Secretion of Unconjugated Androgens and Estrogens by the Normal and Abnormal Human Testis before and after Human Chorionic Gonadotropin

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ABSTRACT The secretion of androgens and estrogens by normal and abnormal testes was compared by determining the concentrations of dehydroepiandrosterone (DHEA), androstenedione (Δ_4A), testosterone (T), estrone (E_1), and 17β-estradiol (E_2) in peripheral and spermatic venous plasma samples from 14 normal men and 5 men with unilateral testicular atrophy. Four normal men and one patient with unilateral atrophy of the testis were given human chorionic gonadotropin (HCG) before surgery. Plasma estrogens were determined by radioimmunoassay; plasma androgens were measured by the double-isotope dilution derivative technique. Peripheral concentrations of these steroids before and after HCG were similar in both the normal men and the patients with unilateral testicular atrophy. In normal men, the mean $\pm$SE spermatic venous concentrations were DHEA, 73.1$\pm$11.7 ng/ml; Δ_4A, 30.7$\pm$7.9 ng/ml; T, 751$\pm$114 ng/ml; E_1, 306$\pm$55 pg/ml; and E_2, 1298$\pm$216 pg/ml. Three of four subjects with unilateral testicular atrophy had greatly diminished spermatic venous levels of androgens and estrogens. HCG treatment increased the testicular secretion of DHEA and T fivefold, Δ_4A threefold, E_1 sixfold, and E_2 eightfold in normal men. In the single subject with an atrophic testis who received HCG, the spermatic venous concentrations of androgens and estrogens were much less than in normal men similarly treated. We conclude that: (*a*) E_1 is secreted by the human testis, but testicular secretion of E_1 accounts for less than 5% of E_1 production in normal men; (*b*) HCG stimulation produces increases in spermatic venous estrogens equal to or greater than the changes in androgens, including testosterone; and (*c*) strikingly decreased secretion of androgen and estrogen by unilateral atrophic human testes cannot be appreciated by analyses of peripheral steroid concentrations.

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INTRODUCTION

Secretion of 17β-estradiol (E_2)[1] by the human testis has recently been documented and compared to the secretion of testosterone (1). To clarify further the role of the normal and abnormal human testis in androgen and estrogen production, we determined the concentrations of dehydroepiandrosterone (DHEA), androstenedione (Δ_4A), testosterone (T), estrone (E_1), and E_2 in peripheral and spermatic venous plasma obtained from 14 normal men and 5 men with unilateral testicular atrophy. In addition, testicular secretory responsiveness was assessed by administering human chorionic gonadotropin (HCG) before surgery to four normal men and one man with an atrophic testis.

[1] *Abbreviations used in this paper:* Δ_4A, androstenedione; DHEA, dehydroepiandrosterone; E_1, estrone; E_2, 17β-estradiol; HCG, human chorionic gonadotropin; T, testosterone.

METHODS

Subjects. Peripheral and spermatic venous blood was drawn simultaneously during an elective inguinal herniorrhaphy (13 subjects) or varicocelectomy (patient 8, Tables I and II) in 14 normal adults. Four subjects received intramuscular HCG before surgery: three 5,000 U/day for 4 days and one 5,000 U/day for 2 days. For comparison, five additional subjects with unilateral testicular atrophy (testis less than 3.0 cm in greatest diameter) were similarly studied during an elective inguinal herniorrhaphy on the side of the atrophic testis. One of these subjects (patient 19, Tables III and IV) also received HCG (5,000 U/day for 4 days) before surgery. Spermatic venous blood was collected as previously described (1). Informed consent was obtained from all subjects.

Steroid analyses. Plasma levels of DHEA, Δ_4A, and T were determined by a modification (2) of the double-isotope dilution derivative technique combined with gas-liquid chromatography (3). Plasma concentrations of E_2 and E_1 were performed by radioimmunoassay. The details of our initial assay of E_2 and the modifications in methodology for simultaneous measurement of plasma E_1 and E_2 have been described in detail (1, 4). Duplicate determinations of an adult female plasma pool (10 assays) averaged 87±8.6 (SD) pg/ml for E_1 and 123±14.1 (SD) pg/ml for E_2. The concentrations of E_1 and E_2 in spermatic venous plasma were determined at four or more different dilutions to ensure parallelism with the standards.

The cross-reactivity of nonphenolic C_{19} and C_{21} steroids is negligible in both E_1 and E_2 radioimmunoassays. For example, T shows 0.001% cross-reactivity in the E_1 assay and less than 0.001% in the E_2 assay. In addition, the Sephadex LH-20 chromatographic system used in the estrogen assays reliably separates C_{19} and C_{21} plasma steroids from the E_1 and E_2 fractions (4). Nonetheless, the remarkably increased concentrations of unconjugated steroids observed in the spermatic venous samples, especially after HCG administration, prompted further validation of the techniques used in this study. DHEA, Δ_4A, T, E_1, and E_2 were quantitatively recovered from a normal saline: absolute ethanol (95:5) solution that contained the following concentrations of unconjugated steroids: DHEA, 500; Δ_4A, 100; T, 2,500; E_1, 2.0; and E_2, 20 ng/ml. The experimentally determined values obtained for these steroids were: (Mean±SD) DHEA, 475±8.0; Δ_4A, 101±2.1, T, 2520 ±36; E_1, 1.99±0.8; and E_2, 15.9±1.8 ng/ml. In addition to the above steroids, the "simulated spermatic venous plasma sample" also contained various other steroids at the following concentrations: 5-androstene-3β, 17α-diol, 100; 5-androstene-3β,17β-diol, 800; 4-androstene-3β,17αdiol, 100; 4-androstene-3β,17β-diol, 800; pregnenolone, 200; progesterone, 200; 17αhydroxypregnenolone, 500; and 17αhydroxyprogesterone, 250 ng/ml.

RESULTS

Normal human testis

Androgen secretion before and after HCG. Table I lists the results of peripheral and spermatic venous concentrations in 14 normal adults, 21–60 yr old. The values for DHEA in peripheral plasma and the concentrations of Δ_4A in peripheral and spermatic venous plasma agree closely with previous reports (2, 5, 6). The mean spermatic vein concentration of DHEA is somewhat higher but this was influenced greatly by three subjects, 3, 4, and 7, who had remarkably high

TABLE I

Peripheral and Spermatic Venous Plasma Concentrations of DHEA, Androstenedione and Testosterone in Normal Man

Subject	Age	DHEA		Androstenedione		Testosterone	
		Peripheral	Spermatic	Peripheral	Spermatic	Peripheral	Spermatic
no.	*yr*	*ng/ml*		*ng/ml*		*ng/ml*	
1	60	3.8	20.9	1.1	13.2	2.8	365
2	44	1.9	74.1	1.0	32.6	3.8	984
3	45	5.1	116.0	1.3	18.1	2.8	362
4	35	1.4	138.0	0.5	20.7	3.7	767
5	21	7.2	57.3	0.6	11.4	11.0	878
6	24	3.7	30.1	0.8	54.0	9.7	738
7	31	7.8	101.0	1.5	89.9	6.8	1,550
8	23	3.3	48.3	0.9	9.9	5.4	350*
9	50	2.4	77.8	0.5	20.3	3.3	695*
10	53	1.9	66.8	0.7	36.8	6.3	821*
Mean concn (±SE)		3.9±0.7	73.1±11.7	0.9±0.1	30.7±7.9	5.6±0.9	751±114
HCG treatment‡							
11	40	6.5/7.2§	300	0.6/0.8	56	7.8/17.6	2,870
12	24	3.8/3.9	380	0.5/0.9	89	10.2/23.8	5,780
13	31	2.5/3.4	343	1.6/2.1	117	5.1/11.0	3,030
Mean concn (±SE)		4.3±1.2/4.8±1.4	341±23.1	0.9±0.4/1.3±0.4	87±18	7.7±1.5/17.5±3.7	3,890±944
14	27	1.5/1.9	179	0.5/0.9	44	7.1/11.3	1,210

* Testosterone values reported previously (1).
‡ Subjects 11, 12 and 13: 5,000 U daily for 4 days; subject 14: 5,000 U daily for 2 days.
§ Before/after HCG.

TABLE II

Peripheral and Spermatic Venous Plasma Concentrations of Estrone and Estradiol in Normal Men

Subject	Age	Estrone		Estradiol	
		Peripheral	Spermatic	Peripheral	Spermatic
no.	*yr*	*pg/ml*		*pg/ml*	
1	60	107	135	33	448
2	44	52	162	23	709
3	45	69	665	34	2,550
4	35	52	288	34	2,158
5	21	48	227	37	790
6	24	54	167	23	900
7	31	46	398	20	1,900
8	23	*	*	21	1,235‡
9	50	*	374	16	1,260‡
10	53	*	340	13	1,030‡
Mean concn (±SE)		61±8	306±55	25±2.3	1,298±216
		HCG treatment§			
11	40	53/62‖	1,397	42/94	10,800
12	24	27/57	1,423	15/101	11,800
13	31	58/72	1,900	32/116	8,500
Mean concn (±SE)		46±10/64±4	1,573±164	30±8/104±7	10,367±977
14	27	*/30	963	*/43	7,419

* Volume not sufficient.
‡ Estradiol values reported previously (1).
§ Subjects 11, 12, and 13: 5,000 U daily for 4 days; subject 14: 5,000 U daily for 2 days.
‖ Before/after HCG.

values. The range and mean spermatic venous T closely agree with values previously reported by us (1) and by Laatikainen, Laitinen, and Vihko (6) but is higher than values reported by Hudson, Coghlan, Dulmanis, and Wintour (7) and by Jeffcoate, Brooks, Lim, London, Prunty, and Spathis (8). After HCG administration to three subjects, 11, 12, and 13, changes in mean peripheral levels of DHEA (4.3 ng/ml to 4.8 ng/ml) and Δ_4A (0.9 ng/ml to 1.3 ng/ml) were much smaller compared with T (7.7 ng/ml to 17.5 ng/ml). However, mean spermatic venous DHEA, Δ_4A, and T increased similarly, approximately 3–5-fold. In subjects 14, only 2 days of HCG clearly increased the concentrations of DHEA, Δ_4A, and T in spermatic venous plasma.

Estrogen secretion before and after HCG. Table II lists the peripheral and spermatic venous concentrations of E_1 and E_2 in normal subjects. Peripheral E_1 levels in early morning plasma samples ranged between 46 pg/ml and 107 pg/ml, while the mean concentration was 61±8 (SE) pg/ml. These values are comparable to other reports (9–11) but are slightly higher than concentrations in randomly obtained specimens in adult males not undergoing surgery (range 20–69 pg/ml; mean±SE, 40±5) (4). The concentrations of E_2 in peripheral plasma agree with values previously reported by us (1, 4) and others (9, 11, 12). Spermatic venous E_1 concentrations ranged from 135 pg/ml to 665 pg/ml. Concentrations of spermatic venous E_2 are slightly higher than in our previous report (1). This increase, similar to the changes in DHEA, was influenced greatly by subjects 3, 4, and 7. Concentrations of spermatic venous E_2 were approximately fourfold greater than spermatic venous E_1 levels. HCG, in subjects 11, 12, and 13, produced a slight increase in peripheral venous E_1 but a greater, approximately threefold, increase in E_2, while testicular gradients (spermatic minus peripheral) of E_1 and E_2 increased six- and eightfold, respectively. In subject 14 after only 2 days of HCG, four- and sixfold increases, respectively, in testicular gradients of E_1 and E_2 were observed.

Abnormal human testis

Androgen secretion before and after HCG. Table III lists the peripheral and spermatic venous concentrations of DHEA, Δ_4A, and T in five subjects, each with an atrophic testis. Peripheral DHEA, Δ_4A, and T were

TABLE III

Peripheral and Spermatic Venous Plasma Concentrations of DHEA, Androstenedione, and Testosterone in Men with a Unilateral Atrophic Testis

Subject	Age	DHEA		Androstenedione		Testosterone	
		Peripheral	Spermatic	Peripheral	Spermatic	Peripheral	Spermatic
no.	*yr*	*ng/ml*		*ng/ml*		*ng/ml*	
15	28	2.3	14.3	1.1	3.7	6.8	95.3*
16	31	5.5	10.7	0.9	2.3	4.1	76.0
17	22	6.6	12.6	1.3	4.0	5.2	99.8*
Mean concn (±SE)		4.7±1.2	12.5±1.0	1.1±0.1	3.3±0.5	5.2±0.6	89±7.1
18	26	5.6	57.8	1.1	29.1	3.0	996*
			HCG treatment‡				
19	23	3.7/4.4§	25.0	1.5/1.8	12.0	4.7/11.0	260

* Testosterone values reported previously (1).
‡ Subject 19: 5,000 U daily for 4 days.
§ Before/after HCG.

normal. Spermatic venous DHEA, Δ_4A, and T were markedly reduced in three subjects, 15, 16, and 17, while levels in subject 18 were normal. After HCG, a normal increase in the peripheral concentrations of DHEA, Δ_4A, and T was found in subject 19. However, the spermatic venous concentrations of DHEA, Δ_4A, and T were much less than in normal men treated with HCG.

Table IV lists the peripheral and spermatic venous E_1 and E_2 concentrations in five subjects, each with an atrophic testis. Peripheral concentrations of E_1 and E_2 were normal. Like the concentrations of androgens, spermatic venous concentrations of E_1 and E_2 were strikingly reduced in subjects 15, 16, and 17. In subject 18, E_1 and E_2 spermatic venous concentrations were low-normal. In subject 19, the spermatic venous concentrations of E_1 and E_2 were much less than the levels found in normal men similarly treated with HCG; however, HCG produced a normal rise in peripheral E_1 and E_2.

DISCUSSION

The concentrations of unconjugated androgens and estrogens in peripheral and spermatic venous plasma have been compared in men with normal and abnormal testes before and after HCG. In normal subjects concentrations of unconjugated Δ_4A and T in spermatic venous plasma

TABLE IV

Peripheral and Spermatic Venous Plasma Concentrations of Estrone and 17β-Estradiol in Men with a Unilateral Atrophic Testis

Subject	Age	Estrone		17β-Estradiol	
		Peripheral	Spermatic	Peripheral	Spermatic
no.	*yr*	*pg/ml*		*pg/ml*	
15	28	66	84	24	280*
16	31	31	61	25	104
17	22	36	102	28	186*
Mean concn (±SE)		44±11	72±15	26±1	190±51
18	26	‡	164	23	416*
		HCG treatment§			
19	23	72/196‖	555	34/99	1,849

* Estradiol values reported previously (1).
‡ Volume not sufficient.
§ Subject 19: 5,000 U daily for 4 days.
‖ Before/after HCG.

and the responses to HCG are in agreement with values reported by Laatikainen et al. (6). However, the concentration of DHEA in spermatic venous plasma was higher. This difference can be explained, in part, by the remarkably high values in subjects 3, 4, and 7.

After HCG, peripheral levels of DHEA and Δ_4A increased 50% or less, while spermatic venous concentrations increased five- and threefold, respectively. Since the major source of these steroids is adrenal and not testicular, the small increase in peripheral plasma values would be expected.

Recently secretion of E_2 by the normal human testis was established (1, 13), which allowed us to estimate that testicular secretion of E_2 accounts for at least one-fourth of E_2 production (1). We now present direct evidence for the testicular secretion of E_1 with a testicular E_1 gradient of 245 pg/ml. Utilizing the mean blood production rate of T, 7 mg/day for normal adult males (14), and the experimentally determined T and E_1 gradients from this study, we estimate testicular E_1 secretion to be 2.5 μg/day. A comparison of this estimate with calculated production rates of E_1 (45 μg/day–80 μg/day) (15, 16) indicates that testicular secretion accounts for less than 5% of E_1 production in normal men. Thus, nearly all of E_1 production is by peripheral hormonal interconversions (17, 18) and extratesticular secretion (19).

Longcope, Widrich, and Swain (20) recently reported the secretion of E_1 by human testes. Perhaps because Longcope et al. obtained spermatic venous plasma via renal vein catheterization and not from the spermatic plexus, their values for the spermatic venous concentrations of E_1, E_2, Δ_4A, and T were lower than those of most previous reports, including the present study.

After HCG, spermatic venous E_1 and E_2 increased strikingly: E_1, sixfold; and E_2, eightfold. These responses were equal to or greater than changes observed in any of the androgens, including T. The slight increase in the peripheral concentrations of E_1 after HCG support our conclusion that the major sources of E_1 are nontesticular. In contrast, the larger rise in peripheral E_2 after HCG (fourfold) also indicates that the testicular secretion of E_2 is greater than E_1.

Spermatic venous concentrations of unconjugated androgens and estrogens in three subjects, 15, 16, and 17, with unilateral testicular atrophy were significantly reduced. The normal peripheral levels in these subjects suggest that the contralateral testis had compensated by secretion of greater than normal amounts of androgens and estrogens. The low values found in the spermatic venous samples in HCG-stimulated and untreated patients with unilateral atrophic testes (four of five) indicate that hormone secretion and secretory reserve are limited in these instances.

Although comparisons between the subjects in this study (treated vs. untreated and normal vs. atrophic testes) seem valid, unqualified extrapolation of these data to the normal state is unwarranted. For example, surgical stress has been shown to decrease plasma T in men (cf. peripheral T values in subjects 1 and 3) (21). Further, the effects of anesthesia on testicular blood flow and on metabolic clearance of unconjugated steroids are unclear.

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REFERENCES

1. Kelch, R. P., M. R. Jenner, R. L. Weinstein, S. L. Kaplan, and M. M. Grumbach. 1972. Estradiol and testosterone secretion by human, simian, and canine testes, in males with hypogonadism and in male pseudohermaphrodites with the feminizing testes syndrome. *J. Clin. Invest.* **51**: 824.
2. Weinstein, R. L., and R. E. Reitz. 1974. Pituitary-testicular responsiveness in males with hypogonadotropic hypogonadism. I. Biochemical observations. *J. Clin. Invest.* In press.
3. Kliman, B., and C. Briefer, Jr. 1967. Collection of carbon-14 and tritium labeled steroids in gas liquid chromatography with application to the analysis of testosterone in human plasma. *In* The Gas Liquid Chromatography of Steroids. J. R. Grant, editor. Cambridge University Press, London. 229.
4. Kelch, R. P., S. L. Kaplan, and M. M. Grumbach. 1973. Suppression of urinary and plasma follicle-stimulating hormone by exogenous estrogens in prepubertal and pubertal children. *J. Clin. Invest.* **52**: 1122.
5. Gandy, H. M., and R. E. Peterson. 1968. Measurement of testosterone and 17-ketosteroids in plasma by the double isotope dilution derivative technique. *J. Clin. Endocrinol. Metab.* **28**: 949.
6. Laatikainen, T., E. A. Laitinen, and R. Vihko. 1971. Secretion of free and sulfate-conjugated neutral steroids by the human testis. Effect of administration of human chorionic gonadotropin. *J. Clin. Endocrinol. Metab.* **32**: 59.
7. Hudson, B., J. P. Coghlan, A. Dulmanis, and M. Wintour. 1964. The measurement of testosterone in biological fluids in the evaluation of androgen activity. *Excerpta Med. Int. Congr. Ser.* **83**: 1127.
8. Jeffcoate, S. L., R. V. Brooks, N. Y. Lim, D. R. London, F. T. G. Prunty, and G. S. Spathis. 1967. Androgen production in hypogonadal men. *J. Endocrinol.* **37**: 401.
9. Baird, D. T. 1968. A method for the measurement of estrone and estradiol-17β in peripheral human blood and other biological fluids using ^{35}S pipsyl chloride. *J. Clin. Endocrinol. Metab.* **28**: 244.
10. Tulchinsky, D., and S. G. Korenman. 1970. A radio-

ligand assay for plasma estrone; normal values and variations during the menstrual cycle. *J. Clin. Endocrinol. Metab.* **31**: 76.

11. Mikhail, G., C. H. Wu, M. Ferin, and R. L. Vande Wiele. 1970. Radioimmunoassay of plasma estrone and estradiol. *Steroids.* **15**: 333.
12. Korenman, S. G., L. E. Perrin, and T. P. McCallum. 1969. A radio-ligand binding assay system for estradiol measurement in human plasma. *J. Clin. Endocrinol. Metab.* **29**: 879.
13. Leonard, J. M., R. H. Flocks, and S. G. Korenman. 1971. Estradiol (E_2) secretion by the human testis. Program of the Endocrine Society, 53rd Meeting, June 24–26, 1971. The Endocrine Society, San Francisco. A-99 (Abstr. 113).
14. Lipsett, M. B. 1969. The testis. *In* Duncan's Diseases of Metabolism. P. K. Bondy, editor. W. B. Saunders Company, Philadelphia. 6th edition. 1174.
15. Lipsett, M. B. 1970. Steroid secretion by the human testis. *In* The Human Testis, Advances in Experimental Medicine and Biology. Vol. 10. E. Rosemberg and C. A. Paulsen, editors. Plenum Publishing Corporation, New York. 407.
16. Longcope, C. 1972. Postural effects on metabolism of ^{3}H-Δ4-androstenedione (Δ_4A) and ^{3}H-estrone (E_1). *Excerpta Med. Int. Congr. Ser.* **256**: 528. (Abstr.).
17. MacDonald, P. C., R. P. Rombaut, and P. K. Siiteri. 1967. Plasma precursors of estrogen. I. Extent of conversion of plasma Δ^4-androstenedione to estrone in normal males and nonpregnant normal, castrate, and adrenalectomized females. *J. Clin. Endocrinol. Metab.* **27**: 1103.
18. Longcope, C., T. Kato, and R. Horton. 1969. Conversion of blood androgens to estrogens in normal adult men and women. *J. Clin. Invest.* **48**: 2191.
19. Baird, D. T., A. Uno, and J. C. Melby. 1969. Adrenal secretion of androgens and estrogens. *J. Endocrinol.* **45**: 135.
20. Longcope, C., W. Widrich, and C. T. Swain. 1972. The secretion of estrone and estradiol-17β by human testis. *Steroids.* **20**: 439.
21. Carstensen, H., N. Terner, L. Thoren, and L. Wide. 1972. Testosterone, luteinizing hormone, and growth hormone in blood following surgical trauma. *Acta. Chir. Scand.* **138**: 1.