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EFFECT OF TESTOSTERONE ON TESTICULAR STEROIDOGENESIS IN THE HYPOGONADAL (*hpg*) MOUSE

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Summary—Previous studies have shown that androgens have direct inhibitory effects on steroidogenesis in active Leydig cells. It is not clear what effect androgens have on inactive Leydig cell either through direct action on the cell itself or indirectly through stimulation of Sertoli cell activity. The *hpg* mouse has undetectable levels of circulating gonadotrophins and the gonads fail to develop post-natally. The effect of androgen treatment on testicular steroidogenesis and morphology was examined in these animals. Treatment with testosterone propionate for two weeks significantly increased testicular and seminal vesicle weight. Seminiferous tubules showed marked development in androgen-treated animals, indicating increased Sertoli cell activity, but the abnormal Leydig cell morphology of the *hpg* testis was unchanged. Androgen production per testis *in vitro* was low in control *hpg* animals and remained unaffected by treatment with androgen. Similarly, the pattern of [^{3}H]pregnenolone metabolism was not significantly affected by androgen treatment. The androgen content of the testis was higher in androgen-treated animals but this could be accounted for by uptake of administered steroid from the circulaton. It is concluded that androgens have no direct trophic effect on Leydig cells and that stimulation of Sertoli cell activity is not, in itself, sufficient to affect Leydig cell function.

INTRODUCTION

The hypogonadal (*hpg*) mouse lacks gonadotrophin-releasing hormone (GnRH) due to a deletion in the gene encoding the hormone and its associated peptide [1]. The testes of these animals develop, consequently, in an environment lacking significant gonadotrophin stimulation [2]. Androgen production by testes from *hpg* animals is very low or undetectable although treatment with GnRH increases testicular steroidogenesis [3] while luteinising hormone (LH), over a ten day period, will increase androgen production to the level seen in the normal adult animal [4]. Androgens have been shown to affect the function of both the Sertoli and Leydig cells of the testis [5–10]. Sertoli cells contain androgen receptors [6] although it is now becoming clear that at least some of the effects are mediated through release of a paracrine factor, P-Mod-S, from the peritubular cells [11]. In Leydig cells it has been shown that androgens have direct, receptor-mediated, inhibitory effects on steroidogenesis [7–10]. These effects have been shown either using isolated Leydig cells from normal animals or using animals recently hypophysectomised. It is not clear from either of these models whether androgens may also have trophic effects on Leydig cell activity either through paracrine effects on Sertoli cells or directly on the Leydig cells themselves. Direct trophic effects might not be observed where the Leydig cells are already active (such as shortly after hypophysectomy) and paracrine effects would, clearly, not be observed in isolated Leydig cells. Strong evidence now exists that Sertoli cells influence Leydig cell activity through paracrine mechanisms [12, 13] and that stimulation of Sertoli cell function through the action of follicle-stimulating hormone (FSH) will have trophic effects on Leydig cell activity [14, 15]. The *hpg* mouse is a good model with which to test for trophic effects of androgens on Leydig cell function. The activity of these cells is extremely low and previous studies have shown that testosterone treatment causes marked growth of the *hpg* testis strongly suggesting that there is stimulation of Sertoli cell function [16]. In this study we have treated adult *hpg* males with androgen and have examined the effect on testicular steroidogenesis and morphology.

EXPERIMENTAL

Materials

[4,7-^{3}H]Pregnenolone for metabolism studies was purchased from Amersham International (Amersham, Bucks, England) and was purified by thin-layer chromatography before use. Non-radioactive steroids were purchased from Sigma Chemical Co. (Poole,

Dorset, England) or Steraloids Ltd (Croydon, Surrey, England). Other reagents and chemicals, unless stated, were from the Sigma Chemical Co.

Animals and treatments

Hypogonadal mice were bred and reared at the Royal Veterinary College from breeding stock initially provided by Dr H. M. Charlton, University of Oxford. The animals were originally derived from F1 hybrids of two inbred strains C3H/HeH and 101/H. Animals were used when aged 90–150 days. Animals were treated with a suspension of testosterone propionate in arachis oil (100 μg/ml) administered via subcutaneous 2 cm silastic implants. These implants were placed in the interscapular region of the mice under anaesthesia. Control *hpg* animals were implanted with silastic tubing containing oil alone. Implants were left in place for 2 weeks at which time the animals were killed for experimentation.

Tissue incubations

Steroid production. Testes were rapidly removed from the animals following decapitation. The testes were decapsulated and incubated in 0.5 ml Medium 199 (Gibco, Paisley, Strathclyde, Scotland) containing 0.1% bovine serum albumin, pH 7.4 (M199). Incubations were for 6 h at 32°C under 5% CO_2 in air. One testis from each animal was incubated under basal conditions while the contralateral testis was stimulated with hCG (200 mIU/ml). Medium from each incubation was stored frozen at −20°C until assayed for androgen content by radioimmunoassay (RIA). At the end of the incubation androgens were extracted from the testes by homogenisation in ethanol containing trace amounts of [^{3}H]testosterone to monitor recovery. The homogenates were centrifuged and supernatants dried under air. Steroids were reconstituted in assay buffer and stored frozen until assayed. Steroid production *in vitro* is expressed as the sum of the steroid content of the incubation medium and the testis.

Pregnenolone metabolism. Whole testes from control *hpg* animals and pieces of tissue weighing about 3 mg from androgen-treated animals were preincubated for 1 h at 32°C. At the end of this preincubation 0.5 μCi [^{3}H]pregnenolone was added in 15 μl dimethyl sulphoxide and the incubation continued for a further 2 h. These conditions were chosen to limit pregnenolone metabolism [17]. The steroid metabolites formed were extracted and separated as previously described [17, 18].

Tissue and serum extraction

Androgens present in testicular and lung tissue were extracted as described above. Serum was extracted using diethyl ether and treated as above.

Radioimmunoassay

Androstenedione, testosterone, 5α-androstane 3α,17βdiol (androstanediol) and progesterone were measured by separate specific RIAs as previously described [3]. Total androgen production is expressed as the sum of androstenedione, testosterone and androstanediol as measured by individual RIAs.

Testis morphology

Testes were fixed and processed as previously described [4].

Statistics

Results were analysed using analysis of variance. Metabolites formed from [^{3}H]pregnenolone have been expressed as a percentage of the total [^{3}H]pregnenolone metabolised. This removes differences in the total percentage of substrate metabolised due to differences in activity between individual samples [17].

RESULTS

Tissue weights and androgen content

Treatment of *hpg* males with androgen for 2 weeks caused the testes and seminal vesicles to increase markedly in weight (Table 1). This was associated with an increase in serum testosterone levels from undetectable (<0.1 pmol/ml) in control *hpg* animals ($n = 6$) to 218.8 ± 8.5 pmol/ml ($n = 7$) in androgen-treated animals. The androgen content of the testis increased significantly from 70 ± 20 fmol/testis in *hpg* controls to 900 ± 100 fmol/testis ($n = 4$) (equivalent to 61.6 fmol/mg tissue) in treated animals. This increase in testicular androgen content is likely to be due to uptake of administered androgen since the androgen content of the lungs of androgen-treated animals was 118 ± 8 fmol/mg ($n = 5$). The androgen content of control *hpg* lungs was undetectable. Progesterone was undetectable in all tissues.

[^{3}H]Pregnenolone metabolism

To examine the effect of androgen on the pattern of steroid metabolism testes were incubated with [^{3}H]pregnenolone. In control *hpg* animals pregnenolone was metabolised mainly to progesterone with some further metabolism to the C_{19} steroids (Table 2). Treatment of the animals with testosterone had no significant effect on this pattern of steroid metabolism.

Androgen production in vitro

Testicular androgen production *in vitro* by control and androgen-treated *hpg* mice is shown in Fig. 1.

Table 1. Testis and seminal vesicle weights in control and androgen-treated *hpg* mice

Animal	Organ weight (mg) Testis	Seminal vesicle
Control *hpg* ($n = 6$)	2.35 ± 0.07	1.68 ± 0.30
hpg plus testosterone ($n = 7$)	14.6 ± 0.8	58.1 ± 6.1

Values are mean ± SEM

Table 2. Testicular [^{3}H]pregnenolone metabolism after androgen treatment of *hpg* mice for 2 weeks

Metabolite	Steroid formed (% of [^{3}H]pregnenolone metabolised) *hpg*	*hpg* + testosterone
17α-Hydroxypregnenolone	1.6 ± 0.5	ND
Progesterone	60.3 ± 15.0	64.9 ± 3.5
17α-Hydroxyprogesterone	2.0 ± 0.9	0.6 ± 0.5
Androstenedione	9.1 ± 0.9	10.4 ± 3.2
Testosterone	3.0 ± 0.5	4.9 ± 1.1
Androstanediol	9.8 ± 5.4	ND
Dehydroisoandrosterone	ND	1.7 ± 0.9
Unidentified	10.1 ± 1.4	13.9 ± 1.9

Results are mean ± SEM of tissue from 3 animals per group. Only those steroids making up 1% or more of metabolites formed are shown. ND not detectable. 2-Factor analysis of variance showed no significant ($P > 0.05$) effect of testosterone and no interaction between the effects of testosterone and formation of different metabolites.

In both groups androstenedione and testosterone production were similar while androstanediol was undetectable. Addition of hCG had no effect on steroidogenesis in either group. There was no significant difference between total androgen production by testes from control *hpg* animals or animals treated with testosterone. Progesterone was undetectable in all groups.

Testis morphology

The morphology of the testes from untreated *hpg* animals and animals treated with testosterone is shown in Fig. 2. Following androgen treatment the seminiferous tubules showed marked development compared to untreated *hpg* animals and this was associated with proliferation of the germ cells. In contrast the Leydig cells showed no significant differences from control *hpg* animals with persistence of large lipid droplets and few other organelles.

Discussion

Testosterone is required for the maintenance of spermatogenesis in normal animals and this effect is mediated through the Sertoli cells [19, 20]. In this study androgen treatment caused a marked increase in testis weight which was associated with stimulation of spermatogenesis. It is likely, therefore, that androgen treatment of *hpg* animals causes stimulation of Sertoli cell function. The results reported here suggest very strongly, however, that androgens have no trophic effects on testicular steroidogenesis in the *hpg* mouse. The pattern of pregnenolone metabolism and androgen production *in vitro* were unchanged while the increase in intra-testicular testosterone following testosterone treatment is likely to be due to uptake from the circulation. It is unlikely that testosterone is having an indirect trophic effect and a direct inhibitory effect on *hpg* Leydig cells since testosterone inhibits androgen production by inhibiting activity and synthesis of cytochrome $P450_{17\alpha}$ [13] which would lead to accumulation of progesterone within the cells. In this study progesterone was undetectable in all tissues. It is likely, therefore, that androgens have no stimulatory effect on Leydig cell function either directly or through stimulation of Sertoli cell activity.

It is generally accepted that Leydig and Sertoli cells interact through paracrine mechanisms. Stimulation of Sertoli cell function generally has a trophic effect on Leydig cells [16, 17] although a seminiferous tubule-derived inhibitory factor has also been described [21]. The lack of effect on androgens on Leydig cell function strongly suggests that Sertoli cell-mediated effects on Leydig cells are controlled by specific factors, such as FSH, and not simply by changes in Sertoli cell activity.

Following androgen treatment, Leydig cell morphology did not differ significantly from control *hpg* animals, both groups being marked by the presence of large lipid droplets. Treatment of *hpg* animals with LH stimulates testicular steroidogenesis and causes significant depletion of lipid droplets in the Leydig

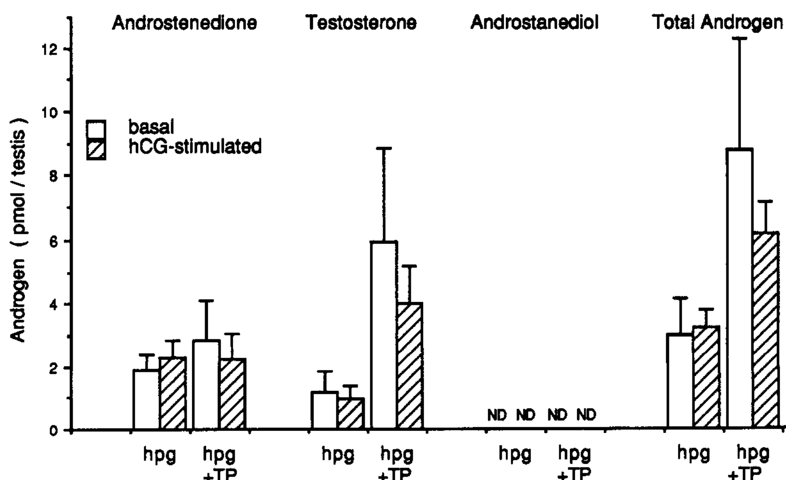

Fig. 1. Effect of testosterone propionate (TP) treatment on androgen production *in vitro* by testes from *hpg* animals. Testes were incubated for 6 h with or without hCG and steroids formed were measured by RIA. Mean ± SEM is shown ($n = 4$). ND, not detectable.

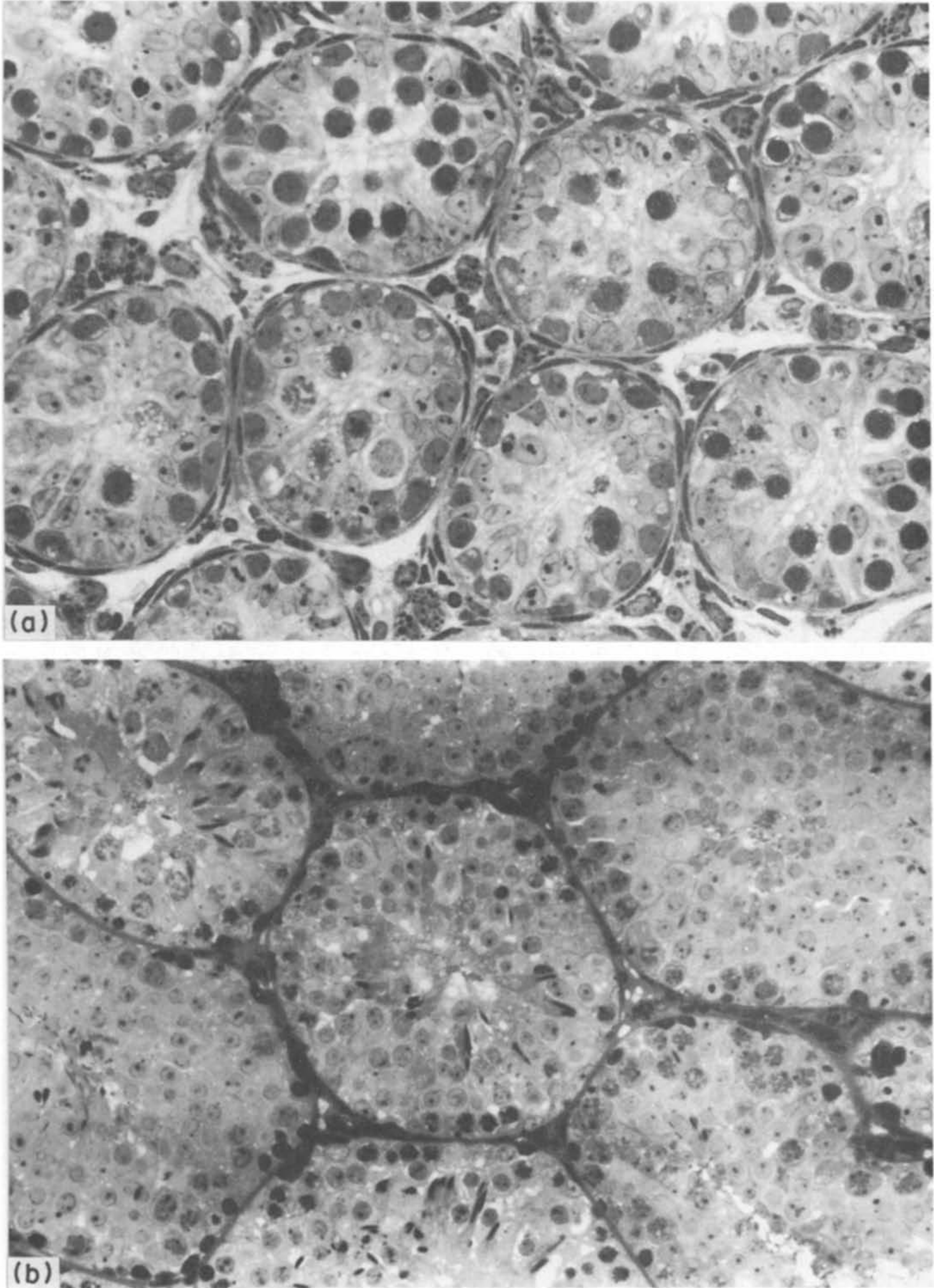

Fig. 2 (a and b)

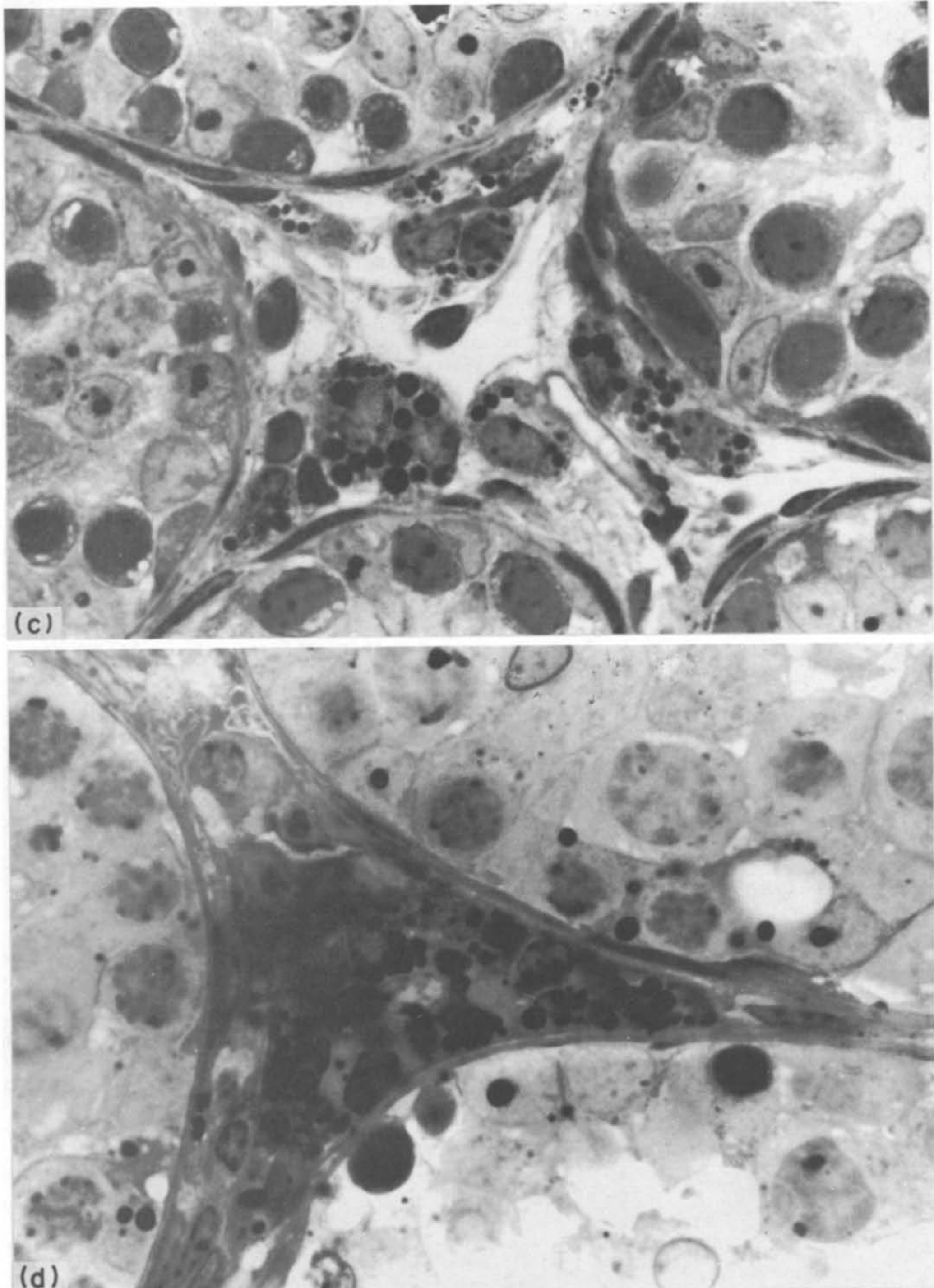

Fig. 2 (c and d)

Fig. 2. Morphology of testes from control *hpg* animals and animals treated with testosterone propionate. Low power magnification showing the seminiferous tubule epithelium in control animals (a) and marked development following androgen treatment (b) (a × 350, b × 200). Higher power magnification showing interstitial tissue with Leydig cells containing large lipid droplets in both control (c) and androgen-treated (d) animals (× 1250).

cells suggesting an inverse relationship between Leydig cell activity and lipid droplet number and size [4]. It is likely, therefore, that these lipid droplets represent accumulation of cholesterol esters within the inactive Leydig cells. Androgen production *in vitro* by both control *hpg* mice and androgen-treated animals was very low and not affected by the presence of hCG. The testes are steroidogenically active *in vitro*, however, since androgens measured in the testis and medium following incubation were significantly greater than in non-incubated testes.

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