Histochemical Location of 17β -Hydroxysteroid Oxidoreductase in The Adult and Infantile Human Testis

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Abstract

17β -Hydroxysteroid oxidoreductase in the human testis was investigated histochemically using tissues obtained from seven patients with undescended testis or varicocele at the time of orchiopexy or high ligation of spermatic vein. Formazan precipitates were formed from nitro-blue tetrasolium in the tissue utilizing hydrogen released by oxidation of testosterone, which is catalyzed by the reductase function of 17β -hydroxysteroid oxidoreductase. The precipitates were formed specifically in the presence of 17β -hydroxy- C_{19} -steroids under the conditions employed in the present study. In infantile testes, the precipitates were formed in cytoplasm of immature Sertoli cells, while in pubertal or adult testes, marked formazan precipitates were found in cytoplasm of both Sertoli and Leydig cells. The results indicate the presence of two distinct 17β -hydroxysteroid oxidoreductases in the human testis; one in Sertoli cells and detectable independent of age and the other only in functional Leydig cells.

17β -Hydroxysteroid oxidoreductase is one of enzymes indispensable for biosynthesis of testosterone in the testis and localizes in seminiferous tubules as well as in interstitial tissues (Rivalora *et al.*, 1973). The authors and other investigators have found that the enzyme is highly active in the human immature testis where no apparent Leydig cells are present and seminiferous tubules are still immature (Mizutani *et al.*, 1977, Oshima *et al.*, 1980). The finding raises a question on intercellular localization of 17β -hydroxysteroid oxidoreductase in the human testis and the present study has been carried out to obtain an answer to this question.

Materials and Methods

Three boys aged 5 to 11 years with undescended testes and four infertile men aged 24 to 36 years with varicocele were selected for the present study. A piece of the testis tissue was obtained by biopsy at the time of either orchiopexy or high ligation of spermatic vein.

Immediately after the removal, the tissue was placed on ice and divided into two portions. A portion was fixed in Bouin's solution. The other portion was cut into small pieces with a razor blade and immersed in 0.5 mM NADP solution for 20 minutes to stabilize the enzyme (Inano *et al.*, 1977). Then, 17β -hydroxysteroid oxidoreductase was histochemically detected by the modified method of Levy *et al.* (1959) for 3β -hydroxysteroid dehydrogenase coupled with Δ^5 - Δ^4 isomerase. Briefly, the specimens were incubated for 40 minutes at 37°C in the reaction mixture indicated in Table 1. Control incubations were carried out in the above medium except for the absence of steroid precursors. After incubation, specimens were rinsed 2 to 3 times in

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Table 1. Composition of the reagent mixture

Testosterone (288 mg/100 ml acetone)	1.0
Androstenediol* (200 µg/1 ml acetone)	0.1
Propyleneglycol	0.5
Nitro-blue tetrasolium (1.0 mg/1 ml aq. dest.)	1.0
Nicotinamide (1.6 mg/1 ml aq. dest.)	0.7
NADP (3 mg/8.5 ml 0.1 M phosphate buffer, pH 8.0)	0.8
0.1 M Phosphate buffer (pH 8.0)	4.0
Total	8.1 ml

*: 5-androstene-3 β , 17 β -diol

0.1 M phosphate buffer (pH 8.0), and fixed in a mixture of 40 % formol in water and 95 % alcohol (10: 90, v/v). The fixed specimen was dehydrated by gradual alcohol solution, immersed in chloroform and embedded in paraffin in a conventional way. Five to six thick sections (about 10 µm) were prepared from each block, deparaffinized and observed under light microscopy either with or without Kernechterot counterstaining.

Steroids and NADP was purchased from Sigma Chemical Co. (St. Louis, Mo.) and other chemicals were of analytical grade. All organic solvents were redistilled before use.

Results

Fig. 1 shows specific precipitation of formazan to immature testis tissues by the reaction catalized by 17 β -hydroxysteroid oxidoreductase. No obvious formazan precipitates are recognized in the absence of steroid substrates (Fig. 1a), while remarkable fine and granular deposits are present in cytoplasm of Sertoli cells in the presence of steroid precursors (Fig. 1b and 1c). In these testis tissues, no apparent Leydig cells are recognized and immature Sertoli cells and gonocytes are present in seminiferous tubules. In contrast, marked formazan precipitates are recognized in both Leydig cells in interstitial spaces and Sertoli cells in seminiferous tubules of the adult testis in the presence of 17 β -hydroxysteroid (Fig. 2).

Histochemical localization of 17 β -hydroxysteroid oxidoreductase in the present study is summarized in Table 2. No obvious

Table 2. Intercellular localization of 17 β -hydroxysteroid hydroxysteroid oxidoreductase in infantile and adult testes.

No.	Age (yrs)	Leydig cells under light microscopy	Formazan precipitates	
			Interstitial spaces	Seminiferous tubules
1	5	—	—	Sertoli cells
2	9	—	—	Sertoli cells
3	11	+	Leydig cells	Sertoli cells
4	24	+	Leydig cells	Sertoli cells
5	30	+	Leydig cells	Sertoli cells
6	32	+	Leydig cells	Sertoli cells
7	36	+	Leydig cells	Sertoli cells

formazan precipitates are observed in interstitial spaces unless mature Leydig cells are present. The formazan deposits, however, are constantly present in Sertoli cells in seminiferous tubules.

Discussion

In the present study, 17 β -hydroxysteroid oxidoreductase in the human testis is specifically visualized utilizing deposits of insoluble formazan at the sites where the reaction catalized by the oxidase function of the oxidoreductase takes place. The hydrogen formed by the oxidation of 17 β -hydroxy C₁₉-steroids binds to nitro-blue tetrasolium resulting in the formation of insoluble formazan at the site of the reaction. For this purpose, optimal pH of 8.0 for the oxidase function of 17 β -hydroxysteroid oxidoreductase (Oshima *et al.*, 1977) was adopted for the present incubations. Nitro-blue tetrasolium can also act as a donor of hydrogens supplied by the oxidation of NADPH to NADP coupled with the reduction of 17-oxo-steroids to corresponding 17 β -hydroxysteroids. The oxidation of NADPH to NADP, however, may be accompanied with many kinds of other enzymic reactions as well as the reduction of 17-oxo steroids to 17 β -hydroxysteroids. Therefore, the oxidation of 17 β -hydroxysteroids has been selected for the histochemical demonstration of 17 β -hydroxysteroid oxidoreductase in the present study,

Fig. 1. Localization of 17β -hydroxysteroid oxidoreductase in the immature human testis. a: Control. The section was prepared from the testis specimen of a 9 year-old boy (case 2) which had been incubated in the incubation medium lacking testosterone and androstenediol. b: The section of the same tissue prepared after incubation with steroid precursors as described in the text. Fine to coarse granular deposits of formazan are positive in Sertoli cells of seminiferous tubules but negative in interstitial spaces. Without counterstaining. c: Infantile testis of a 5 year-old boy (case 1) with counterstaining. Fine granular formazan precipitates are recognized only in seminiferous tubules.

Fig. 2. Localization of 17β -hydroxysteroid oxidoreductase in the adult human testis (case 4). a: Control staining. b: Staining of 17β -hydroxysteroid oxidoreductase without counterstaining. c: Staining of the oxidoreductase with counterstaining using the same testis tissue. Formazan deposits are present in the cluster of Leydig cells as well as in the lining of Sertoli cells. The latter occurs distinctly in the inner portion of Sertoli cell cytoplasm. The spermatogonial layer lies beneath the formazan-positive zone.

although the reduction of 17-oxo-steroid is physiologically carried out to form testosterone in the testis.

In the present study, the activity of 17 β -hydroxysteroid oxidoreductase is found in Sertoli cells of the testis tissue of boys who are supposed to have still low gonadotropin levels, while the enzyme activity is detected in both Leydig cells and Sertoli cells of testis of a pubertal boy and adults in whom gonadotropin secretion has been started. The results are consistent with the fact that 17 β -hydroxysteroid oxidoreductase is active in both infantile and adult human testes (Oshima *et al.*, 1980, Mizutani *et al.*, 1977). In a bird testis, however, 17 β -hydroxysteroid oxidoreductase has been described to localize in only Leydig cells histochemically (Baillie *et al.*, 1966).

Since the oxidoreductase in seminiferous tubules is present in still immature Sertoli cells and the one in interstitial spaces is present only in Leydig cells which are morphologically apparent in the presence of gonadotropins, it appears most likely that the oxidoreductases in Leydig cells and Sertoli cells are distinct and differently regulated; the former activity depends on gonadotropins and the latter is independent of gonadotropin levels. 17 β -Hydroxysteroid oxidoreductase activity in rat and human testes has been recognized in both Leydig cells and seminiferous tubules (Muroso & Payne, 1976, Rivalora *et al.*, 1973) and has a curious property of product activation (Oshima & Ochiai, 1973), while the oxidoreductase partially purified from porcine testes reveals only product inhibition by testosterone. (Inano & Tamaoki, 1974). In a rat study, the product activation has been found only for the 17 β -hydroxysteroid oxidoreductase in Sertoli cells but not for the one in Leydig cells (Muroso & Payne, 1976), also indicating the presence of two distinct 17 β -hydroxysteroid oxidoreductase in the rat testis.

In conclusion, there are two distinct 17 β -hydroxysteroid oxidoreductases in the human

testis; one histochemically localizes in both immature and functional Sertoli cells and therefore, appears to be gonadotropin independent, and the other localizes only in mature Leydig cells and therefore, appears to be gonadotropin dependent.

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