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Effect of Age on Testis Δ^5-3β-Hydroxysteroid Dehydrogenase in the Rat[1] (37983)

JAMES H. LEATHEM AND EUGENE D. ALBRECHT[2]

Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08903

Function of the mammalian testis appears to be influenced by advancing age which in the human is signalled by a decrease in testosterone production (1) and in plasma free testosterone concentration (2). In the rabbit, testosterone secretion decreased significantly with age when testes were perfused with an artificial medium containing added FSH and ICSH (3). However, little is known of steroid metabolism in the testes of aged mammals. Axelrod (1) examined the metabolic pathways of steroid biosynthesis in testes from a 16-yr-old and a 61-yr-old individual and noted a reduced desmolase activity but no change in Δ^5-3β-hydroxysteroid dehydrogenase (3β-OHSD). Nevertheless, the dehydrogenase does exhibit an age-related decline in activity in the rat adrenal (4) but has not been examined in the rat testis, thus presenting this aspect for current investigation.

Materials and Methods. Long–Evans strain rats, born and raised in our laboratory were used. All animals were of known birth date and were fed Purina fox chow ad lib supplemented weekly with cod liver oil on bread. The rats were housed in groups of three to five in a temperature (78°F)-regulated room with a light–dark cycle of 12:12 hr. Untreated male rats of 4–24 mo of age were studied. Additional males, 24 mo of age, were given 20 IU of human chorionic gonadotropin (sc) daily for 5 days and autopsied on the next day. All rats were sacrificed by decapitation between 8 and 10 AM and their testes immediately removed, weighed, then frozen on dry ice until assayed within 24 hr. Testis Δ^5-3β-hydroxysteroid dehydrogenase (3β-OHSD) was estimated biochemically (5) using dehydroepiandrosterone as the substrate. Activity was reported as micrograms of androstenedione formed per minute or per milligram of protein (6). Total activity is presented as the androstenedione formed in micrograms per minute per testis. Significant differences were estimated by the Student's t test and a P value of 0.01 was considered significant.

Results. Actual testis weight (one testis) was unchanged between 6 and 24 mo of age but testis weight to body weight ratios did decline between 6 and 12 mo of age but were unchanged thereafter (Table I).

A significant decline in testis 3β-OHSD activity/mg protein occurred between 12 and 18 mo of age with no further decline noted in 24-mo-old animals. Total testis 3β-OHSD activity was greatest at 12 mo of age and declined significantly thereafter (Table I). Total testis protein was also maximal at 1 yr of age.

Treatment of 24-mo-old rats with human chorionic gonadotropin (20 IU) daily for 5 days resulted in a significant stimulation of 3β-OHSD activity and an increase in total protein. 3β-OHSD activity/mg protein was increased to a level that exceeded that of the 4- to 12-mo-old animals. Furthermore, total enzyme activity was significantly greater than the peak activity noted at 1 yr of age for untreated rats.

Discussion. The decrease in Δ^5-3β-hydroxysteroid dehydrogenase activity in the testis with aging in the older rat contrasts with the lack of an aging effect on this en-

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[2] N.D.E.A. Title IV Predoctoral Fellow.

TABLE I. Effect of Aging and Chorionic Gonadotropin on Testis Δ^5-3β-Hydroxysteroid Dehydrogenase Content of Male Rats.[a]

Age (mo)	Treatment	No. of rats	Body wt (g)	Testis (one) wt (g)	Δ^4 Androstenedione (µg/min) mg	mg/protein	Total (per organ)	Protein mg/100 ml	Total
4	None	8	344	1.315 ± 0.143	0.012 ± 0.001	0.17 ± 0.01	15.23 ± 2.16	7.0 ± 0.1	89.62 ± 9.01
6	None	8	356	1.535 ± 0.037	0.011 ± 0.001	0.17 ± 0.01	16.86 ± 1.18	6.6 ± 0.2	100.68 ± 3.33
12	None	8	507*	1.584 ± 0.037	0.014 ± 0.001	0.18 ± 0.01	22.75 ± 1.42	7.7 ± 0.3	122.78 ± 2.74
18	None	13	538	1.581 ± 0.065	0.008** ± 0.002	0.12** ± 0.02	12.73** ± 2.52	7.0 ± 0.1	111.09 ± 5.09
24	None	10	509	1.670 ± 0.115	0.008 ± 0.001	0.12 ± 0.02	12.87 ± 3.12	6.9 ± 0.3	113.63 ± 6.39
24	HCG[b]	8	536	1.997* ± 0.068	0.016** ± 0.001	0.24 ± 0.02	33.60** ± 2.95	6.9 ± 0.2	137.62* ± 7.26

[a] Values expressed as means ± SEM. Significance of differences from rats of the prior age group: $*P = 0.05$, $**P = 0.01$.

[b] Twenty units of human chorionic gonadotropin injected (sc) daily × 5 days.

zyme in the human testis (1). However, the human study included only one individual of 61 yr of age. Since degenerative changes have been observed in the testes of the older rat (7), an adverse effect on the enzyme may have been anticipated. Nevertheless, the sharp increase in testis 3β-OHSD activity in response to chorionic gonadotropin attests to the responsiveness of the 24-mo-old rat testis to gonadotropin. Since hypophysectomy reduced testis 3β-OHSD levels to 22% of normal (8) and LH restored the activity (9), it would appear that depressed serum LH levels were responsible for the testis changes. Indeed a decrease in pituitary and serum levels of gonadotropins have been noted in older male rats (10). On the other hand, prolactin is elevated in the serum of senescent female rats (11). Despite the lack of similar measures in male rats, it should be noted that testis dehydrogenase is responsive to prolactin (9). However, in a preliminary study we have failed to note a response of the depressed enzyme level to administered prolactin suggesting that a change in testis sensitivity might also occur in aging.

Summary. Testis Δ^5-3β-hydroxysteroid dehydrogenase activity and protein content were determined in rats 4–24 mo of age. Activity of the enzyme per milligram of protein declined between 12 and 18 mo of age and remained depressed through 24 mo of age. Total testis enzyme activity was maximal at 12 mo of age. Chorionic gonadotropin increased testis 3β-OHSD activity significantly in 2-yr-old rats.

1. Axelrod, L. R., Biochim. Biophys. Acta **97**, 551 (1965).

2. Vermeulen, A., Stoica, T., and Verdonck, L., J. Clin. Endocrinol. **33**, 759 (1971).

3. Ewing, L. L., Johnson, B. H., Desjardins, C., and Clegg, R. F., Proc. Soc. Exp. Biol. Med. **140**, 907 (1972).

4. Shapiro, B. H., and Leathem, J. H., Proc. Soc. Exp. Biol. Med. **136**, 19 (1970).

5. Rubin, B. L., Deane, H. W., Hamilton, J. A., and Driks, E. C., Endocrinology **72**, 924 (1963).

6. Lowry, O. H., Rosebrough, M. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

7. Berg, B. N., *in* "Pathology of Laboratory Rats and Mice" (E. Cotchin and F. J. C. Roe, eds.), p. 749. Davis, Philadelphia (1967).

8. Samuels, L. T., and Helmreich, M. L., Endocrinology **58**, 435 (1956).

9. Hafiez, A. A., Philpott, J. E., and Bartke, A., J. Endocrinol. **50**, 619 (1971).

10. Debeljuk, L., Arimura, A., and Shally, A. V., Endocrinology **90**, 585 (1972).

11. Clemens, J. A., and Meites, J., Neuroendocrinology **7**, 249 (1971).

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