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Effect of injection of gonadotrophin-releasing hormone on testicular steroidogenesis in the hypogonadal (*hpg*) mouse

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Summary. Hypogonadal (*hpg*) mice were injected once daily with 10 ng, 50 ng or 1 μg GnRH for 5, 10 or 20 days or 12 times daily with 4·2 ng GnRH for 5 days. Basal and hCG-stimulated production *in vitro* of androstenedione, testosterone and 5α-androstane-3α,17β-diol (androstanediol) were measured by radioimmunoassay. All doses of GnRH increased testicular weight and in-vitro androgen production although seminal vesicle weights were unchanged and serum testosterone concentrations remained undetectable. After 5 days' treatment androstenedione and androstanediol were the dominant androgens produced, the latter indicating the presence of high levels of 5α-reductase. By 20 days testosterone production was predominant after treatment with higher doses of GnRH. Total androgen production (androstenedione + testosterone + androstanediol) after 5 and 10 days was similar at all concentrations of GnRH used. After 20 days' treatment total androgen production was significantly greater with 50 ng GnRH/day than with 10 or 1000 ng/day. Multiple daily injections of 4·2 ng GnRH (total dose 50 ng/day) had no greater effect on androgen production *in vitro* compared to single daily injections of 50 ng. This suggests that under the conditions used in this study the testis does not require pulsatile release of the gonadotrophins. The pattern of [^{3}H]pregnenolone metabolism was measured after 5 days injection of 50 ng GnRH/day. Compared to control *hpg* animals there was a significant increase in formation of C_{19} steroids, synthesis being solely through the 4-ene pathway. These results show that GnRH treatment of *hpg* mice will induce testicular steroidogenesis. The changes which occur after GnRH treatment show similarities to those in the normal animal around puberty.

Keywords: hypogonadal; testis; GnRH; androgen; mouse

Introduction

The hypogonadal (*hpg*) mouse contains a deletion in the gene encoding for gonadotrophin-releasing hormone (GnRH) (Mason *et al.*, 1986) which results in a severe deficiency of pituitary and circulating gonadotrophins. The gonads of the *hpg* mouse develop, consequently, in an environment free of significant gonadotrophin stimulation. The testes of the *hpg* mouse remain abdominal throughout life and testicular growth is severely restricted from the perinatal period onwards. The pattern of steroidogenesis in the testis of the *hpg* adult is unlike that at any age in the normal animal and, presumably, represents basal testicular steroidogenic activity (Sheffield & O'Shaughnessy, 1988). It is unclear what effect trophic stimulation has on Leydig cell function in the *hpg* animal. Single daily injections of *hpg* mice with GnRH stimulate testicular growth and spermatogenesis and multiple daily injections will increase seminal vesicle weight, indicating an increase in serum androgens (Charlton *et al.*, 1983a). In this study we have examined the effect of GnRH injections

on both the pattern of testicular steroidogenesis and the capacity for androgen synthesis in the *hpg* mouse.

Materials and Methods

Materials

The radioactive steroids [1,2,6,7-^{3}H]testosterone, [1,2,6,7-^{3}H]androstenedione, 5α-[1,2(n)-^{3}H]androstane-3α,17β-diol for radioimmunoassay (RIA) and [4,7-^{3}H]pregnenolone for metabolism studies were purchased from Amersham International (Amersham, Bucks, UK). [^{3}H]Pregnenolone was purified by thin-layer chromatography before use. Non-radioactive steroids were purchased from Sigma Chemical Co. (Poole, Dorset, UK) or Steraloids Ltd (Croydon, Surrey, UK). Organic solvents were purchased from BDH (Poole, Dorset, UK); other chemicals and hormones, including GnRH and hCG, were purchased from Sigma Chemical Co.

Animals and treatments

Adult *hpg* mice were provided by Dr H. M. Charlton, Department of Human Anatomy, University of Oxford. Normal animals from the same inbred strain were reared in the Royal Veterinary College from breeding stock provided by Dr H. M. Charlton (Sheffield & O'Shaughnessy, 1988). Animals were maintained at 24°C with light–dark cycle of 12 h light (lights on at 07:00 h). Animals (3 per group) were injected s.c. with GnRH, at the appropriate dose, in 0·2 ml saline (0·9% NaCl, w/v) or with saline alone. In the first set of experiments animals were injected once daily, between 10:00 and 11:00 h, and were killed 24 h after the final injection. In another set of experiments animals (3 per group) were treated as above or were injected every 2 h for 5 days and were killed 2 h after the final injection. Animals were killed by decapitation and blood was collected to measure serum testosterone concentrations by RIA.

Tissue incubations

Androgen production. Testes were rapidly removed from animals after decapitation. Whole testes were incubated at 32°C for 6 h in 0·5 ml Medium 199 (Gibco, Paisley, Strathclyde, UK) containing Hepes (25 mmol/l), $NaHCO_3$ (350 mg/l) and 0·1% (w/v) bovine serum albumin, pH 7·4 (M199). One testis from each animal was incubated under basal conditions while the contralateral testis was stimulated with hCG (200 m i.u./ml). The tunica of each testis was cut in several places with fine scissors to allow access of medium and hormones to the tissue parenchyma. Testes from normal adult animals were decapsulated before incubation and the incubation volume was increased to 2 ml. Medium from each incubation was stored at −20°C until assayed for androgen content by RIA. Tissue content of androgen was not measured in these experiments but preliminary results indicated that more than 75% of androgens produced are secreted into the medium and that medium content of androgen reflects total androgen production.

Pregnenolone metabolism. Hemitestes were preincubated in 0·5 ml M199 for 1 h under basal conditions or in the presence of hCG (200 m i.u./ml). After the preincubation, 0·5 μCi [^{3}H]pregnenolone was added in 15 μl dimethyl sulphoxide and the incubation was continued for a further 2 h (GnRH-treated) or 5 h (control *hpg*). These conditions were chosen to limit substrate metabolism to less than 40% during the period of the incubation (Table 4). Metabolites formed were extracted and separated as previously described (Mannan & O'Shaughnessy, 1988; Sheffield & O'Shaughnessy, 1988).

Radioimmunoassays

The androgen content of the incubation medium was measured by RIA without previous extraction. Serum testosterone concentrations were measured after extraction with diethyl ether using [^{3}H]testosterone to monitor recovery. Testosterone was measured by RIA using antiserum purchased from Guildhay Antisera (Surrey, UK). The limit of detection of the assay, measured as the smallest quantity of testosterone which gives rise to a response significantly different from zero, was 100 fmol/ml. The intra- and inter-assay coefficients of variation were 5·9 and 8·3% respectively. Cross-reactivity with androstenedione and 5α-androstane-3α,17β-diol (androstanediol) was 0·3 and 3·9% respectively. Androstenedione and androstanediol were measured by RIA using antisera provided by Dr M. Dowsett (Chelsea Hospital for Women, London, UK). The limit of detection of the assay for androstenedione was 125 fmol/ml and cross-reactivity was 1·1% for testosterone and <0·1% for androstanediol. The intra- and inter-assay coefficients of variation were 9·6 and 8·3% respectively. The limit of detection of the assay for androstanediol was 250 fmol/ml and cross-reactivity was 5·0% for testosterone and 9·8% for androstenedione. The intra- and inter-assay coefficients of variation were 10·6 and 16·4% respectively. Values are presented in this study without correction for cross-reactivity. Total androgen production is expressed as the sum of androstenedione, testosterone and androstanediol production as measured by individual RIAs.

Statistics

The effects of hCG were analysed by paired *t* tests. Other effects were analysed by analysis of variance and the Newman–Keul test. Metabolites formed from [^{3}H]pregnenolone have been expressed as a percentage of the total [^{3}H]pregnenolone metabolized. This removes differences in the total percentage of substrate metabolized due to differences in activity between individual samples.

Results

Effect of single daily injections of GnRH

Daily injections of all doses of GnRH caused an increase in testicular weight (Table 1). The changes in testicular weight were both time- and dose-dependent with the maximum increase observed using 1 μg GnRH for 20 days. No significant changes in seminal vesicle weight were observed at any time and serum testosterone concentrations remained undetectable throughout. In control *hpg* mice synthesis of C_{19} steroids *in vitro* was very low (in some cases undetectable) and was not enhanced by the addition of hCG (Table 2). All doses of GnRH treatment increased both basal and hCG-stimulated androgen production *in vitro*. After 5 days of treatment with GnRH, androstenedione and androstanediol were the major androgens produced under basal conditions and after hCG stimulation (Table 2). By 10 days the concentrations of all three C_{19} steroids were similar at each dose of GnRH tested except for basal androstanediol production which was undetectable after treatment with 50 ng/day. After 20 days treatment, androstenedione production had declined markedly while testosterone synthesis was high after treatment with 50 ng/day. Androstanediol and testosterone production were similar after treatment with 10 ng or 1000 ng/day for 20 days. In normal adult animals testosterone was the major androgen produced by the testes *in vitro* with markedly lower production of androstenedione and androstanediol (Table 2).

Table 1. Effect of GnRH treatment on testicular weight in *hpg* males

Time (days)	GnRH (ng)	Testis weight (mg) (N = 3)*	Body weight (g) (N = 3)*
0	0	1·6 ± 0·3	25·3 ± 1·2
20	0	2·1 ± 0·7	29·3 ± 0·8
5	10	2·2 ± 0·3	23·8 ± 1·9
	50	3·5 ± 0·5	26·0 ± 0·8
	1000	3·8 ± 0·4	23·0 ± 0·9
10	10	4·2 ± 0·7	24·1 ± 0·4
	50	7·2 ± 0·2	26·9 ± 1·2
	1000	6·2 ± 0·4	21·4 ± 1·0
20	10	7·9 ± 0·6	24·8 ± 1·6
	50	12·0 ± 0·8	24·2 ± 1·0
	1000	12·9 ± 1·1	25·6 ± 1·1

Values are mean ± s.e.m.
*In the normal 60-day-old animal testicular weight is 107 ± 3 mg and bodyweight is 33 ± 2 g (N = 6).

Total androgen production after GnRH treatment for up to 20 days is shown in Table 3. At all times after GnRH treatment the testes were sensitive to hCG stimulation *in vitro*. After 5 days the total androgen production was similar at all 3 doses of GnRH tested. Between 5 and 10 days there was a decline in hCG-stimulated androgen production at all doses of GnRH. At the lowest dose of GnRH (10 ng/day) this decline in androgen production continued up to 20 days. In contrast, there

Table 2. Androgen production *in vitro* by whole testes from *hpg* mice after daily GnRH injections

Time (days)	GnRH (ng)	Steroid production (pmol/testis)					
		Androstenedione		Testosterone		Androstenediol	
		Basal	hCG	Basal	hCG	Basal	hCG
0*	0	0·20 ± 0·10	0·21 ± 0·09	0·21 ± 0·10	0·19 ± 0·06	ND	0·18 ± 0·09
20	0	0·25 ± 0·07	0·18 ± 0·04	0·14 ± 0·05	0·21 ± 0·06	0·15 ± 0·04	0·27 ± 0·10
5	10	3·68 ± 0·99	10·8 ± 3·79	0·87 ± 0·12	4·30 ± 1·45	1·33 ± 0·78	7·11 ± 2·83
	50	2·50 ± 0·71	8·13 ± 4·85	1·64 ± 0·31	2·58 ± 1·07	1·93 ± 0·68	9·62 ± 3·93
	1000	2·42 ± 0·93	8·67 ± 2·72	1·78 ± 0·16	2·98 ± 0·79	4·47 ± 1·20	8·87 ± 3·05
10	10	1·88 ± 0·40	5·37 ± 1·29	2·41 ± 0·33	2·76 ± 0·14	2·00 ± 0·88	2·95 ± 0·50
	50	2·98 ± 1·44	4·45 ± 0·77	1·16 ± 0·67	7·31 ± 1·06	ND	3·01 ± 0·92
	1000	1·73 ± 0·15	4·18 ± 1·48	0·98 ± 0·41	3·51 ± 1·51	0·71 ± 0·35	3·52 ± 1·21
20	10	0·16 ± 0·08	0·23 ± 0·05	3·97 ± 1·38	2·93 ± 1·58	0·77 ± 0·14	4·02 ± 1·03
	50	0·21 ± 0·04	ND	6·61 ± 0·93	40·8 ± 7·63	2·80 ± 0·83	4·33 ± 0·12
	1000	0·11 ± 0·04	0·51 ± 0·09	4·26 ± 1·05	9·14 ± 1·32	4·30 ± 1·80	8·07 ± 2·23
Normal adult males		32 ± 4	252 ± 40	239 ± 48	1568 ± 140	17 ± 4	111 ± 25

Values are mean ± s.e.m. for 3 males/group.
ND, not detectable.
*This group contained 5 animals and androgen production was undetectable in 2: the values reported are for the remaining 3 animals.

Table 3. Total androgen production by testes from *hpg* mice after daily GnRH injections

Time (days)	GnRH (ng)	Total androgen production (pmol/testis)	
		Basal	hCG-stimulated
0	0	0·41 ± 0·15	0·58 ± 0·20
20	0	0·54 ± 0·19	0·66 ± 0·24
5	10	5·88 ± 1·82[a]	22·2 ± 6·44[a]
	50	6·07 ± 1·71[a]	20·3 ± 9·87[a]
	1000	8·67 ± 2·20[a]	20·5 ± 5·61[a]
10	10	6·29 ± 1·32[a]	11·1 ± 3·12[a]
	50	4·14 ± 1·52[a]	14·8 ± 2·52[a]
	1000	3·42 ± 1·23[a]	11·2 ± 0·53[a]
20	10	4·93 ± 1·44[a]	7·2 ± 2·94[a]
	50	9·62 ± 2·00[a]	45·1 ± 11·8[b]
	1000	8·67 ± 2·71[a]	17·7 ± 1·10[a]
Normal adult male		288 ± 40	1931 ± 130

Values are mean ± s.e.m. for 3 animals per group.
Within a single time point and treatment regimen *in vitro*, values with different superscripts are significantly ($P < 0·05$) different. The effects of hCG *in vitro* were significant as asssessed by paired *t* tests at each time of GnRH treatment.

was an increase in androgen production between 10 and 20 days following treatment with 50 and 1000 ng/day although the effect of 50 ng/day was significantly greater. Androgen production in *hpg* male animals treated with GnRH, although greater than in controls, remained markedly lower than in normal adult animals (Table 3).

Table 4. [^{3}H]Pregnenolone metabolism after GnRH treatment (50 ng/day) of *hpg* mice for 5 days

	Steroid formed (% of [^{3}H]pregnenolone metabolized)			
	Control animals		GnRH-treated animals	
Metabolite*	Basal	hCG-stimulated†	Basal	hCG-stimulated†
$17OHP_5$	12·0 ± 7·5	7·1 ± 0·7	ND	ND
Progesterone	38·7 ± 9·9	52·3 ± 5·7	25·0 ± 4·6	21·3 ± 6·5
$17OHP_4$	4·9 ± 0·4	5·1 ± 1·4	3·4 ± 0·2	3·7 ± 0·6
Androstenedione	13·4 ± 3·8	10·3 ± 1·1	31·3 ± 5·5	17·0 ± 4·9
Testosterone	1·1 ± 0·1	1·4 ± 0·2	29·9 ± 3·1	24·3 ± 4·3
Androstanediol	4·7 ± 1·1	5·8 ± 1·2	7·7 ± 4·5	17·7 ± 7·7
Androsterone	0·4 ± 0·3	1·5 ± 0·1	3·4 ± 2·7	1·1 ± 0·6
$5\alpha DHP_4$	6·7 ± 1·6	2·5 ± 1·0	ND	ND
Allopregnenolone	2·7 ± 2·2	0·4 ± 0·3	0·8 ± 0·7	3·2 ± 2·5
Unidentified 1	ND	ND	ND	11·4 ± 7·3
2	11·9 ± 4·1	7·4 ± 1·9	ND	ND
Pregnenolone metabolized‡	33·3 ± 3·5	29·8 ± 4·6	24·0 ± 4·2	24·2 ± 5·2

Results are mean ± s.e.m. of tissue from 3 animals per group.
ND, not detectable.
*Only those metabolites which made up more than 3% of [^{3}H]pregnenolone metabolized in at least one group are shown. $17OHP_5$, 17α-hydroxypregnenolone; $17OHP_4$, 17α-hydroxyprogesterone; $5\alpha DHP_4$, 5α-dihydroprogesterone.
†200 mi.u./ml.
‡Percentage of added [^{3}H]pregnenolone metabolized in each group.

The pattern of [^{3}H]pregnenolone metabolism by testes from mice injected with 50 ng/day for 5 days is shown in Table 4. In control *hpg* mice progesterone was the major metabolite formed from exogenous pregnenolone as previously described (Sheffield & O'Shaughnessy, 1988). After GnRH treatment there was a marked increase in metabolism beyond progesterone to the C_{19} steroids. Metabolism of pregnenolone was almost exclusively via the 4-ene pathway with significant formation of the 5α-reduced steroids androsterone, allopregnenolone and androstanediol, confirming the presence of high 5α-reductase. Acute stimulation with hCG had no significant effect on the pattern of pregnenolone metabolism in control *hpg* animals but caused a significant increase in the percentage conversion to androstanediol in GnRH-treated animals.

Effect of multiple daily injections of GnRH

The effect of 12 injections daily with 4·2 ng GnRH (total daily dose 50 ng) were compared with single injections daily of 50 ng GnRH. Neither treatment affected seminal vesicle weights and serum testosterone remained undetectable. The effects of single and multiple daily injections of GnRH on total androgen production *in vitro* are shown in Fig. 1. Both basal and hCG-stimulated androgen production were greater after multiple injections of GnRH than single daily injections but this difference was not significant ($P > 0·05$). In this experiment addition of hCG *in vitro* caused a significant ($P < 0·05$) increase in androgen production by testes from control animals although the level of androgen synthesis was markedly lower than in GnRH-treated animals.

Discussion

It is clear from these experiments that GnRH treatment of *hpg* mice causes a marked and rapid increase in testicular androgen production. This enhanced steroidogenesis probably helps to

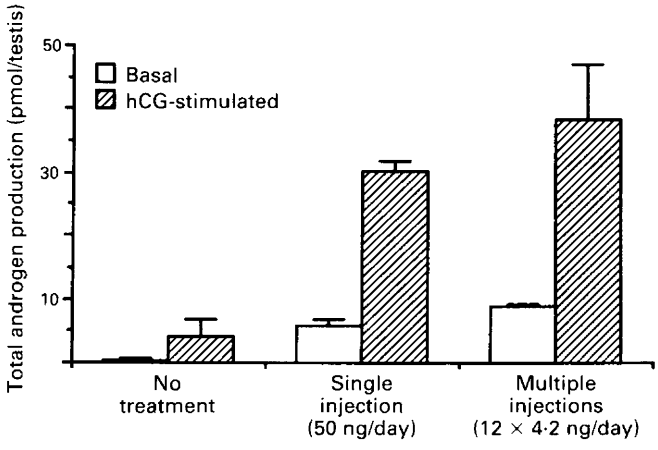

Fig. 1. Effect of single or multiple daily injections of GnRH on total androgen production by testes from *hpg* mice. Animals were injected once daily with 50 ng GnRH or 12 times daily with 4·2 ng GnRH or saline. After 5 days treatment androgen production was measured *in vitro*. Results from 3 separate animals in each group are shown as mean ± s.e.m.

stimulate development of the seminiferous tubules and testicular growth although blood androgen concentrations are clearly not increased to a level which affects seminal vesicle weights. Charlton *et al.* (1983a) have shown that the treatments used in this study will cause a marked increase in pituitary FSH content but that they have little or no effect on pituitary LH. Injection of GnRH to *hpg* mice does, however, cause a rapid transient increase in plasma LH and FSH values and testes in treated animals would, therefore, be exposed to elevated concentrations of both gonadotrophins. It is unlikely that any of the effects observed here are due to direct actions of GnRH on the testis since mouse Leydig cells have been shown to be insensitive to this hormone (Hunter *et al.*, 1982).

Testes from GnRH-treated *hpg* mice contained high levels of 5α-reductase, particularly during the first 5 days of treatment, if compared to the normal adult animal (Sheffield & O'Shaughnessy, 1988). High levels of enzyme activity occurred in the metabolism of [^{3}H]pregnenolone to 5α-reduced steroids, particularly in the presence of hCG, and in the high output of androstanediol, as measured by RIA. It is likely that this high level of activity is due to induction of enzyme synthesis by the GnRH treatment although there appear to be significant amounts of 5α-reductase in testes of control *hpg* mice. During development in the normal mouse 5α-reductase activity starts to show a marked increase between 15 and 20 days and then declines again by about 35 days (Tsujimura & Matsumoto, 1974; Chase & Payne, 1983; Sheffield & O'Shaughnessy, 1988). During this time there is also a significant increase in Leydig cell androgen production (Chase & Payne, 1983). Treatment of adult *hpg* mice with GnRH therefore appears to stimulate changes in testicular function similar to those which occur during normal development. In addition, androgen production in *hpg* testes, after GnRH treatment, was almost entirely via the 4-ene pathway which resembles the immature normal mouse (15–25 days) but is in contrast to the adult animal (Sheffield & O'Shaughnessy, 1988). The total androgen production in GnRH-treated *hpg* animals remained markedly lower than in normal adult animals. This is similar to the 20- and 30-day-old mouse which produces only 1–2% of the adult level of androgen per testis (Anakwe & Moger, 1984). The amount of androgen produced per testis after GnRH treatment of *hpg* mice is very similar to that in the normal 20-day-old animal reported by Anakwe & Moger (1984).

There is strong evidence that 5α-reductase activity in the immature rat is regulated by LH (Nayfeh *et al.*, 1975; Murono & Payne, 1979; Chase & Payne, 1985) and that prolactin can augment the effect of LH (Chase & Payne, 1985; Takeyama *et al.*, 1986). The pituitary content of prolactin is

normal in adult *hpg* males (Charlton *et al.*, 1983b) and high levels of activitiy of 5α-reductase in the *hpg* testis following GnRH treatment may reflect, therefore, increased stimulation by LH. Leydig cells which develop after ethylene dimethanesulphonate treatment of adult rats also go through a transient period in which 5α-reductase activity is high (Vreeburg *et al.*, 1988). It is possible, therefore, that once Leydig cell development is stimulated an early event is expression of high levels of 5α-reductase, provided the hormonal environment is suitable.

In this study the concentration of [^{3}H]pregnenolone used as substrate to measure the pattern of steroidogenesis was not limiting. The marked change in the pattern of [^{3}H]pregnenolone metabolism following GnRH treatment suggests, therefore, that the effects observed are due, at least partly, to changes in Leydig cell activity rather than solely a change in cell number per testis. The increase in progesterone metabolism, after GnRH, suggests that 17α-hydroxylase/C_{17}–C_{20} lyase activity is increased per cell. This would be consistent with the results of Purvis *et al.* (1973), O'Shaughnessy & Payne (1982) and Anakwe & Payne (1987) which have shown that this enzyme is under gonadotrophin regulation. The marked increase in androgen production which occurs in the testes of *hpg* mice after GnRH treatment suggests that changes in the activity of other steroidogenic enzymes have occurred. Activity of both 3β-hydroxysteroid dehydrogenase and cholesterol side-chain cleavage has also been shown to be under gonadotrophin regulation (Murono & Payne, 1979; O'Shaughnessy & Payne, 1982; Funkenstein *et al.*, 1983; Anakwe & Payne, 1987). Activity of 17-ketosteroid reductase appears to be less sensitive to gonadotrophin regulation (Murono & Payne, 1979; O'Shaughnessy & Payne, 1982) which may explain why relatively high concentrations of androstenedione were produced after 5 and 10 days' treatment.

The pituitary–testicular axis in adult *hpg* males appears to be very sensitive to GnRH since all three doses caused the same marked increase in steroidogenesis during the first 5 days. Similarly, hCG-stimulated androgen secretion declined at all doses of GnRH between 5 and 10 days of treatment. The reason for this decrease is not clear but may be due to desensitization to GnRH at the pituitary or to the gonadotrophins at the testis. Large doses of GnRH are known to cause pituitary desensitization with a subsequent decrease in gonadotrophin secretion (Haynes *et al.*, 1977; de Konig *et al.*, 1978; Fraser & Lincoln, 1980). Similarly, testicular responsiveness to both LH and FSH can be desensitized by exposure to elevated concentrations of the hormone (Sharpe, 1977; O'Shaughnessy, 1980; Quinn & Payne, 1985). Alternatively, the decline in responsiveness to the lowest dose of GnRH may be due to the inability of this concentration of GnRH to sustain pituitary function after the initial surge in activity. With higher doses of GnRH there was an increase in steroidogenic activity between 10 and 20 days, the effect being significantly greater at 50 ng/day than 1000 ng/day. There was no difference in testicular weight after 20 days' treatment with 50 or 1000 ng/day which suggests that, while androgen production is lower after 1000 ng/day, perhaps due to tissue desensitization, it is sufficient to maintain testicular growth.

Gonadotrophins are known to be released from the pituitary in a pulsatile manner and this reflects secretion of GnRH from the hypothalamus (Clarke & Cummins, 1982; Levine *et al.*, 1982). The episodic pattern of LH secretion is important for events such as initiation of puberty (Foster *et al.*, 1978) and control of the oestrous cycle (Marut *et al.*, 1981). Little is known, however, about the effect of changing GnRH pulse-frequency on the pituitary–gonadal axis in the male. The importance of pulsatile secretion of LH to Leydig cell function is uncertain, there is little difference in responsiveness of perfused cultured Leydig cells to constant exposure to LH or pulsatile exposure (Verhoeven *et al.*, 1986). In addition Chase *et al.* (1988) have shown that a pulsatile pattern of LH secretion in rams is not necessary for its trophic actions. In the present study there was no significant difference between treating *hpg* animals once daily or 12 times daily. It is unlikely that the dose used during multiple injections (4·2 ng) is too low to affect the pituitary since 10 ng was as effective as 50 ng and 1000 ng in stimulating steroidogenesis during the first 5 days of treatment. The lack of effect of multiple injections may be due to artefacts of the system used, such as frequency of injection or doses of GnRH used, or it is possible that a pulsatile pattern of GnRH may be unimportant in stimulating testicular function.

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