Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 337–339. Pergamon Press. Printed in Great Britain

PRODUCTION AND SECRETION OF 5α-REDUCED TESTOSTERONE (DHT) BY MALE REPRODUCTIVE ORGANS*

KRISTEN B. EIK-NES

Institute of Biophysics, University of Trondheim, N.T.H., Trondheim 7034, Norway

SUMMARY

Production and secretion of 5α reduced metabolites of testosterone by male reproductive organs have been discussed. In spite of the fact that such organs appear to need androgens for proper biological function, secretion of dihydrotestosterone takes place by the infused testis, prostate and epididymis of the dog. Regulation of this type secretion is currently unknown. Seminiferous tubules as well as Leydig cells from testes of mature rats convert testosterone to dihydrotestosterone and progesterone is a potent inhibitor for this biotransformation. Data from experiments show that testicular metabolites of dihydrotestosterone have androgenic effects.

In 1956 Dorfman and Shipley published that DHT possessed androgenic properties [1]. The total androgenic potency of DHT has, however, been debated [2], but when DHT is implanted in testes of hypophysectomized, mature rats, advanced stages of spermatogenesis can be observed in such testes 4 and 8 weeks after implantation even when less than 10 γg DHT per day are consumed by the implanted organ [3]. Moreover, when hypophysectomized, mature, male rats are injected with large doses of DHT (s.c.) for a long period of time, these animals will mate and father normal offspring [4]. Finally, administration of DHT to castrated, mature, male rats will inhibit the excess secretion of LH and FSH seen in gonadectomized animals [5, 6]. It is difficult, then, to escape the conclusion that DHT possesses all the classical properties of *an androgen* in experiments *in vivo*. In order to understand hypothalamic-pituitary regulation of male reproductive organs, the question must be asked: do such organs secrete DHT and contribute significant amounts of this steroid hormone to systemic blood?

Over the past 10 years our laboratory has developed techniques for infusing an isolated organ with the animal's own arterial blood *via* the main artery and collecting effluent blood from the infused tissue [7, 8]. In these experiments the input of arterial blood is kept constant and the organ is maintained at physiological temperature during the experiment. This technique suffers distinct shortcomings [8], but secretion of steroids cannot be determined in experiments *in vitro*. Utilizing this preparation *in vivo* it can be demonstrated that the infused canine prostate and epididymis will secrete DHT in effluent, venous blood [9, 10]. In experiments *in vitro* these organs will convert radioactive testosterone (T) to radioactive DHT [11]. The infused canine testis secretes DHT in spermatic venous blood [12] and testes infused with radioactive T *via* the spermatic artery show measurable quantities of radioactive DHT in testicular lymph [13]. The concentration of radioactive DHT per ml testicular lymph is comparable to that per ml of spermatic venous blood plasma [13], but the flow of lymph is much slower than that of spermatic effluent blood. Thus, most of the DHT produced in the testis and available for secretion, is delivered from this organ *via* the spermatic vein. Secretion of DHT is not a unique property of the dog testis since it also takes place in the human testis [14].

The rates of DHT secretion by the canine prostate, epididymis and testis are, however, low. Thus, in systemic blood [12] of normal dogs the concentration of T is 10–15 times higher than that of DHT. This is also the situation in normal men [15]. Many organs in male animals [16] including the adrenal gland [17] contain a C_{19}-steroid 5α-reductase, but the ability of these organs to contribute DHT to systemic blood is much lower than that of testicular contribution of T. It must, however, be remembered that DHT is a substrate for many steroid metabolic enzymes [18] and unless all DHT metabolites from the action of such enzymes are measured, no accurate estimate is possible of the capacity of an organ to form DHT. Moreover, much work in many laboratories has been devoted to measurement of DHT formation by looking at conversion of T to DHT. Metabolism leading to DHT has been recorded in testes of immature rats not involving T or 4-androstenedione [19].

There is a good deal of experimental evidence to suggest that DHT exhibits biological action on organs like the prostate, the epididymis and the testis. Whether secretion of DHT by these organs [9, 10, 12] reflects excess DHT production or a distinct mechanism of release from specific androgen binders in the organs of biosynthesis await, however, documentation.

At day 19·5 of intrauterine existence the fetal rat testis will produce DHT from acetate [20]. Before

* The work reviewed from the author's laboratories was supported by research grant HD-4193 and research contracts 69-2097 and 42 812 from National Institutes of Health, Bethesda, Md., U.S.A.

maturity the activity of a C_{19}-steroid 5α-reductase in testes of rats is high [21–23]. At this period of gonadal development in the rat, the major metabolism of radioactive T to radioactive DHT takes place in the cells of Leydig[24]. Using a standard procedure for the separation of seminiferous tubules from interstitial tissue [25], it is clear from experiments that in testes from mature rats, both cell fractions can convert radioactive T to radioactive DHT [24, 26]. Moreover, the activity of the C_{19}-steroid 5α-reductase is about the same in tubular elements and cells of Leydig from such rats [24] and the major 5α-reduced metabolites of radioactive T are radioactive androstandiols [18]. Under our conditions of incubation, however, seminiferous tubules from testes of mature rats either produce more radioactive DHT from radioactive T than the interstitial tissue [24] or also this latter tissue has a faster rate of metabolism of formed DHT to the androstandiols [18]. It is not known whether this tendency of seminiferous tubules to accumulate formed radioactive DHT in experiments *in vitro* is connected with the presence of specific androgen binders in these cells [27]. It has been observed that metabolism of progesterone to 20α-reduced progesterone by purified human placental 20α-hydroxysteroid dehydrogenase is inhibited by transcortin [28]. When evaluating data on steroid metabolism by "distinct" cell fractions from testicular tissue, it must be kept in mind that such fractions [25] are not pure but suffer probably enzymic cross-contamination.

Rivarola *et al.*[29] have recorded that the onset and development of meiosis in seminiferous tubules from rat testes are associated with increasing ability of these cells to convert radioactive T to radioactive 5α-androstan-3α,17β-diol. This latter steroid may be an important compound in the androgenic household of male animals. Androstandiol will support spermatogenesis in the hypophysectomized mouse [30] and 5α-androstan-3α,17β-diol is a potent inhibitor for LH secretion in castrated male rats [31]. When given in high doses this steroid will also lower blood serum concentrations of FSH in such animals (Table 1). Evidence that these effects of androstandiol are produced by the steroid as such and not via conversion to DHT has, however, not been delivered. Metabolism of 5α-androstan-3α-,17β-diol to DHT will occur in male reproductive organs [18, 33].

Testes from mature dogs will augment secretion of T when infused with HCG via the spermatic artery [7], but the concomitant increment of DHT secretion is minute [12]. Testes from mature rats exposed to HCG for 18 h contain less DHT than testes from control animals [34]. HCG will, however, increase testicular C_{19}-steroid 5α-reductase activity in 20 day old rats [35], fails to do so in older animals [35], but HCG injections enhance testicular C_{19}-steroid 5α-reductase activity in 28–42 day old rats subjected to large doses of testosterone propionate [36]. Testes from mature rats treated with FSH show no significant increase in ability to convert radioactive T to radioactive DHT [34]. This type of conversion is, however, decreased by testes from mature rats subjected to HCG *in vivo* or *in vitro* [34]. It has been observed that progesterone will inhibit C_{19}-steroid 5α-reductase in pituitary tissue [37]. When progesterone or 17α-hydroxyprogesterone are incubated with teased testes from mature rats, less radioactive DHT and radioactive 5α-androstan-3α,17β-diol are formed from radioactive T than in control incubations (Table 2). Teased testicular tissue from mature rats can form 5α-reduced metabolites of progesterone in experiments *in vitro* [38]. It is thus possible that progesterone acts as a competitive inhibitor in these experiments (Table 2). The presence of an 11α or an 11β-hydroxyl group on progesterone may interfere with the substrate-enzyme interaction whereas 17α or 6β-hydroxyl groups on progesterone are inert in this respect. At the concentration of 5 γg per 100 mg teased testicular tissue, corticosterone will not inhibit the C_{19}-steroid 5α-reductase and 11β-hydroxytestosterone may actually stimulate this enzymic activity (Table 2).

Table 1. Mean blood serum concentrations of LH (ng NIAMD rat LH RP-1) and FSH (ng NIAMD rat FSH RP-1) in castrated, mature, male rats injected (s.c.) with DHT or 5α-androstan-3α,17β-diol (5α-diol) in oil every day for 7 consecutive days. All injections were started on the day of castration

Treatment: (γg steroids/100 g b.w.)	Mean blood serum gonadotrophins (ng/ml) FSH	LH
Oil only	1222 ± 225*	255 ± 83*
6·25 γg DHT	1365 ± 84	300 ± 70
12·5 γg DHT	1478 ± 86	307 ± 77
25·0 γg DHT	1320 ± 64	85 ± 29
50·0 γg DHT	930 ± 174	16 ± 4
100·0 γg DHT	434 ± 124	Less than 5
6·25 γg 5α-diol	1395 ± 104	188 ± 47
12·5 γg 5α-diol	1432 ± 97	138 ± 35
25·0 γg 5α-diol	1160 ± 29	88 ± 19
50·0 γg 5α-diol	1085 ± 120	79 ± 25
100·0 γg 5α-diol	277 ± 40	Less than 5

* One standard deviation of mean value. The data are from an investigation by Verjans and Eik-Nes[32] and 3 animals or more were used in each group.

Table 2. Mean conversion of H^3-T to H^3-DHT and H^3-5α-androstan-3α,17β-diol (5α-diol) by teased testicular tissue from mature rats

Compound added to the incubation	Metabolites produced (d.p.m./mg tissue) DHT	5α-Diol
No addition	2380 ± 270*	12,420 ± 1450*
Progesterone	690 ± 90	1460 ± 190
17α-Hydroxyprogesterone	590 ± 70	1200 ± 160
11β-Hydroxyprogesterone	1650 ± 310	11,600 ± 1400
11α-Hydroxyprogesterone	2080 ± 300	9370 ± 1000
6β-Hydroxyprogesterone	980 ± 110	6640 ± 980
Corticosterone	2160 ± 300	11,900 ± 1290
11β-Hydroxytestosterone	2450 ± 430	16,110 ± 2100

100 mg teased testicular tissue from mature rats were incubated in 3 ml Krebs-Ringer bicarbonate buffer (pH 7·4) for 1 hr at 37°C in 95% O_2 and 5% CO_2. Each incubation flask contained 25 μc H^3-T (SA: 54·8 mCi/micromole). When used 5 γg of the different steroids listed were added to the incubation flask. The data are from work by Haltmeyer and Eik-Nes[38] and 6 independent incubation replications were employed.

* One standard deviation of mean value.

Testicular progesterone and 17α-hydroxyprogesterone could thus *in part* regulate production of C_{19}-5α-reduced steroids in the male gonad. The effects we have observed with HCG treatment on 5α-reduction of T in testis of mature rats could be due to HCG stimulated progesterone and 17α-hydroxyprogesterone concentrations in this tissue [34]. The mature dog testis produces more DHT per g than the mature rat testis [12, 34]. It is now recognized that testis of the mature rat forms T via progesterone and 17α-hydroxyprogesterone while the mature dog testis produces T predominantly via the 5-en-intermediates [7]. One may ask whether the inhibitory effect of progesterone on 5α-reduction of T (Table 2) is connected with the depression of spermatogenesis and accessory gland function seen in animals treated with progesterone [39]. Also, data are available on meiotic chromosome alterations in dog testes infused with progesterone via the spermatic artery [40]. If DHT and/or 5α-androstan-3α,17β-diol are needed for proper testicular function, any compound curbing testicular production of these steroids may promote changes in the physiological capacity of the male gonad.

REFERENCES

1. Dorfman R. L. and Shipley R. A.: *Androgens, Biochemistry, Physiology and Clinical Significance* (1956) pp. 117–119. Wiley, New York.
2. Jeffcoate W. J. and Short R. W.: *J. Endocr.* **48** (1970) 199–204.
3. Ahmad N., Haltmeyer G. C. and Eik-Nes K. B.: *Biol. Reprod.* **8** (1973) 411–419.
4. Ahmad N., Haltmeyer G. C. and Eik-Nes K. B.: In press (1975).
5. Swerdloff R. S., Walsh P. C. and Odell W. D.: *Steroids* **20** (1972) 13–22.
6. Verjans H. L., Eik-Nes K. B., Aafjes J. H., Vels F. J. M. and Van Der Molen H. J.: *Acta endocr., Copenh.* **77** (1974) 643–654.
7. Eik-Nes K. B.: *Recent Prog. Horm. Res.* **27** (1971) 517–535.
8. Eik-Nes K. B.: *Perfusion Techniques* (1972) pp. 270–285 (Edited by E. Diczfalusy). Forum, Copenhagen.
9. Sowell J. G. and Eik-Nes K. B.: *Proc. Soc. exp. Biol. Med.* **141** (1972) 827–830.
10. Haltmeyer G. C. and Eik-Nes K. B.: *Acta endocr., Copenh.* **69** (1972) 394–402.
11. Gloyna R. E. and Wilson J. D.: *J. clin. Endocr. Metab.* **29** (1969) 970–977.
12. Folman Y., Haltmeyer G. C. and Eik-Nes K. B.: *Am. J. Physiol.* **222** (1972) 653–656.
13. Haltmeyer G. C. and Eik-Nes K. B.: *J. Reprod. Fert.* **36** (1974) 41–47.
14. Pazzagli M., Borrelli D., Forti G. and Serio M.: *Acta endocr., Copenh.* **76** (1974) 388–392.
15. Ito T. and Horton R.: *J. clin. Endocr. Metab.* **31** (1970) 362–371.
16. Robel P., Corpechot C. and Baulieu E. E.: *FEBS Lett.* **33** (1973) 218–221.
17. Maynard P. V. and Cameron E. H. D.: *Biochem. J.* **132** (1973) 293–300.
18. Sowell J. G., Folman Y. and Eik-Nes K. B.: *Endocrinology* **94** (1974) 346–354.
19. Yamada M. and Matsumoto K.: *Endocrinology* **94** (1974) 777–784.
20. Warren D. W., Haltmeyer G. C. and Eik-Nes K. B.: *Biol. Reprod.* **7** (1972) 94–97.
21. Nayfeh S. N., Barefoot S. W. and Baggett B.: *Endocrinology* **78** (1966) 1041–1048.
22. Inano H., Hori Y. and Tamaoki B.-I.: *Ciba Found. Colloq. Endocr.* **16** (1967) 105–119.
23. Ficher M. and Steinberger E.: *Steroids* **12** (1968) 491–506.
24. Folman Y., Ahmad N., Sowell J. G. and Eik-Nes K. B.: *Endocrinology* **92** (1973) 41–47.
25. Christensen A. K. and Mason N. R.: *Endocrinology* **76** (1965) 646–656.
26. Rivarola M. A. and Podesta E. J.: *Endocrinology* **90** (1972) 618–623.
27. Hansson V., Reusch K., Trygstad O., Torgersen O., Ritzen E. M. and French F. S.: *Nature New Biol.* **246** (1973) 56–57.
28. Billiar R. B., Tanaka Y., Knappenberger M., Hernandez R. and Little B.: *Endocrinology* **84** (1969) 1152–1160.
29. Rivarola M. A., Podesta E. J. and Chemes H. E.: *Endocrinology* **91** (1972) 537–542.
30. Nelson W. D. and Merckel C. E.: *Proc. Soc. exp. Biol. Med.* **38** (1938) 315–317.
31. Zanisi M., Motta M. and Martini L.: *J. Endocr.* **56** (1973) 315–316.
32. Verjans H. L. and Eik-Nes K. B.: Unpublished (1975).
33. Baulieu E. E. and Robel P.: *Some Aspects of the Aetiology and Biochemistry of Prostatic Cancer* (1970) pp. 74–81 (Edited by K. Griffiths and C. G. Pierrepoint). Alpha Omega Alpha Printers, Cardiff.
34. Folman Y., Sowell J. G. and Eik-Nes K. B.: *Endocrinology* **91** (1972) 702–710.
35. Shikita M. and Hall P. F.: *Biochim. biophys. Acta* **136** (1967) 484–497.
36. Oshima H., Sarada T., Ochiai K. and Tamaoki B.-I.: *Endocrinology* **86** (1970) 1215–1224.
37. Massa R., Stupnicka E., Kniewald Z. and Martini L.: *J. steroid Biochem.* **3** (1972) 385–393.
38. Haltmeyer G. C. and Eik-Nes K. B.: Unpublished (1975).
39. Ericsson R. J. and Dutt R. H.: *Endocrinology* **77** (1965) 203–208.
40. Williams D. L., Runyan J. W. and Hagen A. A.: *Nature* **220** (1968) 1145–1147.