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THE EFFECTS OF FOLLICLE STIMULATING HORMONE AND LUTEINIZING HORMONE ON STEROID METABOLISM BY ISOLATED SEMINIFEROUS TUBULES AND INTERSTITIAL TISSUE FROM HYPOPHYSECTOMIZED AND OESTROGEN-TREATED RATS

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Abstract—The effects of FSH and LH on the metabolism of progesterone, to 17α-hydroxyprogesterone, androstene dione and testosterone by isolated seminiferous tubules and interstitial tissue from hypophysectomized and oestrogen-treated rats were compared. In rats hypophysectomized for 28 days, treatment with either FSH or LH enhanced the production of each metabolite examined, in both the interstitial tissue and seminiferous tubules. In rats receiving ethinyl oestradiol daily for 24 days, treatment with LH from day 28 to day 42 resulted in an increase in testosterone and androstenedione production by both the tubules and interstitium, but this increase was lower than that found in hypophysectomized rats given an equivalent dose. FSH had no effect on the production of any metabolite in either the seminiferous tubules or interstitial tissue from oestrogen-treated rats. It is concluded thay ethinyl oestradiol directly inhibits the stimulatory effects of FSH and LH on the production of testosterone from progesterone precursor by both the seminiferous tubules and interstitial tissue.

There is now evidence to show that the effects of oestrogens on the reproductive system of the male rat are not due entirely to an inhibitory effect on the pituitary but are caused, in part, by a direct effect on the testis. This came initially from the observation that oestrogens induce a reduction in circulating androgens whilst LH levels remain normal [1, 2]. In addition it has been shown that oestrogens produce a partial inhibition in the response of the germinal epithelium to exogenous FSH and completely block the effects of exogenous LH on the accessory sex organs [3]. The latter may result from a reduction of the stimulatory effects of LH on Leydig cell function, as shown by Moger [4]. The mechanism by which oestrogen inhibits the action of FSH on the seminiferous tubules is not clear. Spermatogenesis can be maintained in hypophysectomized rats by testosterone [5, 6]. In addition both spermatogenesis and the levels of testosterone in the rete testis fluid can be maintained in hypophysectomized rats by treatment with various C_{21} steroids [7]. Since the seminiferous tubules have been shown to synthesize testosterone from progesterone and pregnenolone *in vitro* [8, 9] it appears that spermatogenesis could be partly dependant on the intra-tubular metabolism of C_{21} steroids, possibly derived from the insterstitium, to testosterone. FSH has been shown to affect the availability of steroid precursors in the Sertoli cell [10] and to enhance steroid metabolism when added to Sertoli cell cultures [11]. Thus FSH may act by stimulating steroid metabolism within the tubules. If so, then oestrogens may interfere with this process.

To provide further evidence for a direct action of oestrogens on the testis and to investigate a possible mechanism for this, the effects of FSH and LH on steroid metabolism by isolated seminiferous tubules and interstitial tissue from hypophysectomized and oestrogen-treated rats have been compared.

MATERIALS AND METHODS

Animals and experimental design

Hypophysectomized. Eighteen mature male Wistar rats of 220–240 g body weight were hypophysectomized by the parapharyngeal route by Anglia Laboratories Ltd. Twenty-eight days after hypophysectomy the animals were divided into three groups and were treated intramuscularly with either FSH (ovine NIH-FSH-S10, 1.10 FSH-S1 units/mg containing 0.010 LH units/mg) or LH (ovine NIH-LH-S18, 1.03 LH-S1 units/mg containing 0.05 FSH-S1 units/mg) with one group serving as untreated controls. The hormones were given daily for 14 days at a dose of 100 μg/100 g body weight in 0.1 ml of 0.9% (w/v) NaCl. The animals were weighed every 7 days throughout the experimental period and those animals whose growth curves indicated partial hypophysectomy were eliminated from the study.

Oestrogen-treated. Eighteen mature male Wistar rats were given ethinyl oestradiol (Schering Chemicals Ltd.) orally at a dose of 100 μg/100 g body weight daily for 42 days. The oestrogen was administered in 1% sodium carboxymethyl cellulose by using a hypodermic syringe fitted with a short length of plastic

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tubing which was gently inserted into the oesophagus. After 28 days of oestrogen treatment the animals were divided into groups and were treated with either FSH or LH for 14 days, up to day 42. These hormones were given in an identical manner to those given to hypophysectomized rats.

Incubation procedure

On day 42 all animals were killed by decapitation. One testis from each animal was then removed and decapsulated. Two hundred mg wet weight of whole testis tissue was then weighed out and transferred to an open Petri dish containing ice cold Krebs–Ringer bicarbonate buffer (pH 7.4) containing glucose (2 g/l). The seminiferous tubules were then separated from the interstitial tissue by the method of Christensen & Mason [8]. When the tissue had been completely dissected (usually within 1 hr of death), the separate components were transferred to Quickfit tubes containing 2 ml of fresh Krebs–Ringer solution and 0.1 μCi of [4-^{14}C]progesterone obtained from the Radiochemical Centre, Amersham, Bucks. (specific activity 193 μCi/mg). The tubes were incubated in a constant temperature bath at 33° (rat scrotal temperature) with continuous shaking and frequent aeration with a 95% O_2–5% CO_2 mixture. The incubation continued for 2 hr after which 2 vol of ethyl acetate were added to terminate the reaction.

Before extraction commenced, tritiated progesterone, 17α-hydroxyprogesterone, androstenedione and testosterone were added to all tubes so that the losses of these compounds during extraction and subsequent procedures could be calculated. The incubate was then extracted with 2 vol of ethyl acetate. Progesterone, 17α-hydroxyprogesterone, androstenedione and testosterone were then isolated, purified and characterized by methods described previously [9]. The isotopic content of the various compounds was expressed as a percentage of the added precursor after correction for loss during identification procedures. Statistical comparisons were carried out using the Student's '*t*' test. P values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The metabolism of progesterone by isolated interstitial tissue is shown in Table 1 and isolated seminiferous tubules in Table 2.

Interstitial tissue. The yields of 17α-hydroxyprogesterone, androstenedione and testosterone were significantly lower in oestrogen-treated controls than in hypophysectomized rats receiving no therapy ($P < 0.001$ in all cases). However, significantly greater amounts of progesterone were metabolized in oestrogen-treated rats than in hypophysectomized controls ($P < 0.001$). It has been shown that 20α-hydroxypregn-4-en-3-one production is increased in the testis of hypophysectomized rats [12] and in the seminiferous tubules of oestrogen-treated [13] and heat-treated [14] rats. Thus the increased metabolism of progesterone found in oestrogen-treated rats compared with hypophysectomized animals probably results from oestrogen treatment having a greater stimulatory effect on the 20α-hydroxysteroid dehydrogenase enzyme than does hypophysectomy.

There was a significant increase in the yields of 17α-hydroxyprogesterone, androstenedione and testosterone in hypophysectomized rats given LH. This is in accord with the work of Rommerts *et al.* [15] who found that LH stimulated endogenous testosterone production by isolated interstitial tissue from hypophysectomized rats. There was also a marked increase in each metabolite examined in hypophysectomized rats given FSH which suggests that FSH may play a part in controlling testicular steroidogenesis. This hypothesis is further supported by the observation of Johnson and Ewing [16] that FSH increased the secretion of testosterone by perfused rabbit testis already maximally stimulated with LH. Furthermore FSH has been shown to potentiate the action of LH on plasma testosterone levels in adult hypophysectomized rats [17].

In oestrogen-treated rats given FSH there was no increase in the production of 17α-hydroxyprogesterone, androstenedione or testosterone although less

Table 1. Metabolism of progesterone by isolated interstitial tissue from hypophysectomized and oestrogen-treated rats given gonadotrophins

Treatment	No. of animals	Progesterone	17α-Hydroxy progesterone (17α)	Androstenedione (a)	Testosterone (t)	Total products 17α + a + t
Hypophysectomized (hypox.)	6	64.52 ± 3.44	3.79 ± 0.65	6.39 ± 1.09	2.10 ± 0.33	12.82 ± 1.83
Oestrogen-treated (Oe)	6	42.88 ± 5.47	0.18 ± 0.03	0.06 ± 0.02	0.37 ± 0.08	0.60 ± 0.09
Hypox + FSH	4	54.03 ± 4.93	6.39 ± 1.10*	18.60 ± 1.36§	5.121 ± 0.77‡	31.78 ± 2.75§
Oe + FSH	6	71.25 ± 2.68*	0.44 + 0.13	0.13 ± 0.02	0.59 ± 0.20	1.17 ± 0.33
Hypox + LH	6	39.89 ± 2.55§	8.80 ± 1.09‡	21.08 ± 2.28§	15.45 ± 0.70‡	35.34 ± 3.20§
Oe + LH	5	15.73 ± 1.59‡	0.19 ± 0.05	0.20 ± 0.06	6.35 ± 2.31*	6.95 ± 2.54*

Results expressed as mean (±S.E.M.) % added activity per 200 mg wet wt whole tissue.
†* $P < 0.05$.
†‡ $P < 0.01$.
†§ $P < 0.001$.
† Treated vs untreated control.

Table 2. Metabolism of progesterone by isolated seminiferous tubules from hypophyectomized and oestrogen-treated rats given gonadotrophins

Treatment	No. of animals	Progesterone	17α-Hydroxy progesterone (17α)	Androstene dione (a)	Testosterone (t)	Total products 17α + a + t
Hypophyectomized (Hypox)	6	57.52 ± 3.97	0.32 ± 0.10	0.50 ± 0.09	1.00 ± 0.20	2.18 ± 0.30
Oestrogen-treated (Oe)	6	62.10 ± 2.91	0.41 ± 0.12	0.17 ± 0.03	0.33 ± 0.07	1.20 ± 0.16
Hypox + FSH	4	55.16 ± 2.63	1.355 ± 0.24‡	1.08 ± 0.14‡	1.60 ± 0.05*	3.33 ± 0.35*
Oe + FSH	6	65.15 ± 3.40	0.39 ± 0.08	0.17 ± 0.05	0.41 ± 0.06	0.98 ± 0.18
Hypox + LH	6	46.01 ± 7.62	3.57 ± 0.29§	9.25 ± 2.17‡	5.42 ± 0.62§	18.24 ± 2.85§
Oe + LH	5	19.81 ± 3.54§	0.38 ± 0.06	0.50 ± 0.08‡	4.68 ± 0.75§	5.54 ± 0.83§

Results expressed as mean (±S.E.M.) % added activity per 200 mg wet wt whole tissue.
†* $P < 0.05$.
†‡ $P < 0.01$.
†§ $P < 0.001$.
† Treated vs untreated control.

progesterone was metabolized than in oestrogen-treated controls. In oestrogenized rats given LH there was an increase in the metabolism of progesterone but no increase in 17α-hydroxyprogesterone formation and only a slight increase in androstenedione. Hence the figures for the total products of 17α-hydroxylation were significantly lower than in hypophysectomized rats given LH ($P < 0.001$). These results suggest that ethinyl oestradiol has directly inhibited the stimulatory effects of FSH and LH on the production of testosterone from progesterone by the interstitial tissue. The differing patterns of progesterone metabolism found in oestrogenized animals receiving gonadotrophins may represent effects of these hormones on 20α-hydroxysteroid dehydrogenase activity.

Seminiferous tubules. The yields of testosterone and androstenedione were significantly lower in oestrogen-treated controls than in hypophysectomized rats receing no therapy ($P < 0.01$ in each case). In hypophysectomized rats treatment with LH resulted in a marked increase in each metabolite examined. There was also a small but significant increase in these metabolites following the administration of FSH. These results are in general agreement with previous work which has shown increased testosterone in incubations of seminiferous tubules to which gonadotrophins had been added [18]. Furthermore, Van der Vusse *et al.* [19] have reported increased 3 βHSD activity and pregnenolone production in mitochondrial fractions prepared from seminiferous tubules of HCG treated rats. In addition it has recently been shown that there are LH receptors in the seminiferous tubules that are unmasked by homogenization [20]. There have been no previous reports of enhanced steroid metabolism following the administration of FSH *in vivo* although several workers have shown that the seminiferous tubules are the target organ for FSH [21–23].

In oestrogen-treated rats, LH increased the metabolism of progesterone and the production of testosterone and androstenedione. However there was no increase in 17α-hydroxyprogesterone and the increase in androstenedione was significantly less than that found in hypophysectomized rats given LH. Hence the total products of 17α-hydroxylation were significantly lower in oestrogen-treated rats given LH than in hypophysectomized rats given this hormone. In oestrogen-treated rats given FSH there was no increase in any metabolite examined. Thus ethinyl oestradiol appears to have directly inhibited the stimulatory effects of FSH and LH on the production of testosterone from progesterone precursor by the seminiferous tubules. However, the enhanced metabolism of progesterone in oestrogenized rats treated with LH suggests that LH may have exerted an effect on biosynthetic pathways not examined in the present study.

The stimulation of steroid metabolism following gonadotrophin replacement therapy was less in incubations of seminiferous tubules than in incubations of interstitial tissue. This raises the question as to whether the results obtained with the tubules could have been due to contamination by the interstitium. Evidence which suggests that this is unlikely has been presented by Bell *et al.* [9]. Furthermore, in a study of steroid metabolism by isolated interstitial tissue and seminiferous tubules during puberty it has been found that the metabolism of progesterone to testosterone by the tubules is maximum at an age when testosterone production by the interstitum was low. Testosterone production by the tubules then declined as production by the insterstitium was rising [24, 25]. This would suggest that the method used to separate the seminiferous tubules from the interstitial tissue produces preparations in which the cross contamination is minimal. However, in addition to this type of contamination, the purity of hormone preparations has also to be considered. FSH preparations usually contain a small proportion (approximately 1 per cent) of LH activity which may affect results in the present study this represents the

equivalent of administering 2 μg of LH/rat/day. This is unlikely to account for any of the effects of FSH observed since 5 μg of LH has been shown to be ineffective in inducing a significant increase in testosterone levels in hypophysectomized rats [17].

The present work has shown that FSH may act by enhancing steroid metabolism In addition, the ability of the seminiferous tubules to metabolize steroids is influenced by FSH and LH. This suggests that the effects of gonadotrophins on spermatogenesis [6, 26] may be mediated by way of increased metabolism of steroids within the seminiferous tubules. Thus the inhibitory effects of oestrogens on testicular function could result from a part direct inhibition of the stimulatory effects of FSH and LH on steroid metabolism by the seminiferous tubules as well as the interstitial tissue. However, care must be taken in extrapolating results obtained *in vitro* to events occurring *in vivo*. For example, testosterone has been shown to enter the tubules more rapidly than progesterone [27]. Furthermore, the permeability of isolated tubules may well differ from those in the intact animal. The latter are normally exposed to a higher concentration of testosterone than progesterone. It appears that further work is required to determine the exact significance of tubular steroid metabolism but it seems likely that it represents a mechanism for producing a high concentration of androgens in close proximity to the germ cells.

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REFERENCES

1. V. Danutra, M. E. Harper, A. R. Boyns, E. M. Cole, B. G. Brownsey and K. Griffiths, *J. Endocr.* **57**, 207 (1973).
2. M. Chowdhury, R. Tcholakian and E. Steinberger, *J. Endocr.* **60**, 375 (1974).
3. P. C. Sivelle, A. S. McNeilly and P. M. Collins, *Biol. Reprod.* **18**, 878 (1978).
4. W. H. Moger, *Biol. Reprod.* **14**, 115 (1976).
5. Walsh, E. L., Cuyler, W. K. and D. R. McCullagh, *Am. J. Physiol. Rev.* **51**, 1 (1971).
6. Y. Clermont and C. S. Harvey, *Ciba Fdn Colloq. Endocr.* **16**, 173 (1967).
7. M. E. Harris and A. Bartke, *Endocrinology* **96**, 1396 (1975).
8. A. K. Christensen and N. R. Mason, *Endocrinology* **76**, 646 (1965).
9. J. B. G. Bell, G. P. Vinson and D. Lacy, *Proc. R. Soc.* B **176**, 433 (1971).
10. D. Lacy and B. Lofts, *Proc. R. Soc.* B **162**, 188 (1965).
11. M. J. Welsh and J. P. Wiebe, *Biochem. biophys. Res. Commun.* **69**, 936 (1976).
12. R. A. Appell, *Steroids* **22** 3, 352 (1973).
13. D. Lacy, P. Fyson, P. M. Collins, W. N. Tsang and A. J. Pettit, *Adv. in the Biosciences* **10**, 27 (1972).
14. P. M. Collins and D. Lacy, *Proc. R. Soc.* B **186**, 37 (1974).
15. F. F. G. Rommerts, B. A. Cooke, J. W. C. M. Van der Kemp and H. J. Van der Molen, *FEBS Lett.* **33**, 1, 114 (1973).
16. H. B. Johnson and L. L. Ewing, *Science N.Y.* **173**, 635 (1971).
17. S. El Safoury and A. Bartke, *J. Endocr.* **61**, 2, 193 (1974).
18. B. A. Cooke, F. F. G. Rommerts, J. W. C. M. Van der Kemp and H. J. van der Molen *Molec. Cell. Endocr.* **1**, 2, 99 (1974).
19. G. J. Van der Vusse, M. L. Kalkman and H. J. Van der Molen, *Biochim. biophys. Acta* **380**, 473 (1975).
20. T. Braun and S. Sepsenwol, *Molec. Cell. Endocr.* **4**, 3, 183 (1976).
21. J. Dorrington and I. B. Fritz, *Endocrinology* **94**, 2, 395 (1974).
22. P. R. K. Reddy and C. A. Villee, *Biochem. biophys. Res. Commun.* **63**, 4, 1063 (1975).
23. A. R. Means, *Life Sci.* **15**, 371 (1974).
24. W. N. Tsang, D. Lacy and P. M. Collins, *J. Reprod. Fert.* **34**, 351 (1973).
25. W. N. Tsang, D. Lacy and P. M. Collins, *J. Reprod. Fert.* **34**, 513 (1973).
26. *E. Steinberger. Physiol. Rev.* **51**, 1 (1971).
27. T. G. Cooper and G. M. H. Waites, *J. Endocr.* **65**, 195 (1975).