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Testosterone Response of Cryptorchid and Hypophysectomized Rats to Human Chorionic Gonadotrophin (hCG) Stimulation

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Abstract

The testosterone responses to a single injection of hCG (100 i.u.) in hypophysectomized (hypox.), cryptorchid or sham-operated rats were followed over a 5-day period. In sham-operated rats, hCG induced a biphasic rise in serum testosterone, peaks being observed at 2 and 72 h. Reduced testis weights, elevated FSH and LH levels and reduced serum testosterone levels were found after 4 weeks of cryptorchidism, but hCG stimulation resulted in a normal 2 h peak in serum testosterone. However, the secondary rise at 72 h in cryptorchid rats was significantly lower than sham-operated rats.

Reduced testis weight and undetectable serum FSH and LH levels together with decreased testosterone levels were found 4 weeks after hypophysectomy. Serum testosterone levels rose 2 h after hCG in comparison to hypox. controls but this peak was significantly reduced compared with sham-operated rats. The second rise in serum testosterone began on day 2, peaking on day 4 at levels comparable to that seen in sham-operated rats after hCG.

The *in vitro* basal and hCG stimulated secretion of testosterone by cryptorchid testes was greater than that secreted by normal rat testes (518·0 ± 45·9 and 3337·6 ± 304·1 pmol per testis per 4 h compared with 223·6 ± 24·9 and 1312·9 ± 141·4 pmol per testis per 4 h for normal rat testes). In cryptorchid animals a single injection of 100 i.u. hCG resulted in a pattern of *in vitro* refractoriness similar to normal rats, lasting from 12 h to 2 days, during which testosterone secretion was reduced to near basal levels. The *in vitro* basal and hCG-stimulated secretion of testosterone by hypox. rat testes was severely diminished compared with normal rat testes. The temporal pattern of *in vitro* secretion of testosterone from hypox. rat testes mimicked the *in vivo* serum testosterone pattern seen in these animals. This study demonstrates important differences in the *in vivo* and *in vitro* testosterone response to hCG after testicular damage.

Introduction

Recent studies have shown that following a single injection of hCG to rats, serum testosterone levels demonstrate a biphasic pattern (Haour and Saez 1977; Hodgson and de Kretser 1982). The two peaks in serum testosterone occur at 2 and 72 h after hCG and data is available to suggest that the nadir between the peaks is a representation in serum testosterone of the refractoriness of the testis to further hCG stimulation seen *in vitro* (Hseuh *et al.* 1977; Hodgson and de Kretser 1983, 1984*a*). Furthermore, there is evidence to suggest that the occurrence of the second peak during the recovery from refractoriness is due to the long half-life of hCG (Hodgson and de Kretser 1982). Our own data also indicates that this biphasic pattern of testosterone secretion in response to hCG is not seen in young rats but evolves during sexual maturation (Hodgson and de Kretser 1984*b*). A knowledge of the serum testosterone pattern is important in deciding when samples should be collected since the response of the testes to hCG is often used as a test of Leydig cell function in experimental animals.

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In this paper we present data in two different settings concerning the response of testes to hCG stimulation. The first concerns the response of the testis following testicular damage induced by the process of experimental cryptorchidism, which has been shown to cause spermatogenic damage and Leydig cell hypertrophy (Moore 1924; Nelson 1951; van Demark and Free 1970; Kerr *et al.* 1979). The second concerns the response of chronic hypophysectomized testes, a state associated with profound atrophy of the Leydig cells (Vilar 1968; Hauger *et al.* 1977). The resulting patterns of testosterone secretion provide striking contrasts and emphasize the importance of such detailed studies.

Materials and Methods

Animals

Adult male Sprague–Dawley rats were made cryptorchid for 4 weeks. Cryptorchidism was performed under ether anaesthesia through an incision made in the inguinal region (Kerr *et al.* 1979). The testes and epididymides were carefully pushed through the inguinal canal into the abdomen and subsequent descent was prevented by ligation of the inguinal canal. Rats were hypophysectomized under ether anaesthesia using the parapharyngeal method. Immediately following surgery an intramuscular injection of 45 000 units of procaine penicillin (Sigma Chemical Co. Pty Ltd) and an intraperitoneal injection of 3·0 mg hydrocortisone sodium succinate (Solu-Cortef, Upjohn) were administered. The diet of the hypox. rats was supplemented with 5% glucose and 0·9% saline. Rats were killed 4 weeks after hypophysectomy and the completeness of hypophysectomy was confirmed by examination of the sella turcica for remaining fragments of pituitary gland and by measurement of serum testosterone, LH and FSH levels.

Rats received a subcutaneous injection of 0·5 ml of 100 i.u. hCG or saline and were killed by decapitation at intervals up to 6 days after injection. Body and testis weights were determined and trunk blood samples were taken. The serum was stored at −20°C until assayed for testosterone, LH, FSH and hCG by radioimmunoassay. Two groups of five sham-operated rats were used to provide control data for body and organ weights and serum hormonal levels for the cryptorchid rats and hypox. rats. A further group of 70 animals was sham-operated and injected with saline or 100 i.u. hCG to provide control data on the serum testosterone response. As the serum testosterone response of sham-operated animals was not different to that found in normal rats (Haour and Saez 1977; Hodgson and de Kretser 1982) the *in vitro* testosterone response to hCG which had already been measured in normal rats was used as a comparison for the *in vitro* results obtained in cryptorchid and hypox. rats.

In vitro *Testosterone Production*

To determine the *in vitro* secretion of testosterone the testes from each animal were removed, decapsulated and placed in glass vials containing 2 ml Krebs–Ringer bicarbonate glucose (KRBG) buffer or buffer plus 700 mi.u./ml hCG. Testes were incubated at 34°C for 4 h with shaking in a water-bath according to the methods previously described by de Kretser *et al.* (1979). At the conclusion of the incubation the media was removed and centrifuged at 2000 *g* for 20 min to remove cell debris. The supernatant was then diluted and assayed directly for testosterone by radioimmunoassay.

Testosterone Assay

Testosterone levels were measured by radioimmunoassay similar to that described by Corker and Davidson (1978). The antiserum, supplied by Dr R. Cox (Division of Animal Production, CSIRO, Prospect, N.S.W.) was raised in sheep against testosterone-3-carboxymethyloxime conjugated to bovine serum albumin. 5α-Dihydroxytestosterone showed a 98% cross-reactivity. The interassay coefficient of variation was 16–17% and the intra-assay coefficient of variation was 7–9%.

LH Assay

LH was measured by a double antibody radioimmunoassay as previously described by Lee *et al.* (1975). Reagents were kindly supplied by the National Pituitary Agency and purified rat LH (NIAMDD-LH-I4) was used as tracer and standard. Rat LH was iodinated using chloramine-T and purified on an ultragel AcA 54 column (Greenwood *et al.* 1963). The antiserum (NIAMDD-LH-S5) was raised in rabbits against rat LH and showed a cross-reactivity of $<0·03\%$ with hCG. The second antibody was a goat anti-rabbit serum. The sensitivity of the assay was $0·50 \pm 0·13$ ng/ml (mean ± s.d.). The intra-assay and interassay coefficients of variation ranged between 2·4–5·5% and 7·2–9·2%, respectively. Samples from each experiment were measured in the same assay.

FSH Assay

A double antibody radioimmunoassay was used to measure FSH. Purified rat FSH preparation (NIAMDD-FSH-I3) was used as tracer and was iodinated according to Miyachi *et al.* (1972) with lactoperoxidase (EC 1.11.1.7) and then purified on an ultragel AcA 54 column. NIAMDD-rat-FSH-RP1 was used as the standard and the antiserum was produced by Dr V. Lee (Prince Henry's Hospital, Melbourne). The antiserum was raised in rabbits against human FSH (LER-1563) and showed no cross-reactivity with rat prolactin, LH, α-LH or β-LH. The second antibody was a goat anti-rabbit serum. The sensitivity of the assay varied between 70 and 90 ng/ml (mean ± s.d.). The intra- and interassay coefficients of variation were 2·4–3·5% and 10·1–13·8% respectively. Samples from each experiment were measured in the same assay.

hCG Assay

Levels of hCG in the serum of rats after a single injection of hCG were monitored by a double antibody radioimmunoassay using an antibody raised against the β-subunit of hCG. Highly purified hCG (CR-121; 9286 i.u./mg; kindly supplied by NIAMDD) was iodinated and used as tracer. The lower limit of detection varied between 3·6 and 4·3 mi.u./ml second International hCG standard. All samples were measured in a single assay with an intra-assay coefficient of variation of 8%.

Statistical Analysis

Results are expressed as mean ± s.e. Statistical significance in serum hormone levels within groups was determined using analysis of variance in conjunction with Duncan's multiple-range test. Between-group differences were analysed using Student's *t*-test or analysis of variance.

In the present study refractoriness was defined statistically as the point where excess hCG failed to produce a significant increase in the secretion of testosterone.

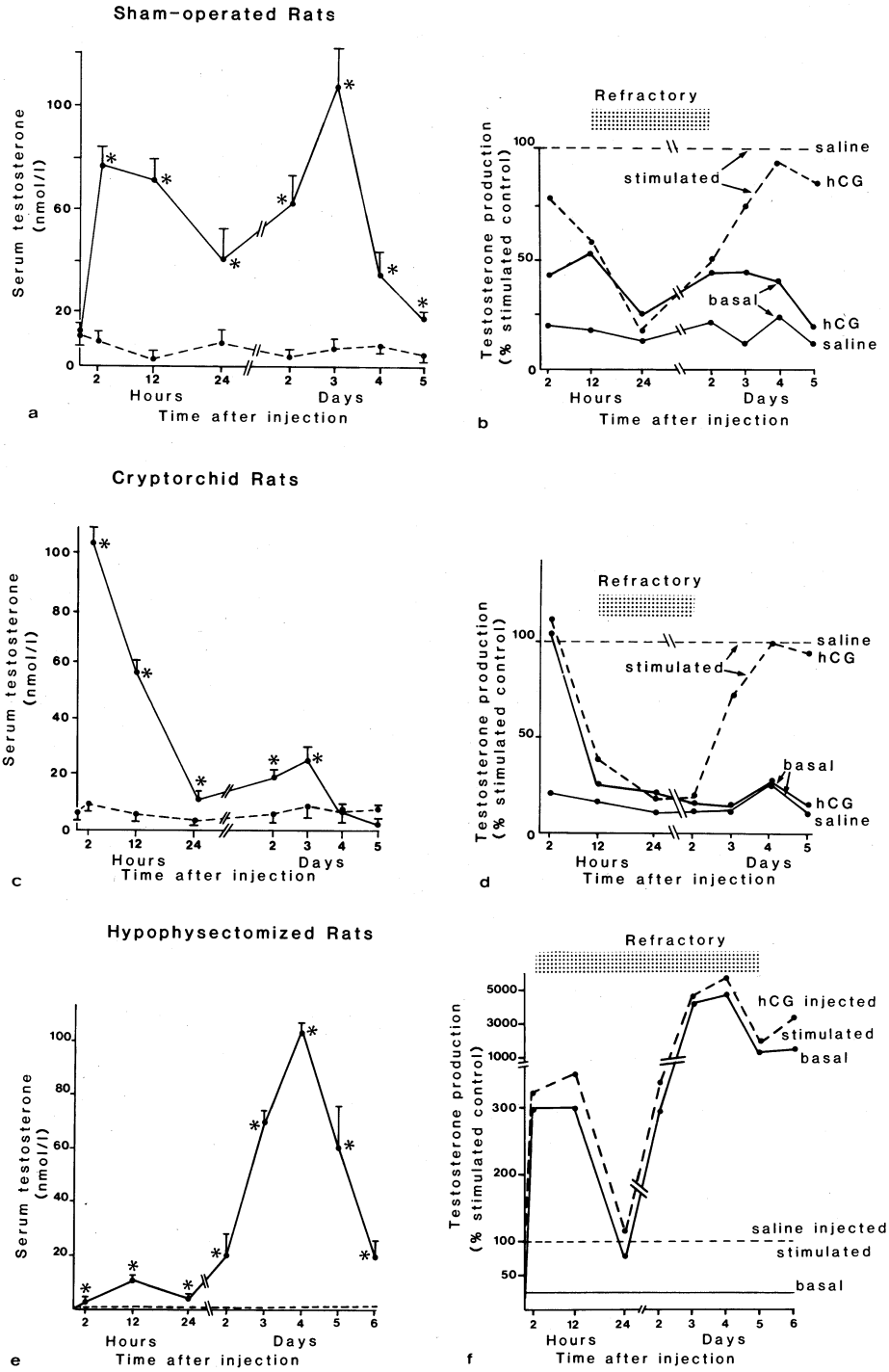
Results

Response of Normal Rats to hCG

The serum testosterone response of sham-operated rats to a single injection of 100 i.u. hCG was biphasic (Fig. 1*a*). Serum testosterone was increased within 2 h (73·71 ± 5·89 nmol/l) and then declined over the next 22 h reaching a nadir of 41·79 ± 10·61 nmol/l 24 h after injection. A second significant increase in serum testosterone (111·37 ± 27·67 nmol/l; $P < 0·05$) was seen at 3 days, following which serum testosterone then declined, but was still significantly increased above saline-injected animals at 4 and 5 days.

The basal *in vitro* secretion of testosterone by testes of normal rats injected with hCG was significantly increased 2 and 12 h after injection (483·0 ± 89·6 and 588·0 ± 261·0 pmol per testis per 4 h compared with 234·5 ± 52·5 and 226·8 ± 36·1 pmol per testis per 4 h for saline-injected controls; $P < 0·05$; Fig. 1*b*). Addition of hCG to the media caused a further increment in testosterone secretion 2 h after hCG injection (from 483·0 ± 89·6 to 891·0 ± 120·4 pmol per testis per 4 h, $P < 0·05$), but the amount of testosterone produced was significantly lower than hCG-stimulated saline-treated controls (1100·4 ± 129·5 pmol per testis per 4 h). From 12 h to 2 days following injection of 100 i.u. hCG the testis was totally refractory to further hCG stimulation, since the amount of testosterone produced during this period could not be increased above the basal production, hCG-stimulated levels of testosterone returned to normal at 3, 4 and 5 days.

Serum hCG levels in sham-operated rats were elevated 2 h after hCG injection. Maximum concentration was found at 12 h (204·0 ± 12·9 mi.u./ml) after which hCG levels began to decrease and became undetectable at 5 days (Fig. 2*a*).


Sham-operated Rats
Serum testosterone (nmol/l)
Hours
Days
Time after injection
a
Refractory
Testosterone production (% stimulated control)
saline
stimulated
hCG
basal
hCG
saline
b
Cryptorchid Rats
c
d
Hypophysectomized Rats
e
hCG injected
stimulated
basal
saline injected
f

Response of Cryptorchid Rats to hCG

Four weeks of cryptorchidism had no effect on body weight, but caused a significant reduction in testis weight and in the serum level of testosterone (Table 1). Serum levels of LH and FSH were significantly higher in cryptorchid rats than in controls ($P < 0{\cdot}05$, Table 1).

A single injection of 100 i.u. hCG to cryptorchid rats produced a significant increase in serum testosterone levels within 2 h ($105{\cdot}63 \pm 6{\cdot}28$ nmol/l compared with $14{\cdot}91$ nmol/l for saline-injected cryptorchid controls; $P < 0{\cdot}05$, Fig. 1*c*). Testosterone levels remained high in cryptorchid rats for 24 h and fell to $8{\cdot}61 \pm 1{\cdot}97$ nmol/l at 1 day. Two and three days after the hCG injection there was a slight, but significant elevation of serum testosterone with values of $19{\cdot}53 \pm 1{\cdot}89$ and $26{\cdot}52 \pm 5{\cdot}29$ nmol/l (compared with $5{\cdot}72 \pm 1{\cdot}63$ and $9{\cdot}98 \pm 2{\cdot}72$ nmol/l for saline-injected cryptorchid controls; $P < 0{\cdot}05$). After the second peak testosterone levels declined and were not different to the cryptorchid control levels.

Table 1. Effect of cryptorchidism and hypophysectomy on body weight, testis weight and serum levels of testerone, LH and FSH

Values are means ± s.e.; $n = 5$. *$P < 0{\cdot}05$

Treatment of rats (4 weeks)	Body weight (g)	Testis weight (g)	Serum testosterone (nmol/l)	Serum LH (ng/ml)	Serum FSH (ng/ml)
Cryptorchid	$437{\cdot}97 \pm 11{\cdot}96$	$1{\cdot}49 \pm 0{\cdot}04$*	$10{\cdot}99 \pm 0{\cdot}70$	$1{\cdot}95 \pm 0{\cdot}13$*	$604{\cdot}60 \pm 49{\cdot}20$*
Hypophysectomized	$297{\cdot}15 \pm 3{\cdot}35$*	$0{\cdot}84 \pm 0{\cdot}02$*	$0{\cdot}29 \pm 0{\cdot}03$*	$<0{\cdot}13$[A]	<70[A]
Sham-operated	$368{\cdot}95 \pm 3{\cdot}34$	$3{\cdot}61 \pm 0{\cdot}05$	$19{\cdot}04 \pm 1{\cdot}26$	$1{\cdot}26 \pm 0{\cdot}06$	$469{\cdot}14 \pm 46{\cdot}35$

[A] Not detectable.

The basal *in vitro* secretion of testosterone by cryptorchid testes ($518{\cdot}0 \pm 45{\cdot}9$ pmol per testis per 4 h) was greatly increased compared with normal rat testes. Addition of hCG (700 mi.u./ml) to the media increased this secretion to $3337{\cdot}6 \pm 304{\cdot}1$ pmol per testis per 4 h, an amount again exceeding that secreted by normal rat testes. Two hours after hCG injection the basal and hCG-stimulated secretion of testosterone were not different from each other ($2612{\cdot}4 \pm 249{\cdot}3$ and $2651{\cdot}2 \pm 187{\cdot}6$ pmol per testis per 4 h respectively) or to the hCG-stimulated production of testosterone by saline-injected cryptorchid rats (Fig. 1*d*). The basal and stimulated concentration of testosterone in the media decreased at 12 h, and the testes were refractory to further hCG stimulation. This refractoriness persisted until day 2 at which point the basal and hCG-stimulated secretion of testosterone from hCG injected animals were not significantly different to the basal testosterone levels of saline-injected cryptorchid rats. By day 3 the basal and hCG-stimulated levels of testosterone in the media of testes from hCG treated animals had returned to normal (Fig. 1*d*).

Fig. 1. Serum testosterone responses (*a*, *c* and *e*) and *in vitro* testosterone secretion by decapsulated testes (*b*, *d* and *f*) following a single injection of saline or 100 i.u. hCG to normal adult sham-operated male rats (*a* and *b*), cryptorchid rats (*c* and *d*) and hypox. rats (*e* and *f*). For serum testosterone each point is the mean ± s.e. of five animals. *Significantly different from saline-injected value (– – –) at the same time point, $P < 0{\cdot}05$. For *in vitro* testosterone secretion, each point represents the mean testosterone production of five testes expressed as a percentage of stimulated saline-injected control levels. Standard error bars omitted for clarity.

Serum hCG levels were significantly increased in cryptorchid rats 2 h after injection to 100 i.u. hCG (48·4 ± 10·58 mi.u./ml, Fig. 2*a*). Peak values were measured after 12 h (315 ± 35·0 mi.u./ml) and were greater than those seen in normal rats. hCG levels were still very high at 2 days (99·2 ± 5·0 m.i.u./ml) but then decreased, so that on day 5 levels of 4·0 ± 0·2 m.i.u. were measured when hCG could not be detected in the serum of saline-injected cryptorchid animals.

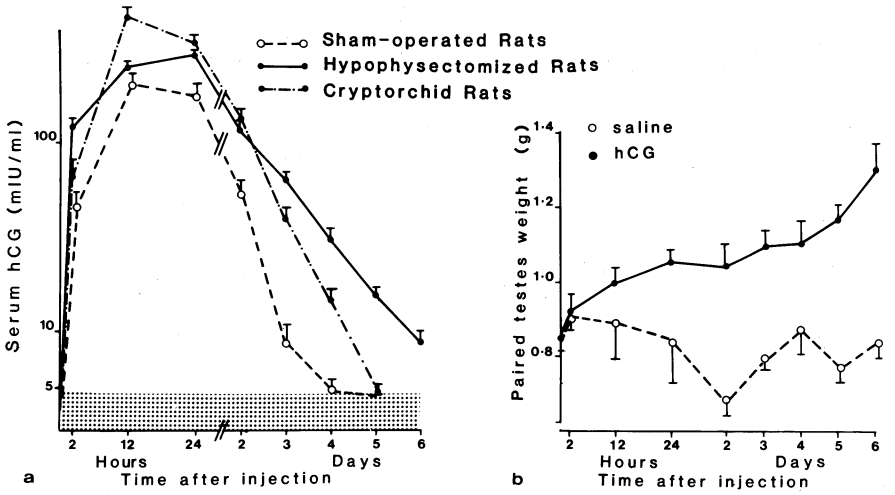


Fig. 2. (*a*) Increase in serum hCG levels produced by a single injection of 100 i.u. hCG in sham-operated rats (○ - - - ○), cryptorchid rats (-·-·-) and hypox. rats (———). hCG was undetectable in the serum of saline-treated rats (hatched area). Each point is the mean ± s.e. of five animals. (*b*) Increase in paired testis weight of hypox. rats following a single injection of 100 i.u. hCG (———). Paired testis weight of saline-treated hypox. rats is shown by the broken line (- - -). Each point represents the mean ± s.e. of five animals.

Response of Hypox. Rats to hCG

Hypophysectomy for 4 weeks caused a significant reduction in body weight and testis weight (Table 1). Serum testosterone concentrations were significantly decreased by hypophysectomy and LH and FSH were undetectable in the serum (Table 1).

A single injection of 100 i.u. hCG had a marked effect on the testis weight (Fig. 2*b*) and on the levels of serum testosterone (Fig. 1*e*). The testis weight following a hCG injection increased significantly at 1 day ($P < 0·05$) and continued to rise so that by 6 days it was 1·5 times the weight of testes from saline-treated hypox. rats (Fig. 2*b*).

Within 2 h of stimulation with 100 i.u. hCG, serum testosterone levels in hypox. rats were significantly elevated (2·94 ± 0·88 nmol/l compared with 0·28 ± 0·14 nmol/l for hypox. rats treated with saline, $P < 0·05$, Fig. 1*e*). Levels continued to increase until 12 h when values of 10·64 ± 0·84 nmol/l were recorded. At 24 h there was a slight, but significant decrease in serum testosterone, although levels were still much higher than saline-injected hypox. controls (3·96 ± 0·95 nmol/l compared with 0·34 ± 0·11 nmol/l). A second increase in serum testosterone occurred at 2 days reaching peak values of 102·55 ± 2·63 nmol/l on day 4. Testosterone levels fell after 4 days, but remained elevated 6 days after the hCG injection.

The amount of testosterone secreted by hypox. rat testes *in vitro* was significantly less than that secreted by normal rat testes. The basal and hCG-stimulated *in vitro* production of testosterone by testes from hypox. rats was 5·34 ± 1·12 pmol per testis per 4 h and 19·78 ± 4·87 pmol per testis per 4 h, respectively. The temporal *in vitro* secretion of testosterone by the testes from hypox. rats following a single injection of 100 i.u. hCG mimicked the *in vivo* serum testosterone pattern. Two hours after the injection there was a significant increase in the basal and stimulated concentration of testosterone in the media (59·96 ± 3·82 and 64·54 ± 1·69 pmol per testis per 4 h, respectively Fig. 1*f*). The basal and stimulated levels of testosterone were not significantly different at this time and at all subsequent times until day 6, indicating that the testis was refractory to any further stimulation. The production of testosterone by hypox. testes 12 h after the hCG injection was essentially the same as that after 2 h (Fig. 1*f*). At 24 h the basal and hCG-stimulated production of testosterone decreased to levels similar to those from saline-treated hypox. rats (19·78 ± 2·80 and 24·29 ± 2·17 compared with 33·60 ± 13·27 pmol per testis per 4 h for stimulated saline-treated hypox. controls). The amount of testosterone produced, basal and hCG-stimulated, by hCG-treated hyox. rat testes increased again at 2 days. A marked rise occurred subsequently with peak production being reached on day 4 (960·75 ± 131·74 basal and 1090·25 ± 11·62 stimulated pmol per testis per 4 h, respectively). These levels of testosterone secretion are comparable to the hCG-stimulated secretion by normal rat testes. The steroidogenic activity of hCG-treated testes decreased on day 5 and although basal and stimulated levels were still not significantly different from each other, they were still greater than the saline-treated hypox. controls. On day 6 the testes responded to further *in vitro* hCG stimulation, as basal levels were 310·10 ± 46·20 pmol per testis per 4 h increasing to 614·60 ± 70·35 pmol per testis per 4 h when hCG was added to the media.

Administration of 100 i.u. hCG to hypox. rats produced elevated serum hCG levels within 2 h (120·0 ± 7·07 mi.u./ml; Fig. 2*a*). Peak concentrations were seen at 1 day when values reached 294·0 ± 10·29 mi.u./ml. Following this, levels declined but they were still above the limit of detection (4·6 mi.u./ml) 6 days after injection (8·8 ± 1·12 mi.u./ml). hCG was present in the serum of hypox. rats for a longer period than sham-operated rats but could not be detected in serum taken from hypox. animals injected with saline.

Discussion

The results described in this study showed that the biphasic serum testosterone response to a single injection of 100 i.u. hCG was modifed by both cryptorchidism and hypophysectomy. In the former, the early or acute increase in serum testosterone, 2 h after injection, was similar to that observed in normal adult rats (Haour and Saez 1977; Hodgson and de Kretser 1982) and is in close agreement with the levels measured by Kerr *et al.* (1979) in cryptorchid animals. However, the second increase in serum testosterone levels 3 days after hCG injection was greatly diminished in comparison with normal adult animals (Haour and Saez 1977; Hodgson and de Kretser 1982). It is unlikely that this diminution represents a direct effect of the decreased steroidogenic enzyme activity which has been reported in cryptorchid testes by a number of workers (Llaurado and Dominguez 1963; Inano and Tamaoki 1968; Niewenhuis 1980) since the initial response of serum testosterone within 2 h of hCG injection was comparable with that seen in normal adult rats (Haour and Saez 1977; Hodgson and de Kretser

1982). Furthermore, the increased secretion of testosterone by cryptorchid testes *in vitro* compared with normal testes does not suggest an enzyme deficiency. However, a reduced availability of hCG to the Leydig cells may result in a decreased degree of restimulation by hCG after recovery from refractoriness. Previous work has demonstrated the dependence of this second testosterone response on elevated circulating levels of gonadotrophin (Hodgson and de Kretser 1982). Although the peripheral serum levels of hCG were higher in cryptorchid than in normal rats at 3 days, the cryptorchid testis is known to have a reduced blood flow (Damber *et al.* 1978) and uptake of hCG (Sharpe 1983). It is unclear why the hCG levels in cryptorchid rats are higher than controls and further studies are necessary to define if the metabolic clearance of hCG is altered in that state.

Despite the quantitative differences in testosterone secretion, the pattern of the *in vitro* steroidogenic activity following a single injection of hCG was similar in cryptorchid and normal testes (Hodgson and de Kretser 1982). Testes showed peak steroidogenic capacity 2 h after hCG and were refractory from 12 h to 2 days, secreting low to basal amounts of testosterone even in the presence of excess hCG. The *in vivo* parallel of this refractoriness occurs during the same interval, when serum testosterone levels decline in the face of elevated serum hCG levels. The uniformity of this response suggests that the mechanism of action of hCG on the Leydig cell is unaltered by cryptorchidism.

In accordance with previous reports (Amatayakul *et al.* 1971; Gomes and Jain 1976; de Kretser *et al.* 1979; Kerr *et al.* 1979) cryptorchidism was associated with elevated serum levels of LH and FSH and subnormal levels of testosterone. An increase in Leydig cell size and number have also been documented following cryptorchidism (Iturriza and Irusta 1969; Kerr *et al.* 1979). Infertile men with Leydig cell hyperplasia also show an acute serum testosterone response similar to normal men and an enhanced *in vitro* production of testosterone when challenged with hCG (Nieschlag *et al.* 1979). Since these men also have elevated levels of LH and FSH with normal and subnormal levels of testosterone their hormonal profile is reminiscent of cryptorchidism. Unfortunately, the serum testosterone response of these men was not monitored beyond several hours and so it is not known whether a second component of the response exists. It is possible that the pattern of the serum testosterone response following hCG administration to cryptorchid rats may not be specific for cryptorchidism but may represent a uniform response of Leydig cells in other forms of testicular damage associated with elevated gonadotrophin levels and Leydig cell hyperplasia/hypertrophy. Further work on other forms of testicular damage is required to support this hypothesis.

In the hypox. rat stimulation of Leydig cell activity via a single hCG injection produced a small increment in serum testosterone at 2 h, which was very much smaller than that of normal intact rats (Hodgson and de Kretser 1982), reflecting the atrophied state of the Leydig cells. The magnitude of the late or secondary response, however, was comparable to that of intact animals similarly treated with hCG. This biphasic serum testosterone pattern following hCG administration to chronic hypox. rats is in agreement with the findings of Haour and Saez (1978) in rats hypophysectomized for 8 days. The pattern is also similar to that found in hypogonadotrophic hypogonadal men (Smals *et al.* 1980). However, in these men the acute increase in serum testosterone was not significant and even during the second phase when serum testosterone levels were significantly elevated, they did not reach the maximum values seen in normal

men (Smals *et al.* 1980). This may reflect the small dose of hCG used in these studies or alternatively, the fact that the Leydig cells of hypogonadotrophic hypogonadal men are in a prepubertal state and therefore have a reduced capacity to produce testosterone.

It is of interest that the biphasic pattern persists in the hypophysectomized state since it is evident that the trophic action of hCG would set in motion stimulatory changes involving the synthesis of enzymes necessary for the steroidogenic process. The *in vitro* pattern of steroidogenesis showed a refractory state that persisted for 5 days following hCG, far longer than that demonstrated in intact rats (Hsueh *et al.* 1976; Hodgson and de Kretser 1983). The reason for this prolonged refractory period may be the result of the atrophic state of the hypophysectomized Leydig cells, particularly, if the view of Quinn *et al.* (1981) is accepted, namely, that the refractoriness is due to lack of substrate for steroidogenesis. The stimulatory changes resulting from the hCG injection could be envisaged as increasing the available substrate in the Leydig cell enabling it to increase basal secretion after 24 h thereby elevating serum testosterone levels. However, with the addition of further hCG *in vitro*, the biosynthetic machinery of the cell is unable to respond further, resulting in the persistence *in vitro* of a refractory state. Alternatively, the lowered clearance rate of hCG in hypox. rats, causing a longer period of elevated hCG levels, may be another factor responsible for the lengthened refractory state. Regardless of the cause for the lengthened refractory state the testis of the hypox. rat undergoes a very large increase in steroidogenic output in a comparatively short length of time.

The testis weight of hypox. rats increased significantly after hCG treatment. An increase in lymph and Leydig cell volume has been found after hCG treatment of hypox. rats (Laws *et al.* 1984; Laws 1985). However, this increase can only account for a maximum of 0·38 g and the hypox. testis weight increment 6 days after hCG injection was 0·58 g. Clearly, other compartments of the testis must contribute to this weight gain. It is possible that part of this increase represents a re-initiation of spermatogenesis in some tubules since previous studies have shown that increased testosterone levels can partially restore spermatogenesis in long-term hypox. rats (Boccabella 1963).

In summary, in both cryptorchidism and hypophysectomy, the pattern of the testicular response to hCG differs from normal and this fact should be noted in the design of future studies. While, ideally, the response in each state should be characterized by dose responses, we would submit that the altered responses are not due to optimal stimulation since, in both states, hCG levels in serum were significantly higher than in control rats.

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