BIOSYNTHESIS AND METABOLISM OF TESTOSTERONE BY SERTOLI CELL-ENRICHED SEMINIFEROUS TUBULES

Martine Tencé and Michel Drosdowsky

Laboratoire de Biochimie Médicale,
C.H.U. Côte de Nâcre, 14000 Caen, France

Received August 5,1976

SUMMARY: Sertoli cell-enriched tubules isolated from rats which had been treated with 1,4-dimethyl sulfonyloxybutane were incubated with either [^{14}C] progesterone or [^{14}C] testosterone for 2 hours. Tubules of normal rats and fragments of Sertoli cell-enriched testes were incubated under the same conditions. Sertoli cell-enriched tubules converted progesterone to 20α-dihydroprogesterone, 17α-hydroxyprogesterone, androstenedione and testosterone. The major metabolite was 20α-dihydroprogesterone. The percentage conversion of progesterone into testosterone corresponded to a production of 10-20 ng testosterone. Sertoli cell-enriched tubules converted testosterone to dihydrotestosterone, androstenedione, 3α-androstanediol and 3β-androstanediol. Under our experimental conditions, dihydrotestosterone was the major 5α-reduced metabolite. Normal tubules converted progesterone and testosterone to the same metabolites as Sertoli cell-enriched tubules. Fragments of Sertoli cell-enriched testes were much more active than isolated tubules in metabolizing progesterone. They produced the same amounts of 5α-reduced metabolites of testosterone.

The seminiferous tubules of rat testis are considered as target organs for T[+], a steroid required for the initiation and the maintenance of spermatogenesis (1). The T concentration in tubular fluid is much higher than in the peripheral circulation (2). The interstitial tissue of the testis is not the only source of intratubular T. The seminiferous tubules are able to synthesize *in vitro* this steroid from P, pregnenolone and dehydroepiandrosterone (3-5). As

---

[+] Abbreviations and trivial names: T, testosterone; P, progesterone; DHT, dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; 3α-DIOL, 3α-androstanediol, 5α-androstan-3α,17β-diol; 20α-H_2P, 20α-dihydroprogesterone, 20α-hydroxypregn-4-ene-3-one ; 17α-OHP, 17α-hydroxyprogesterone, 17α-hydroxypregn -4-ene-3,20-dione; A, androstenedione; androstanedione, 5α-androstan-3,17-dione; 3β-DIOL, 3β-androstanediol, 5α-androstan-3β,17β-diol; 5α-reductase, Δ^4-3-keto steroid 5α-reductase;
Busulphan, Myleran, 1,4-dimethyl sulfonyloxybutane; t.l.c., thin layer chromatography; PPO , 2,5-diphenyl oxazole;POPOP, 1,4-bis- [2-(4-methyl-5-phenyl oxazolyl)] -benzene; KRBG, Krebs Ringer bicarbonate buffer containing 0.2% glucose.

well as other target organs for T, the seminiferous tubules contain a 5α-reductase and can convert T to DHT and 3α-DIOL (6-9). It was shown that DHT can maintain spermatogenesis in hypophysectomized adult rats (10). At the present time, the cellular localization of these tubular activities is not completely known. Several authors have shown that Sertoli cell-enriched preparations isolated from rat testes were able to convert steroids in vitro (11,12,13). In the present report, we have investigated the in vitro metabolism of [^{14}C]-P and [^{14}C]-T by Sertoli cell-enriched tubules isolated from mature rats which had been treated with busulphan during their fœtal life.

MATERIALS AND METHODS

Animals — Aspermatogenic male Wistar rats, provided by Drs J. Gillet and P. Laporte from the University of Poitiers, France, were studied at 90-100 days of age (body wt, 250g; testis wt, 221±43 mg). They came from females which had received an intraperitoneal injection of busulphan (10 mg/kg body wt) on the 20 th day of gestation (14).Normal male rats of the Wistar strain were studied at 80-100 days of age (body wt, 350 g; testis wt, 1500 mg).

Radioactive materials — All radioactive steroids were purchased from the Radiochemical Centre, Amersham, England. Their purity was checked by t.l.c. and recrystallization.

Chromatography — For paper chromatography, the following solvent systems were used: system A, ligroin/propylene glycol, system B, hexane/formamide, system C, hexane:benzene (50: 50)/propylene glycol, system D, petroleum ether:methanol: water (100:80:20), system E, hexane:methanol:water (100:90:10), system F, hexane:methanol:water (100:98:2). For t.l.c., silica gel F-254 plates were used. The following solvent systems were used: system I, benzene:ethyl acetate (60: 40), system II, chloroform:ethyl acetate (135:15), system III, chloroform:ethyl acetate (80:20), system IV, chloroform:diethyl ether (70:30). The Δ^4-3-keto steroids were located under uv light (254 nm). The 5α-reduced reference steroids were visualized by exposure to various reagents (particularly antimony trichloride).

Measurement of radioactivity — Paper chromatograms and t.l.c. plates were scanned for radioactivity on a Packard Radiochromatogram Scanner model 7201. Samples for liquid scintillation countings were dissolved in 10 ml toluene containing 0.4% PPO and 0.01% dimethyl POPOP. Radioactivity was measured in a Packard Tri-Carb model 3385 with an efficiency of 38% for [^{3}H] and 72% for [^{14}C] for double labeled experiments.

Preparation of tissues — Normal and treated rats were sacrificed by decapitation. The testes were removed and placed in ice-cold KRBG at pH 7.0. They were cleaned from adherent fat, the tunica albuginea and the spermatic artery. The tubules were isolated by microdissection (3) as previously described (15). Histological examinations of some of normal and Sertoli cell-enriched tubules confirmed that they were free of interstitial tissue. Undissected Sertoli cell-enriched testes (testes in which interstitial tissue and tubules had not been separated) were also prepared for incubation by removing the tunica albuginea and cutting the organs into 2 portions.

Incubations — Tissue samples of 100-200 mg were incubated in 2 ml KRBG containing 3 µmoles NADP, 37 µmoles glucose 6-phosphate, 5 IU glucose 6-phosphate de-

hydrogenase and 8X10^6 dpm [^{14}C]-P (18 µg, 61.0 mCi/mmole) or 8X10^6 dpm [^{14}C]-T (17 µg, 59.0 mCi/mmole) dissolved in 100 µl propylene glycol. Incubations were performed in a shaking incubator at 31°C in an atmosphere of 95% air-5% CO_2 for 2 hr and were terminated by the addition of 8 ml absolute ethanol.

Separation of steroids – 1) Incubations with [^{14}C]-P – Carrier, unlabeled, steroids (100 µg of each P, 20α-H_2P, 17α-OHP, A and T) and known amounts of [^{3}H]-P, [^{3}H]-17α-OHP, [^{3}H]-A and [^{3}H]-T were added at the end of the incubation procedure. Incubation medium was centrifuged to sediment the tissues. The precipitate was washed several times in 80% ethanol. Ethanolic solutions were pooled and the ethanol was evaporated under nitrogen. The aqueous residue was extracted with 6X10 ml chloroform:diethyl ether (1:3). The extract was chromatographed on paper in system A for 30 hr. The chromatogram showed 3 radioactive areas: area 1 (origin), area 2 (containing 17α-OHP and T), and area 3 (containing 20α-H_2P). The effluent was chromatographed in system D. The chromatogram showed 2 radioactive areas: area 4 (containing A) and area 5 (containing P). Area 1 was not further studied. Area 2 was acetylated with 1 ml pyridine-acetic anhydride (1:1) for 18 hr and chromatographed in system A to separate 17α-OHP and T acetate. Testosterone acetate, 17α-OHP, A and 20α-H_2P were purified separatly by t.l.c. developed in systems II, I, III and I respectively.

2) Incubations with [^{14}C]-T - Carrier, unlabeled, steroids (100 µg of each A, T, DHT, 3α-DIOL, androstanedione and androsterone) and known amounts of [^{3}H]-DHT, [^{3}H]-T and [^{3}H]-A were added at the end of the incubation. The steroids were extracted as described above. The steroid extract was chromatographed in system E for 24 hr. The chromatogram showed 3 radioactive areas: area 1 (containing T and 3α-DIOL), area 2 (containing A and DHT) and area 3 (containing androstanedione). No radioactivity was associated with androsterone. Area 1 was acetylated and chromatographed in system B. The chromatogram showed 3 areas: area 1a (containing T acetate), area 1b and area 1c (containing 3α-DIOL diacetate). Areas 1b and 1c were saponified in 1 ml 1% KOH in methanol for 24 hr and chromatographed in system C for 26 hr. The chromatogram revealed that these 2 areas were heterogenous; area 1b contained a fraction isopolar with 3β-DIOL and area 1c contained a fraction isopolar with 3α-DIOL. Area 2 was acetylated and chromatographed in system F to separate A and DHT acetate. DHT acetate was saponified and chromatographed in system I. Area 3 was submitted to t.l.c. in system IV; after this chromatography, the zone corresponding to androstanedione was free of radioactivity.

Identification of steroids and calculations – Radiochemical purity of the steroid products was established by recrystallization with 15-20 mg of authentic reference steroids to constant specific activity. The specific activity was considered as constant when the values of 3 successive crystallizations were within ± 5 % of the mean (16).

Results are expressed as percentages of initial radioactivity and as ng of product formed using the specific activities of the [^{14}C]-precursors. The levels of radioactivity in the products were corrected for losses in extraction and purification using the [^{3}H]-tracers.

RESULTS AND DISCUSSION

Gillet and Laporte (14) have shown that pregnant female rats injected with busulphan (10 mg/kg) on day 20 of gestation produce male offspring whose gonocytes degenerate completely during the first days of life. The other testicular cells, particularly the supporting cells (immature Sertoli cells) and the Leydig cells , seem to be unaffected by the drug. Indeed, the immature Sertoli cells

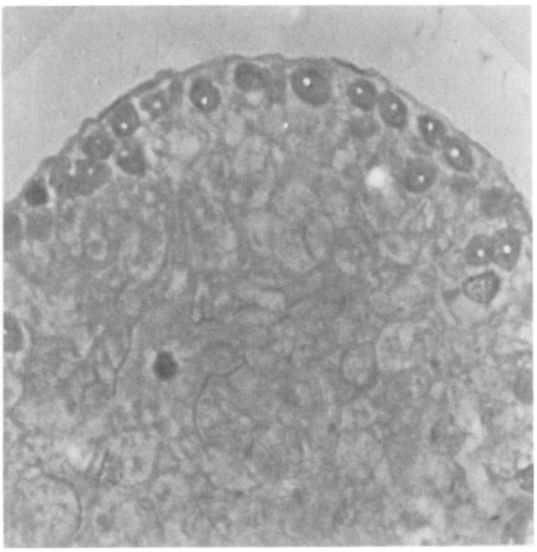

FIGURE 1. Histological cross section of a seminiferous tubule of busulphan-treated rat (Magnification: X350). Note the refringent nucleoli. The tissues were fixed in Carnoy's fixative, embedded in paraffin and 7 µm sections were stained with hematoxylin and indigocarmine

divide normally during the first two weeks of life, after which their number per tubule section remains constant as in a normal testis (14). As shown in Figure 1, the seminiferous tubules of the adult animals are completely deprived of germ cells and consist exclusively of Sertoli cells and peritubular cells. The Sertoli cells examined by electron microscopy show a nucleus with deep indentations, large mitochondria with numerous tubular cristae, cytoplasmic lipid droplets and microtubules. They are separated from each other by specialized junctions whose structure is identical to that seen in mature Sertoli cells of normal rats. This last observation suggests that the Sertoli cell maturation can occur even in the absence of germ cells. The same conclusion was formulated by Tindall et al. (17) who studied prenatally irradiated rats.

We studied the metabolism of $[^{14}C]$-P and $[^{14}C]$-T by Sertoli cell-enriched tubules and Sertoli cell-enriched testes of treated rats, and tubules of normal rats. In confirmation of the work of others (3,11,18), we found that tubules of normal rats incubated with P produced 17α-OHP, A and T in the in-

Table I. *In vitro* metabolism of [^{14}C] progesterone by Sertoli cell-enriched tubules and Sertoli cell-enriched testes of adult rats prenatally treated with busulphan and by seminiferous tubules of normal rats [a]

Metabolites	Percentages of the initial radioactivity [b]					
	SCE tubules [c]		SCE testes		Normal tubules	
	Exp. 1 (19 mg) [d]	Exp. 2 (18mg)	Exp. 1 (16mg)	Exp. 2 (22mg)	Exp. 1 (23mg)	Exp.2 (26mg)
P (unconverted) [e]	82	78	14.0	9.2	89	81
20α-H_2P	2.0 (360)	1.2 (220)	2.1 (400)	2.4 (450)	0.4 (70)	0.5 (90)
17α-OHP	1.2 (220)	0.7 (130)	56.2 (10700)	50.8 (9650)	0.3 (55)	0.4 (70)
A	0.3 (55)	0.1 (20)	4.7 (900)	8.3 (1600)	0.03 (5)	ND [f]
T	0.1 (20)	0.04 (5-10)	17.6 (33 50)	18.3 (3480)	0.05 (10)	0.1 (20)

a- 100-200 mg of isolated tubules and undissected testes were incubated with 8×10^6 dpm [^{14}C]-P in 2 ml KRBG at 31°C in 95% air- 5% CO_2 for 2 hr.
b- Numbers in parentheses: Quantity of product in ng
c- SCE: Sertoli cell-enriched
d- Quantity of tissue (dry wt)
e- Unconverted P was not crystallized in incubations of isolated tubules
f- ND: non detectable

cubation medium. They synthesized also 20α-H_2P (Table I). Hall *et al.* (18) reported that the tubules isolated from two testes produced 50 ng T when incubated with 14 µg P for 1 hour ; we found that 100-200 mg of tubules produced 10-20 ng T when incubated with 17 µg P for 2 hours. Normal tubules converted T to A and 5α-reduced compounds, DHT and 3α-DIOL, which confirms the work of other authors (6-9) (Table II). In contrast to these authors, we found that the main 5α-reduced metabolite was DHT and not 3α-DIOL . This result is probably due to the high quantity of T used as precursor. Indeed, Lloret and Weisz (9) suggested that a high concentration of T could inhibit 3α-hydroxysteroid dehydrogenase activity and therefore give rise to an accumulation of DHT. We observed that normal tubules produced also 3β-DIOL (Table II). Neither androstanedione

Table II. In vitro metabolism of [^{14}C]testosterone by Sertoli cell-enriched tubules and Sertoli cell-enriched testes of adult rats prenatally treated with busulphan and by seminiferous tubules of normal rats [a]

Metabolites	Percentages of the initial radioactivity [b]				
	SCE tubules [c]		SCE testes	Normal tubules	
	Exp. 1 [d] (17mg)	Exp. 2 (18mg)	Exp. 1 (24mg)	Exp. 1 (18mg)	Exp. 2 (17 mg)
T (unconverted) [e]	93	96	90	96	95
A	3.6 (610)	3.3 (560)	8.9 (1600)	5.5 (930)	5.4 (920)
DHT	0.9 (155)	1.2 (200)	0.5 (85)	0.8 (140)	0.9 (155)
3α-DIOL	0.3 (50)	0.2 (35)	0.2 (35)	0.1 (20)	0.2 (35)
3β-DIOL	0.1 (20)	0.1 (20)	0.3 (50)	ND [f]	0.2 (35)

a- 100-200 mg of isolated tubules and undissected testes were incubated with $8X10^6$ dpm [^{14}C]-T in 2 ml KRBG at 31°C in 95% air- 5% CO_2 for 2 hr.
b- Numbers in parentheses: quantity of product in ng
c- SCE: Sertoli cell-enriched
d- Quantity of tissue (dry wt)
e- Unconverted T was not crystallized
f- ND: non detectable

nor androsterone was detected in the incubation medium.

Sertoli cell-enriched tubules produced the same metabolites as normal tubules when incubated with P (Table I). Of the initial radioactivity, 0.04-0.1% was recovered in the form of T (about 15 ng), 0.7-1.2% as 17α-OHP (175ng), 0.1-0.3% as A (40 ng) and 1.2-2% as 20α-H_2P (290 ng). About 80% of the P remained unconverted.

Fragments of Sertoli cell-enriched testes were much more active than isolated tubules (Table I). They converted about 90% of the P. Of the initial radioactivity, 18% was found in T. The major metabolite was 17α-OHP. In contrast to that observed with isolated tubules, only a small percentage of radioactivity was recovered in the form of 20α-H_2P.

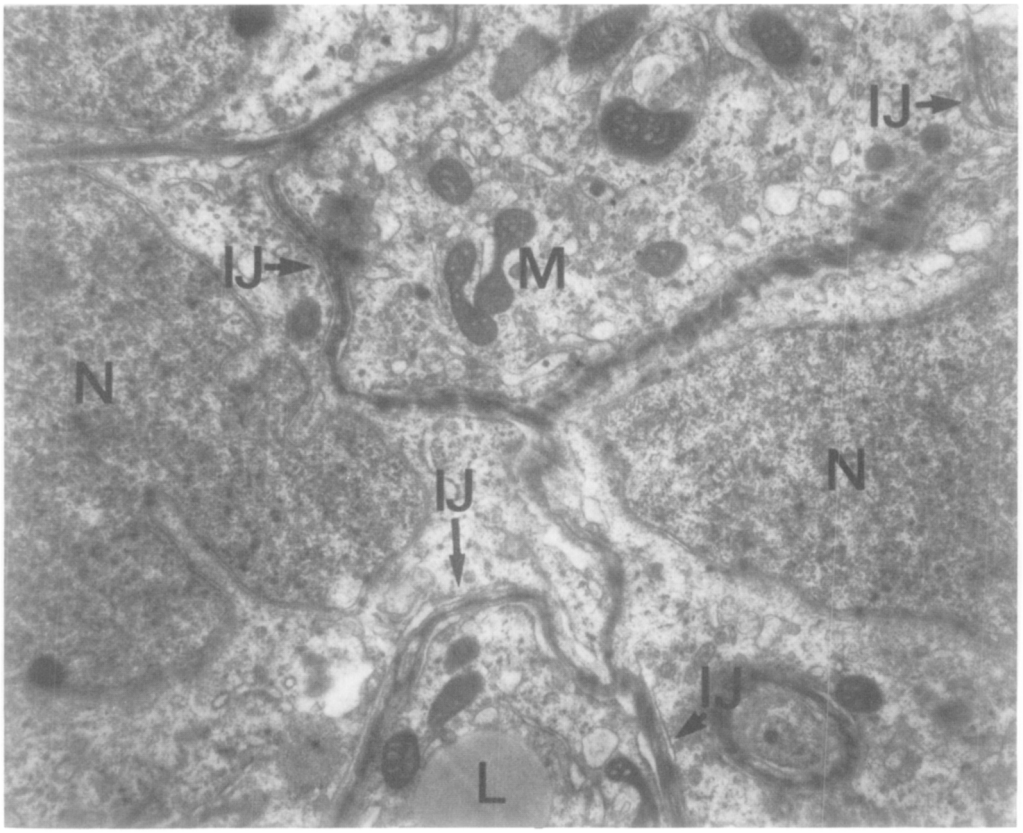

FIGURE 2: Electron micrograph of the Sertoli cells of busulphan-treated rat (Magnification: X8000). IJ= Intercellular junction; M= mitochondria; N= nucleus; L= lipid droplet. The tissues were fixed in 2% glutaraldehyde in a cacodylate buffer containing sucrose and postfixed in 2% osmium tetroxyde in cacodylate buffer. They were embedded in epon and 600 Å sections were contrasted with uranyl acetate and lead citrate.

It is to be noted that the metabolism of P by testes and tubules was sufficiently different to conclude that all the steroids identified after the incubations of tubules, except perhaps T, were synthesized by the tubules themselves and not by contaminating Leydig cells.

Sertoli cell-enriched tubules incubated with T produced the same metabolites as normal tubules. Of the initial radioactivity, 3.3-3.6% was found in A (585 ng), 0.9-1.2% in DHT (175 ng), 0.2-0.3% in 3α-DIOL (40 ng) and 0.1% in 3β-DIOL (20 ng). As in normal tubules, the major 5α-reduced steroid was DHT (Table II).

Sertoli cell-enriched testes produced the same amounts of 5α-reduced metabolites as isolated tubules (Table II).

The present experiments demonstrate that seminiferous tubules deprived of germ cells are capable of metabolizing P to androgens and 20α-H_2P *in vitro*. The physiological significance of the synthesis of 20α-H_2P in the tubules, and in the somatic cells in particular, is not known. It has been proposed that this steroid might play a role in regulating the androgen production (19). The tubular synthesis of androgens does not take place only in the somatic cells; Galena and Terner (20) demonstrated that it takes place also in non flagellate germ cells. Our experiments show that Sertoli cell-enriched tubules from adult rats contain a 5α-reductase; they are able to convert T to 5α-reduced metabolites, particularly DHT, *in vitro*. The 5α-reductase activity is present also in primary spermatocytes and in Sertoli cells from immature rats (12,13). It seems to be absent in round spermatids from adult rats (21).

ACKNOWLEDGMENTS

This work was supported by grants from the "Délégation Générale à la Recherche Scientifique et Technique" (No. 72- 704-81) and from the "Fondation pour la Recherche Médicale Française". The authors are grateful to Drs Laporte and Gillet for providing animals treated with busulphan. They wish to thank Dr Herlin for her help in electron microscopy. The technical assistance of Miss Liégard is gratefully acknowledged.

REFERENCES

1. Steinberger, E. (1971) Physiol. Rev., 51, 1-22
2. Harris, M.E., and Bartke, A. (1975) Endocrinology, 96, 1396-1402
3. Christensen, A.K., and Mason, N.R. (1965) Endocrinology, 76, 646-656
4. Bell, J.B.G., Vinson, G.P., Hopkin, D.J., and Lacy, D. (1968) Biochem. Biophys. Acta, 164, 412-420
5. Richards, G., and Neville, A.M. (1973) Nature New Biol., 244, 359-361
6. Rivarola, M.A., and Podesta, E.J. (1968) Endocrinology, 90, 618-623
7. Yamada, M., Yasue, S., and Matsumoto, K. (1972) Acta Endocrinol. (Kbh), 71, 393-400
8. Folman, Y., Ahmad, N., Sowell, J.G., and Eik-Nes, K.B. (1973) Endocrinology, 92, 41-47
9. Lloret, A.P., and Weisz, J. (1974) Endocrinology, 95, 1306-1316
10. Ahmad,N., Haltmeyer, G.C., and Eik-Nes, K.B. (1973) Biol. Reprod., 8, 411-419
11. Lacy, D., Vinson, G.P., Collins, P., Bell, P., Pudney, J., and Pettit, A.J. (1969) In progress in Endocrinology (Proc. 3 rd. Int. Congr. Endocrinol. Mexico, 1968 (Gual, C., ed.), pp. 1019-1029, Excerpta Medica Foundation, Amserdam

12. Dorrington, J.H., and Fritz, I.B. (1975) Endocrinology, 96, 879-889
13. Welsh, M.J., and Wiebe, J.P. (1976) Biochem. Biophys. Res. Commun., 69, 936-941
14. Gillet, J., and Laporte, P. (1973) Arch. Anat. Microsc. Morphol. Exp., 62, 385-398
15. Tencé, M., Tencé, J.F., and Drosdowsky, M. (1975) C. R. Acad. Sci. Ser.D, 280, 991-994
16. Axelrod, L.R., Mattijssen, C., Goldzieher, J.W., and Pulliam, J.E. (1965) Acta Endocrinol. (Kbh) Suppl. 99
17. Tindall, D.J., Vitale, R., and Means, A.R. (1975) Endocrinology, 97, 636-648
18. Hall, P.F., Irby, D.C., and de Kretser, D.D. (1969) Endocrinology, 84, 488-496
19. Fan, D., Oshima, H., Troen, B.R., and Troen, P. (1974) Biochem. Biophys. Acta, 360, 88-99
20. Galena, H.J., and Terner, C. (1974) J. Endocrinol., 60, 269-276
21. Tencé, M., Tencé, J.F., Barral, I., and Drosdowsky, M. (1975) Ann. Endocrinol. (Paris), 36, 213-214