PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 805–807 (1975)

Alterations with Age in Rat Seminiferous Tubule Monoamine Oxidase Activity when Compared with Whole Testicular Tissue (38636)

RONALD L. URRY, KATHRYN A. DOUGHERTY, AND LEGRANDE C. ELLIS

Division of Urology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642 and Department of Zoology, Utah State University, Logan, Utah 84322

Monoamine oxidase, a deaminating enzyme, (MAO, monoamine: O_2 oxidoreductase [deaminating] EC.1.4.3.4.) is normally present in the rat testes (1–3). Testicular MAO activity has been studied in rats during the late embryonic and the early postnatal periods (4). Seminiferous tubules had more MAO activity during the early postnatal period than did tubules from slightly older animals. The most intense MAO activity was observed in the testes of the neonate and foetuses during the late embryonic period (4). Recently MAO activity was found to be high in testicular preparations from neonatal animals, was reduced in preparations from slightly older animals, was elevated during sexual development, and decreased again with advanced age (after 365 days) (5). Testicular MAO activity was also found to be positively correlated with changes in androgen synthesis and testicular development with respect to aging (5). There have been no attempts to separate the seminiferous tubules from the interstitial elements of the testes and to follow MAO activity simultaneously in these two tissues with age. We therefore desired to determine the relative amounts of MAO activity in these miniferous tubules and whole testicular tissue in animals of various ages. We also wanted to determine the localization of testicular MAO activity.

Materials and Methods. Male rats (Sprague–Dawley strain) were maintained under controlled conditions in an animal laboratory. The temperature was maintained at 72°F and the approximate relative humidity was 32%. Artificial lights were used 12 hr each day followed by 12 hr of darkness. Rats used for the following studies were sacrificed by decapitation and their testes quickly removed, decapsulated, and prepared for later assay as described below.

To determine the localization of MAO activity in the testis, seminiferous tubules were separated from the interstitial elements as previously described (6). Portions of the seminiferous tubules were then placed on thin plates where the contents of the tubules were expressed using flat forceps and a small roller. The tissue was divided into three portions as follows: interstitial cells, seminiferous tubule walls (without contents), and seminiferous tubule contents (without walls). These were collected from several animals, pooled, and then stored at −20° until five samples from each group were assayed for MAO activity as described below.

Experiments to determine the relative amount of MAO activity in seminiferous tubules compared to whole testicular tissue utilized animals with the following average weights: 90, 220, 300, 324, 477, 515, 530, 610, and 667 g (n = 6, 6, 3, 5, 3, 3, 6, and 5 repectively). Aliquots of whole testicular tissue and teased seminiferous tubules were then taken from each animal and assayed for MAO activity.

MAO activity was measured by the amount of radioactively labeled serotonin that was converted into 5-hydroxyindole acetaldehyde and 5-hydroxyindole acetic acid which were then extracted into ethyl acetate, isolated by thin layer chromatography and counted in a scintillation counter (3). The MAO method used was specifically worked out for rat testicular tissue and the amounts of tissue, substrate, and incubation time were chosen so as to give optimal kinetics (3).

Statistical comparison of sample means was made with the standard t test.

Results. Testicular MAO activity (Table I) was higher in the tubule wall than in the

tubule interior, which in turn was higher than in the interstitial cells.

In the aging comparison studies (Table II) MAO activity in the seminiferous tubules was significantly lower than that of the whole testicular tissue in 90 g and 220 g animals (less than 60 days of age). MAO activity then increased in the tubules to a level equal to that of the whole testicular tissue in animals with body wt of 300 g, 324 g, 477 g, and 515 g (60–150 + days of age). Activity in the seminiferous tubules then decreased below the levels of the whole testicular tissue in animals with body wt of 530, 610, and 667 g (200 + days of age).

Discussion. The data in these experiments have shown that the bulk of testicular MAO activity is localized in the walls of the seminiferous tubules with lesser amounts in the tubule interiors and interstitial cells. These findings confirm the observations of other workers who used histochemical techniques and reported that MAO activity was localized primarily in the walls of the seminiferous tubules (4). It appeared from these studies that the changes in testicular MAO activity with age occurred primarily in the seminiferous tubules. Increases also, however, appeared to occur with sexual maturation in the interstitial elements. Testicular MAO activity in the seminiferous tubules of young animals, which were not yet sexually mature, resembled the activity in older animals that were "postsexual maturity". The ratio of MAO activity in the seminiferous tubules compared with whole testicular tissue was in favor of the whole testicular tissue in younger animals that had not yet reached sexual maturity. The ratio changed in sexually mature animals when the activity in the seminiferous tubules became the same as that in whole testicular tissue. The ratio in older animals again shifted so that the seminiferous tubule activity was lower than the activity in the whole testicular tissue.

These experiments suggest a functional role for MAO activity in sexual development and tubular function. MAO activity in the tubules appeared to be positively correlated to sexual maturation and spermatogenic

TABLE I. COMPARISON OF THE RELATIVE MAO ACTIVITY IN VARIOUS LOCATIONS OF TESTICULAR TISSUE. VALUES ARE EXPRESSED AS MEAN ± STANDARD ERROR OF MEAN.

Tissue	MAO activity per mg tissue	
	cpm × 10^3	*P* Value[a]
Tubule-walls	0.77 ± 0.052	<0.001
Tubule-interiors	0.45 ± 0.022	<0.001
Interstitial-cells	0.34 ± 0.017	<0.001

[a] Value when compared with all other groups in the same experiment.

TABLE II. MAO ACTIVITY IN SEMINIFEROUS TUBULE HOMOGENATES AND WHOLE TESTICULAR HOMOGENATES IN ANIMALS OF VARIOUS AGES. VALUES ARE EXPRESSED AS MEAN ± STANDARD ERROR OF MEAN.

Average body wt			Seminiferous tubule MAO activity	Whole testicular tissue MAO activity		MAO activity ratio (Whole testicular tissue)
(g)	(n)[a]	Approx. age days	cpm × 10^3/mg tissue	cpm × 10^3/mg tissue	*P* Value[b]	(Seminiferous tubules)
90	6	30	6.38 ± 1.20	16.79 ± 3.68	<0.01	2.63
220	6	50	6.73 ± 0.92	17.85 ± 1.48	<0.05	2.65
300	6	65	58.60 ± 7.29	60.35 ± 4.07	>0.50	1.03
324	3	75	33.82 ± 2.24	27.87 ± 2.01	<0.1	0.82
477	5	105	27.69 ± 2.44	26.11 ± 1.95	>0.50	0.94
515	3	150+	32.43 ± 1.84	30.83 ± 2.62	>0.50	0.95
530	3	200+	50.05 ± 1.32	70.80 ± 2.02	<0.01	1.41
610	6	365+	19.36 ± 1.22	28.14 ± 2.05	<0.01	1.45
667	5	365+	18.93 ± 0.39	28.82 ± 0.60	<0.001	1.52

[a] Number of animals in group.

[b] *P* Value of seminiferous tubule-MAO activity compared-with that in whole testicular tissue.

onset. The data also suggest a menapausal like effect in MAO activity in the seminiferous tubules with age. The localization of testicular MAO activity in the walls of the seminiferous tubules suggests a functional and perhaps protective role for the tubules. MAO activity may be part of the blood-testis barrier and normally prevent the penetration of potentially damaging amines into the seminiferous tubules. Biogenic amines, such as serotonin, have been shown to have detrimental effects on tubular function and spermatogenesis (7–11). The decline in seminiferous tubule MAO activity with advancing age also suggests that the tubules of older animals are more susceptable to biogenic amine damage than the tubules of sexually mature animals. Studies of other workers have recently demonstrated that serotonin implants adversely affected the testes of older rats (10), but did not effect the testes of younger animals (12). Additional studies are now under way to determine the effect of biogenic amines on spermatogenesis and to determine the relationship of monoamine oxidase to normal testicular function and spermatogenesis.

We thank Miss Phyllis Taylor and Mrs. Lynn Emilson for their help in these studies.

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Received May 20, 1974. P.S.E.B.M. 1975, vol. 148.