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STEROID SULPHATES IN HUMAN ADULT TESTICULAR STEROID SYNTHESIS

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SUMMARY

Our studies on the steroid composition and steroid secretion by human testis are summarized. Using gas-liquid chromatography and gas chromatography–mass spectrometry 24 neutral steroids were identified and measured in human testis tissue obtained from cadavers or in connexion with orchiectomy due to prostatic cancer. The compounds were partly present in unconjugated form and partly as mono- and disulphates. The main compounds present were unconjugated testosterone, pregnenolone and its sulphate, dehydroepiandrosterone sulphate and 5-androstene-3β,17β-diol monosulphate. C_{19} steroid diol monosulphates with a 17β-hydroxyl carried their sulphate group at carbon 3, whereas those with a 17α-hydroxyl had the sulphate group at carbon 17. The main unconjugated compound secreted by human testis was testosterone followed by 5-androstene-3β,17β-diol, 17α-hydroxyprogesterone, pregnenolone, 5-androstene-3β,17α-diol, 17α-hydroxypregnenolone, androstenedione and dehydroepiandrosterone. The monosulphates of pregnenolone, 5-androstene-3β,17β-diol and testosterone were also secreted. The secretion of all these compounds was increased during the administration of human chorionic gonadotropin (HCG) and dehydroepiandrosterone sulphate secretion also became evident. These results, when considered in the light of the findings of other authors strongly suggest that the 5-ene pathway is the quantitatively more important one in the formation of testosterone in human testis. Further, it is possible that sulphated steroids also act as precursors and that testosterone biosynthesis could be regulated through regulation of testicular steroid sulphatase activity.

INTRODUCTION

In human testis, testosterone can be synthesized by one of two pathways, the 4-ene pathway or the 5-ene pathway. In the former, the 3β-hydroxy-5-ene structure of pregnenolone is first converted to a 3-keto-4-ene structure (pregnenolone→progesterone→17α-hydroxyprogesterone→androstenedione→testosterone). In the 5-ene pathway, degradation of the side chain precedes the 3β-hydroxysteroid dehydrogenase catalyzed reaction, which is the final step in the pathway (pregnenolone→17α-hydroxypregnenolone→dehydroepiandrosterone→5-androstene-3β,17β-diol→testosterone).

The operation of both of these pathways in human testis has been demonstrated in a number of *in vitro* investigations (for review articles, see [1–3]). Recent research, to be summarized in this report, stresses the importance of the 5-ene pathway, previously considered to be, quantitatively, the less important one. Further, the role of sulphate-conjugated 3β-hydroxy-5-ene steroids as potential precursors of testosterone has led to renewed thinking on some aspects of steroidogenesis in human testis, especially because sulphate-conjugated steroids have, in the past, been considered mainly to be end products of steroid metabolism.

EXPERIMENTAL

Human adult testes were obtained either from cadavers and were dissected out shortly after death or from patients undergoing orchiectomy due to prostatic cancer and not receiving any hormonal treatment before the operation. Samples of testicular venous blood were obtained from volunteers undergoing operation for inguinal hernia. Heparinized blood samples were immediately centrifuged and the plasma separated. All the samples were stored at −20°C until analyzed.

Steroids in testis tissue as well as in testicular and peripheral venous blood were determined as described previously [4, 5]. The identification of the compounds was performed by gas–liquid chromatography and gas chromatography–mass spectrometry. Quantitation was carried out by gas–liquid chromatography. The position of the sulphate group was determined as described by Cronholm[6].

RESULTS

Table 1 summarizes the steroids present in human testis tissue and their concentrations. Both unconjugated, monosulphated and disulphated steroids were found. As could be expected, the main free steroid was testosterone. Pregnenolone was present in similar concentrations followed by 5α-androst-16-en-3β-ol. In the material so far investigated [4, 7, 8], however, the main steroids detected were sulphate-conjugated. Of the monosulphates, pregnenolone sulphate was the main compound found followed by dehydroepiandrosterone, 5-androstene-3β,17β-diol and testosterone sulphates. In addition to monosulphates, certain disulphates were also detected. Altogether seven disulphates were identified but all of them were present in concentrations less than 10% of the principal monosulphates (Table 1).

The steroid diol monosulphates found in testis tissue were sulphated at either of the two hydroxyl

Table 1. Concentrations of neutral steroids in human testis tissue. The values are mean concentrations (μg/100 g tissue wet weight) and have been calculated from groups of 3-10 testes (4,7,8).

Compound	Form of conjugation[a]	Concentration	
		Cadaver testis	Orchiectomy testis
Androsterone	M	5.8	7.8
Epiandrosterone	M	6.1	2.3
Dehydroepiandrosterone	F	0.8	1.3
	M	134	41
Testosterone	F	37	55
	M	37	6.8
Androstenedione	F	b	b
Epitestosterone	M	b	b
5α-16-Androsten-3α-ol	F	0.8	c
	M	0.6	c
5α-16-Androsten-3β-ol	F	29	c
	M	8.6	c
5,16-Androstadien-3β-ol	F	14	c
	M	13	c
5α-Androstane-3α,17β-diol	F	1.5	0.5
	M	1.5	2.1
	D	c	0.6
5α-Androstane-3β,17α-diol	M	2.0	c
5α-Androstane-3β,17β-diol	F	6.2	8.3
	M	4.5	2.8
	D	c	0.8
5-Androstene-3β,17α-diol	F	0.7	0.5
	M	28	15
	D	5.2	5.5
5-Androstene-3β,17β-diol	F	3.6	3.0
	M	80	19
	D	6.6	6.7
5-Androstene-3β,16α,17β-triol	M	b	b
Pregnenolone	F	53	25
	M	174	51
3β-Hydroxy-17α-5-pregnen-20-one (isopregnenolone)	M	15	c
17α-Hydroxypregnenolone	M	45	3.5
Progesterone	F	b	c
17α-Hydroxyprogesterone	F	10	c
20α-Hydroxy-4-pregnen-3-one	F	8	c
5α-Pregnane-3α,20α-diol	M	0.8	1.1
	D	0.5	0.8
5β-Pregnane-3α,20α-diol	M	0.6	1.0
	D	0.5	0.5
5-Pregnene-3β,20α-diol	F	3.4	4.3
	M	24	4.9
	D	3.1	3.9

a F = unconjugated steroid, M = monosulphate, D = disulphate
b Impurities or other steroids disturb exact quantification
c Not determined

groups. The position of the sulphate group was determined for the monosulphates of 5α-androstane-3α,17β-diol, 5α-androstane-3β,17α-diol, 5α-androstane-3β,17β-diol, 5-androstene-3β,17α-diol and 5-androstene-3β,17β-diol [8]. All the compounds carrying a 17β-hydroxyl group were conjugated at C-3, whereas those with a 17α-hydroxyl group were conjugated at C-17. In addition, the sulphate group of 5-pregnene-3β,20α-diol was exclusively esterified at C-3.

The extensive biosynthesis of steroids in human testis tissue is reflected in their secretion into spermatic venous blood. The excretion of a number of sulphate conjugates has also been detected. In Fig. 1 the unconjugated steroids detected and the amounts secreted are summarized. Testosterone was by far the quantitatively most important steroid secreted, its mean concentration being 74 μg/100 ml of spermatic venous blood in a group of five normal males. Intermediates in the 4-ene pathway, 17α-hydroxyprogesterone and androstenedione were also detected and their secretion was clearly increased during HCG (human chorionic gonadotropin) stimulation (Fig. 1). During

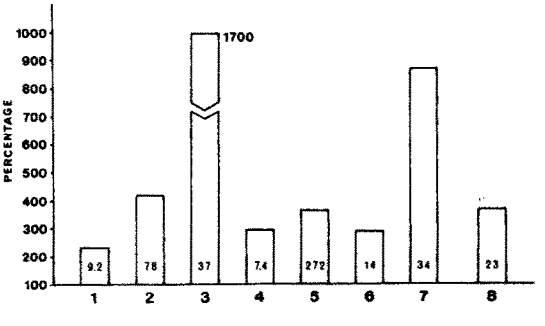

Fig. 1. Mean concentrations in three men of unconjugated neutral steroids in spermatic venous blood plasma during administration of HCG [9]. The values are expressed in percentages being compared to corresponding values from five nontreated individuals (= 100%). The figures in the columns show the actual concentration values (μg/100 ml of blood plasma) in the subjects receiving HCG. 1 = 5-Androstene-3β,17α-diol, 2 = 5-androstene-3β,17β-diol, 3 = dehydroepiandrosterone, 4 = androstenedione, 5 = testosterone, 6 = pregnenolone, 7 = 17α-hydroxypregnenolone, 8 = 17α-hydroxyprogesterone. HCG (Pregnyl, Organon, Oss, The Netherlands) was administered intravenously daily (5000 U) for 5 days. Sample taken on day 5.

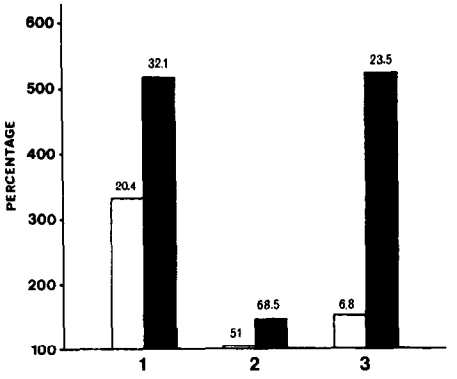

Fig. 2. Secretion of steroid sulphates by human testis. The 100% level represents steroid sulphate concentrations in peripheral blood plasma, the columns, corresponding concentrations in spermatic venous blood plasma without (open columns) and during (black columns) HCG administration. The figures represent the actual concentration values (μg/100 ml of blood plasma). 1 = Pregnenolone sulphate, 2 = dehydroepiandrosterone sulphate, 3 = 5-androstene-3β,17β-diol monosulphate. In addition, testosterone sulphate was secreted by human testis in concentrations of 1·8 and 3·3 μg/100 ml of spermatic venous blood plasma under normal conditions and during HCG administration, respectively [9, 10]. HCG administration, see legend to Fig. 1.

HCG stimulation, progesterone secretion, albeit in very small amounts, was also demonstrated. All the intermediates of the 5-ene pathway (pregnenolone, 17α-hydroxypregnenolone, dehydroepiandrosterone and 5-androstene-3β,17β-diol) were secreted as unconjugated compounds and the secretion of all these compounds was greatly increased during HCG treatment. 5-Androstene-3β,17α-diol was also secreted and in increased amounts during HCG treatment (Fig. 1).

The secretion of certain sulphate conjugates was also demonstrated (Fig. 2). Under basal conditions, the secretion of pregnenolone, 5-androstene-3β,17β-diol and testosterone sulphates was evident and their secretion was clearly increased during HCG administration (Fig. 2). Under these conditions, the secretion of dehydroepiandrosterone sulphate was also demonstrable.

DISCUSSION

The presence and secretion of intermediates of the 5-ene pathway, from pregnenolone to testosterone, in human adult testis tissue strongly suggest that these compounds are utilized in testosterone production *in vivo*. Studies *in vitro* had already demonstrated the existence of the 5-ene pathway together with the 4-ene pathway but the latter one has been considered to be quantitatively more important [1, 2]. Together with testosterone, the 3β-hydroxy-5-ene steroids are the main compounds present in testis tissue and secreted into spermatic venous blood. Alone, this does not provide adequate proof of the 5-ene pathway being quantitatively the more important one. However, when considered together with the results of incubation studies with adult human testis [11] which demonstrate that testosterone is formed more readily from pregnenolone than from progesterone, from 17α-hydroxypregnenolone more readily than from pregnenolone, progesterone or 17α-hydroxyprogesterone and from dehydroepiandrosterone more readily than from 17α-hydroxyprogesterone or 17α-hydroxypregnenolone, it can be concluded that in human adult testis tissue the 5-ene pathway is favoured in the biosynthesis of testosterone.

A remarkable feature of the steroid composition of testis tissue and spermatic venous blood is the presence of a number of sulphate-conjugated neutral steroids. To date, altogether 20 monosulphates and 7 disulphates have been identified in human adult testis tissue. Together with testosterone, the monosulphates of pregnenolone, dehydroepiandrosterone and 5-androstene-3β,17β-diol are the main endogenous compounds in human testis [4, 8]. In addition, pregnenolone, 5-androstene-3β,17β-diol and testosterone sulphates are secreted by normal testis tissue [10]. This is indirect evidence that sulphates play a role in the testicular biosynthesis of testosterone, which is further supported by the finding that the secretion of the eventual precursor sulphate conjugates is clearly increased during HCG administration [9]. In a number of *in vitro* studies both with human and animal tissue the conversion of certain sulphated 3β-hydroxy-5-ene precursors to testosterone has been amply demonstrated [12–15].

The immediate precursor of testosterone in the postulated sulphated pathway would be 5-androstene-3β,17β-diol, present, preferably as a monosulphate, in human testis. The sulphate group in this compound is exclusively conjugated at carbon 3, whereas the 17α-hydroxy diols are conjugated at carbon 17 [8]. Thus, the formation of testosterone and its sulphate, which is also found in testis tissue, from the 3β-sulphated diol would necessitate hydrolysis of the precursor. This sulphatase reaction could efficiently regulate the formation of testosterone. Following this line of argument, Jaffe, Payne and coworkers have shown that in man, there exists an excellent correlation between testicular steroid sulphatase and 3β-hydroxysteroid dehydrogenase-isomerase activities and serum testosterone levels [16]. Further, the cleavage of pregnenolone, dehydroepiandrosterone and 5-androstene-3β,17β-diol 3β-yl sulphates seems to be effected by the same testicular sulphatase [17], which is effectively inhibited by unconjugated 5-pregnene-3β,20α-diol and 5α-androstane-3α,17β-diol, both of which are found in human testis tissue [17]. Thus, the regulation of human testicular sulphatase activity could be effected through endogenous unconjugated steroids. It must be considered, however, that using minced human testicular tissue, Yanaihara and Troen [18] came to the conclusion that 5-androstene-3β,17β-diol 3β-yl sulphate may not function as a significant precursor of testosterone. Thus, it is clear that no definite conclusions can be drawn as yet concerning the possible importance of sulphated neutral steroids as precursors of testosterone in human testis tissue. However, the question assumes even greater interest

because sulphate-conjugated neutral steroids have not been shown to have any major physiological importance in the adult human organism and these conjugates have been mostly considered to be end products of metabolism (see 19). The only biological role so far found for a sulphated steroid has been the demonstration that dehydroepiandrosterone sulphate has slight androgenic as well as estrogenic effects in man [20]. Therefore, the exploration of the potential importance of sulphated steroid conjugates in steroid-producing tissues is very attractive.

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REFERENCES

1. Eik-Nes K. B.: In *The Androgens of the Testis* (Edited by K. B. Eik-Nes). Marcel Dekker, Inc., New York (1970) pp. 1–47.
2. Hall P. F.: In *The Testis* (Edited by A. D. Johnson, W. R. Gomes and N. L. Vandemark). Academic Press, Inc., New York, Vol. II (1970) pp. 1–71.
3. van der Molen H. J., de Bruijn H. W. A., Cooke B. A., de Jong F. H. and Rommerts F. F. G.: In *The Endocrine Function of the Human Testis* (Edited by V. H. T. James, M. Serio and L. Martini). Academic Press, New York, Vol. 1 (1973) pp. 459–491.
4. Ruokonen A., Laatikainen T., Laitinen E. A. and Vihko R.: *Biochemistry* **11** (1972) 1411–1416.
5. Jänne O., Vihko R., Sjövall J. and Sjövall K.: *Clin. chim. Acta* **23** (1969) 405–412.
6. Cronholm T.: *Steroids* **14** (1969) 285–296.
7. Ruokonen A.: *Biochim. biophys. Acta* **316** (1973) 251–255.
8. Ruokonen A. and Vihko R.: *Steroids* **23** (1974) 1–16.
9. Laatikainen T., Laitinen E. A. and Vihko R.: *J. clin. Endocr. Metab.* **32** (1971) 59–64.
10. Laatikainen T., Laitinen E. A. and Vihko R.: *J. clin. Endocr. Metab.* **29** (1969) 219–224.
11. Yanaihara T. and Troen P.: *J. clin. Endocr. Metab.* **34** (1972) 783–792.
12. Aakvaag A., Hagen A. A. and Eik-Nes K. B.: *Biochim. biophys. Acta* **86** (1964) 622–627.
13. Dixon R., Vincent V. and Kase N.: *Steroids* **6** (1965) 757–769.
14. Payne A. H. and Jaffe R. B.: *J. clin. Endocr. Metab.* **87** (1970) 316–322.
15. Raheja M. C. and Lucis O. J.: *J. Endocr.* **46** (1970) 21–28.
16. Payne A. H., Jaffe R. B. and Abell M. R.: *J. clin. Endocr. Metab.* **33** (1971) 582–591.
17. Payne A. H.: *Biochim. biophys. Acta* **258** (1972) 473–483.
18. Yanaihara T. and Troen P.: *J. clin. Endocr. Metab.* **34** (1972) 793–800.
19. Baulieu E.-E., Corpéchot C., Dray F., Emiliozzi R., Lebeau M. C., Mauvais-Jarvis P. and Robel P.: *Recent Prog. Horm. Res.* **21** (1965) 411–500.
20. Drucker W. D., Blumberg J. M., Gandy H. M., David R. R. and Verde A. L.: *J. clin. Endocr. Metab.* **35** (1972) 48–54.