 rats both *in vivo* and *in vitro*.

Leydig cell tumors originate as a hyperplasia of the Leydig cells, which then can progress to a neoplasia. Although these tumors seldom become malignant in other rats, they do in the majority of Fisher 344 rats (Jacobs and Huseby, 1967). The hyperplasia could represent a compensatory mechanism related to the gradual decline in steroidogenesis in aged rats. The hyperplasia, in fact, might effectively overcome the effects of this decline for

a certain period of time, but does not appear to permanently compensate for the age-related deficiencies in testicular function. This compensation was observed in CDF rats with Leydig cell hyperplasia, in which plasma androgen levels were similar to those in non-tumor-bearing animals (Sweeney et al, 1983). Tumor-bearing CDF rats had high testicular LH receptor levels and a higher number of Leydig cells than did non-tumor-bearing animals (Amador and Sweeney, unpublished observations), possibly representing a mechanism compensating for the alterations in steroidogenesis (Sweeney et al, 1983). In the present study, this compensatory mechanism has obviously ceased to operate due either to a failure of the Leydig cells or to their malignant transformation. The changes in testicular function observed in old Fisher 344 rats appear to be either related to the presence of Leydig cell tumors or may represent a strain-specific characteristic, since, in old Wistar rats, the decrease of serum testosterone levels with age is smaller and testicular LH receptor concentration is only slightly decreased (Geisthövel et al, 1981).

The endocrine profile of the rats in the present study differs from that reported by Turek and Desjardins (1979), in that their animals did not exhibit a decrease in serum testosterone, LH or prolactin, but rather had an increase in serum prolactin levels with age and a transient increase in serum LH at age 18 months. The difference in testosterone levels between our animals and those examined by Turek and Desjardins (1979) could be due to the heterogeneity of Leydig cell neoplasias, as reported by Jacobs and Huseby (1968). These authors reported that not all tumors had the same steroidogenic capacity, and their results suggest a possible relationship between the degree of differentiation of the tumor and its steroidogenic capacity. Thus, Turek and Desjardins (1979) might have been using rats with more differentiated Leydig cell tumors than those found in our animals. Furthermore, hyperprolactinemia has been reported to inhibit hyperplasia of the Leydig cells (Sweeney et al, 1983), and thus it may be reasonable to expect that the degree of differentiation of Leydig cell tumors might also be modulated by prolactin. Prolactin levels were elevated in rats studied by Turek and Desjardins (1979) but were reduced in our animals.

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