Effects of photoperiod and hCG on the regulation of testicular LH/hCG receptors in Syrian hamsters (*Mesocricetus auratus*)*

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Summary. The regulation of testicular LH/hCG receptors was studied in Syrian (golden) hamsters with testicular atrophy induced by exposure to short photoperiod (5L:19D) and in gonadally active hamsters kept in a long photoperiod (14L:10D). By 24 h after injection of hCG, long-photoperiod hamsters showed a dose-related decrease in the number of testicular LH/hCG receptors. At 48 and 72 h, there was a recovery from this 'down-regulation'. The recovery was much faster than has been reported for the rat and mouse, and it resulted in elevation of testicular LH/hCG receptor concentrations above basal values. Hamsters with short photoperiod-induced testicular atrophy showed an increase in testicular LH/hCG receptors after injection of hCG, except for animals injected with a very high dose. The hCG-induced increase in testicular LH/hCG binding in these animals was associated with reappearance of testosterone responses to subsequent hCG stimulation. Response of testicular LH/hCG receptors to hCG in prepubertal hamsters resembled that measured in animals with short photoperiod-induced gonadal atrophy.

Introduction

Syrian (golden) hamsters (*Mesocricetus auratus*) are seasonal breeders, i.e. they exhibit annual cycles of gonadal activity and quiescence. In this species, testicular atrophy induced by pineal-mediated effects of short photoperiod (less than 12.5 h light per 24 h) is associated with a pronounced decrease in plasma prolactin and gonadotrophin concentrations (Reiter & Johnson, 1974; Bex, Bartke, Goldman & Dalterio, 1978) and loss of testicular LH receptors (Bex & Bartke, 1977). Prolactin has been shown to influence the total content and concentration of testicular LH/hCG receptors in different rodent species, including the Syrian hamster (Bohnet & Friesen, 1976; Bex & Bartke, 1977; Aragona, Bohnet & Friesen, 1977; Zipf, Payne & Kelch, 1978; Klemcke, Bartke & Borer, 1984), and to modulate their autoregulation in the rat (Sharpe & McNeilly, 1979; Zipf, 1980). This study was undertaken to determine effects of short photoperiod-induced testicular atrophy on the autoregulation of testicular LH/hCG receptors in the Syrian hamster. Regulation of LH/hCG receptors in immature animals and the relationship between the effects of hCG on LH/hCG receptors and on plasma testosterone levels were also examined.

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Materials and Methods

Animals and sample collection. Syrian hamsters (Lak:LVG[SYR]) were purchased from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) or raised in our animal colony from Charles River animals, and were maintained since birth in a long photoperiod (14 h light:10 h dark; lights on at 07:00 h CST). To produce atrophy of the testes, some of the adult males were transferred to a short photoperiod (5L:19D; lights on at 08:00 h). The temperature was maintained at $22 \pm 2^\circ\text{C}$, and the animals had free access to food (Wayne Breeder Blox, San Antonio, TX, U.S.A.) and water.

Hamsters were killed by decapitation and trunk blood was collected in beakers containing 0.25 ml 6% EDTA (Sigma Chemical Co., St Louis, MO, U.S.A.). Plasma was obtained by centrifugation at 4°C and frozen (-20°C) until assayed. Testes were removed, weighed and placed in ice-cold PBS buffer (0.01 M-sodium phosphate buffer, pH 7.4, 0.15 M-NaCl, 0.01% merthiolate) for measurement of hCG binding.

Experiment 1: effects of different doses of hCG. Adult hamsters (>3 months of age) which had been kept in a long photoperiod (14L:10D) or exposed to a short photoperiod (5L:19D) for 8 weeks were injected s.c. with 0.1, 0.3 or 0.9 i.u. hCG (A.P.L. Ayerst, New York, NY, U.S.A.) in 0.01 ml commercial isotonic saline (Abbott Labs, North Chicago, IL, U.S.A.) per g body weight or with saline (0.15 M-NaCl) alone. The animals were killed 24 h after injection, and testes and trunk blood were collected.

Experiment 2: time course of response to hCG. Adult hamsters were exposed to a long or short photoperiod as in Exp. 1 and injected with hCG (0.3 i.u./g body wt.) or saline and were killed 24, 48 or 72 h later. Trunk blood and testes were collected.

Experiment 3: effects of hCG in immature hamsters. Immature, 34-day-old hamsters, raised and maintained in a long photoperiod (14L:10D), were injected with hCG (0.3 i.u./g body wt) or saline and killed 24 h later. Blood and testes were collected.

Experiment 4: effects of hCG in hCG-primed hamsters. Adult hamsters maintained in a long or short photoperiod as in Exp. 1 were injected with hCG (0.3 i.u./g body wt) or saline. After 24 h, half of the animals from each treatment group received an injection of 0.3 i.u. hCG/g body weight and the others were injected with saline. The hamsters were killed 2 h after the second injection and blood was collected.

Measurement of hCG binding and testosterone. Unoccupied testicular LH/hCG receptors were measured by the procedures previously described (Bartke, Klemcke & Amador, 1981a; Klemcke, Bartke & Goldman, 1981), with the following modifications: (1) tissue homogenates were initially centrifuged at 100 g for 5 min, and the supernatant was then recentrifuged at 1786 g for 20 min; (2) 1% bovine serum albumin (Miles Labs, Naperville, IL, U.S.A.)-PBS was used as incubation buffer. The ^{125}I -labelled hCG (CR-121, NIH) used had an average maximum binding of 47% with an average specific activity of 54 $\mu\text{Ci}/\mu\text{g}$. The concentration of protein in testicular membrane preparations used for measurement of hCG binding was determined by a modification of Lowry's method (Markwell, Haas, Bieber & Tolbert, 1978), using bovine serum albumin as the standard.

Testosterone concentrations were measured by radioimmunoassay after extraction of plasma samples with hexane-ethyl acetate (9:1 v/v). The antiserum used (Endocrine Sciences, Tarzana, CA, U.S.A.) showed 44% cross-reactivity with 5α -dihydrotestosterone. Results are reported as testosterone because of the very low levels of 5α -dihydrotestosterone in peripheral circulation of male golden hamsters (Lau, Saksena, Dahlgreen & Chang, 1978). Recovery of internal trace was 72–96%. The intra- and inter-assay coefficients of variation were 4.7% and 15%, respectively. The average sensitivity of this radioimmunoassay was 138 pg/ml plasma.

Statistics. All data were examined for main effects and interaction by use of two-way or multiway analysis of variance. All data were tested for homogeneity of variance by Bartlett's test

and for normality of distribution using the Kolmogorov–Smirnov test and log or square-root transformed as indicated (Sokal & Rohlf, 1981).

Results

Experiment 1: effects of different doses of hCG

As anticipated, saline-injected hamsters kept in 5L:19D had a higher concentration, but lower total content, of LH receptors than did the corresponding hamsters in 14L:10D (Text-fig. 1). The 'low' dose of hCG (0.1 i.u./g) appeared to increase the concentration and content of LH/hCG receptors in both groups, but these changes were small and not statistically significant as compared to saline-injected controls. In contrast, injection of the 'intermediate' dose of hCG (0.3 i.u./g) produced marked and differential responses in both groups. In the gonadally-active animals in 14L:10D, the concentration of testicular LH/hCG receptors was significantly reduced after injection of this dose of hCG, whereas in the animals in 5L:19D, with atrophic testes, it was significantly increased by the same treatment. The parallel changes in the total content of LH/hCG receptors in the testes of these animals did not reach statistical significance. The 'high' dose of hCG (0.9 i.u./g) resulted in reductions in concentration of LH/hCG receptors in both groups of animals and in content of LH/hCG receptors in long photoperiod hamsters.

Text-fig. 1. Testicular hCG binding and plasma testosterone concentrations in hamsters as a function of dose of injected hCG. Values are mean $\pm$ s.e.m. for 6–8 animals /group except for control adults in the long photoperiod ($N = 20$) and in the short photoperiod ($N = 23$). Any values with a letter in common are not significantly different ($P > 0.05$ using the Student–Newman–Keuls procedure of the multiple range test).

Experiment 2: time course of response to hCG

In hamsters in 14L:10D, the concentration of LH/hCG receptors was reduced 24 h after injection with 0.3 i.u. hCG/g (Text-fig. 2). By 48 h after injection, there was an increase in the concentration of LH/hCG receptors and by 72 h there was an increase both in concentration and total content. In hamsters in 5L:19D a substantial and statistically significant increase in LH/hCG receptor concentration and total content was measured at all three times after hCG administration; concentration of LH/hCG receptors in hamsters in 5L:19D was significantly greater than in the 14L:10D hamsters.

Text-fig. 2. Testicular hCG binding and plasma testosterone concentrations in hamsters as a function of time after hCG injection. Values are mean $\pm$ s.e.m. for 6–8 animals/group except for saline-injected hamsters in the long photoperiod ($N = 21$) and in the short photoperiod ($N = 24$). Any values with a letter in common are not significantly different ($P > 0.05$ using the Student–Newman–Keuls procedure of the multiple range test).

Experiment 3: effects of hCG in immature hamsters

In immature hamsters in 14L:10D injection of 0.3 i.u. hCG/g produced a slight, although not significant increase in concentration and total content of LH/hCG receptors (Text-fig. 1). Comparison with saline-injected groups indicated that basal concentrations of testicular LH/hCG receptors were higher in immature hamsters than in adult animals regardless of their photoperiod exposure, whereas total content was comparable to that measured in adult males in 14L:10D (Text-fig. 1).

Effects of treatments on plasma testosterone concentrations

In the 14L:10D hamsters in Exp. 1, peripheral plasma testosterone concentrations were not significantly elevated 24 h after injection of 0.1 i.u. hCG/g, but were significantly increased by 0.3

i.u./g (Text-fig. 1). In the 5L:19D animals, the doses of 0.1 and 0.3 hCG produced an increase in plasma testosterone concentrations. Injection of the high dose of hCG (0.9 i.u./g) had no effect on plasma testosterone concentrations measured 24 h later in either group. As expected, the hamsters in 5L:19D had significantly lower basal plasma testosterone values than did those in 14L:10D.

In Exp. 2, an increase in plasma testosterone concentrations was observed in 5L:19D hamsters at 24, 48 and 72 h after treatment with 0.3 i.u. hCG/g, whereas an increase was measured only at 24 and 72 h after injection in hamsters in 14L:10D (Text-fig. 2).

In immature hamsters, basal plasma testosterone concentrations were significantly lower than in gonadally active adults in 14L:10D (Text-fig. 1). Plasma testosterone concentrations in immature hCG-treated hamsters were elevated and comparable to those measured in similarly treated adults in 5L:19D. However, they remained below plasma testosterone values measured in hCG-injected animals in 14L:10D.

Experiment 4: effects of hCG in hCG-primed hamsters

By 26 h after injection of hCG, plasma testosterone concentrations were significantly elevated in 14L:10D but not in 5L:19D hamsters (Table 1). Similarly, 2 h after the injection of hCG in saline-preinjected hamsters, plasma testosterone concentrations were increased only in animals in 14L:10D. In hamsters in 14L:10D, pretreatment with hCG had no effect on the acute testosterone response to the second hCG injection. In contrast, in hamsters in 5L:19D a significant elevation in plasma testosterone concentrations 2 h after hCG injection was observed only in animals preinjected with hCG.

Table 1. Plasma testosterone concentrations in hamsters maintained in a long (14L:10D) or a short (5L:19D) photoperiod and injected with hCG (0.3 i.u./g body weight) and/or saline 26 h and 2 h before being killed

Treatment		Testosterone conc. (ng/ml)	
- 26 h	- 2 h	14L:10D	5L:19D
Saline	Saline	0.67 $\pm$ 0.30 ^a (6)	0.83 $\pm$ 0.23 ^a (7)
Saline	hCG	6.03 $\pm$ 1.02 ^c (6)	1.24 $\pm$ 0.17 ^a (7)
hCG	Saline	3.38 $\pm$ 0.46 ^b (6)	0.89 $\pm$ 0.26 ^a (7)
hCG	hCG	5.14 $\pm$ 0.28 ^c (6)	2.43 $\pm$ 0.34 ^b (7)

Values are mean $\pm$ s.e.m. for the no. of animals in parentheses. In each column, values with a different superscript are significantly different ($P < 0.05$).

Discussion

The loss of unoccupied LH/hCG receptors after administration of hCG could reflect an apparent loss of receptors due to their occupancy by injected hCG (Dufau & Catt, 1980), a true loss of occupied and unoccupied receptors via internalization processes (Sharpe, 1979), or a combination of both phenomena. Extrapolation of the findings of Dufau & Catt (1980) for the rat suggests that the decrease in LH/hCG receptor numbers after injection of 0.9 i.u. hCG/g body weight in the present study may reflect mainly, if not exclusively, receptor occupancy. However, the loss of LH/hCG receptors measured 24 h after injection of 0.3 i.u. hCG/g body weight in hamsters in 14L:10D is unlikely to involve merely occupancy, because the same dose of hCG injected into hamsters in 5L:19D resulted in a significant increase in testicular LH/hCG receptors. Since hamsters kept in a short photoperiod have a reduced number of LH receptors (Bex & Bartke, 1977;

Klemcke *et al.*, 1981; present observations), the effect of occupancy in these animals should have been more, rather than less, pronounced.

Our results therefore indicate that, in hamsters, hCG can cause both an increase and a decrease of its own testicular receptors (positive and negative autoregulation) as has been previously reported for rats and mice (Huhtaniemi, Katikineni & Catt, 1981a; Huhtaniemi, Katikineni, Dufau & Catt, 1981b; Amador & Bartke, 1982; Huhtaniemi, Nozu, Warren, Dufau & Catt, 1982).

The time course of changes in concentration of LH/hCG receptors after hCG administration observed in the hamsters in the present study differed from that reported for the rat (Sharpe, 1976; Tsuruhara, Dufau, Cigorraga & Catt, 1977; Saez, Haour, Tell, Gallet & Sanchez, 1978; Huhtaniemi *et al.*, 1982) and mouse (Amador & Bartke, 1982). In long-photoperiod hamsters, the initial decline in LH/hCG receptor concentration at 24 h after hCG treatment was followed by a recovery 48 h after hCG, and an increase over the basal levels at 72 h after hCG. In short-photoperiod hamsters, hCG increased the concentration of its receptors 24 h after treatment, with even greater increases occurring at 48 and 72 h. The Syrian hamster, a cricetid rodent, therefore recovers from negative autoregulation of testicular LH receptors faster than do the rat and mouse, murid rodents, in which recovery takes 7–10 days.

Administration of hCG to immature hamsters produced an increase rather than a reduction in LH/hCG receptors, a result resembling the findings for immature mice and adult hamsters in short photoperiods (Amador & Bartke, 1982) (Text-fig. 1). This, together with the high concentration of LH/hCG receptors in immature hamsters, indicates that, with respect to testicular LH binding, hamsters in short photoperiods may be comparable, although not identical, to immature animals. The concentration of FSH receptors is elevated in adult hamsters with short photoperiod-induced gonadal regression and in immature animals (Bartke, Klemcke, Amador & Van Sickle, 1981b).

Although the effects of hCG on plasma testosterone concentrations in different groups of hamsters can be related to changes induced by hCG treatment in testicular LH/hCG receptors in the same animals, our results do not provide a complete evaluation of testicular steroidogenic responses to gonadotrophic stimulation because blood samples for assay of testosterone were collected when the animals were killed, i.e. 24 h after injection of hCG in Exps 1–3 and 2 or 26 h after injections of hCG in Exp. 4. Although the dose-related effects of hCG on plasma testosterone levels were qualitatively similar in hamsters exposed to long and short photoperiods, some differences were also apparent. After injection of each of the three doses of hCG, both the relative increase in and the absolute concentrations of plasma testosterone were lower in short-photoperiod hamsters with regressed gonads. The substantial increase in plasma testosterone values observed in long photoperiod animals after injection of 0.3 i.u. hCG/g body weight was accompanied by a decrease in LH/hCG receptors. In contrast, in short-photoperiod hamsters, the same dose of hCG caused only a modest elevation of plasma testosterone concentrations and a large increase in LH/hCG receptors. This could be due to a partial uncoupling of LH/hCG receptors and testicular steroidogenesis in short photoperiod hamsters. More likely, these findings reflect a disruption of testosterone biosynthesis at a step(s) distal to interaction with the LH/hCG receptor. We have previously observed that exposure of adult hamsters to a short photoperiod drastically reduces the ability of their testes to produce testosterone *in vitro* in response to hCG stimulation (Bartke, Goldman, Klemcke, Bex & Amador, 1980; A. Bartke, M. P. Hogan & H. G. Klemcke, unpublished observations). Lack of elevations in plasma testosterone concentrations after injection of 0.9 i.u. hCG/g in either group of hamsters may have been due to induction of a defect in the steroidogenic pathway by this large dose of hCG, an effect known to occur in the rat testis (Catt *et al.*, 1980).

The relationships of temporal patterns of responses of testicular LH/hCG receptors and plasma testosterone levels to hCG suggest additional functional differences between the long and short photoperiod-exposed hamsters. In long-photoperiod hamsters, the initial decline and subsequent recovery of LH/hCG receptors were accompanied by a biphasic response of plasma testosterone concentrations with increases occurring at 24 and 72 h. In contrast, in short-photoperiod hamsters an increase in plasma testosterone values persisted throughout this time, with no evidence of an

intervening decline. Moreover, when short photoperiod hamsters received two injections of hCG, in Exp. 4, plasma testosterone concentrations after the second injection were markedly increased, but the same treatment of long-photoperiod hamsters produced an increase similar to that observed after one hCG injection. In hamsters kept in a short photoperiod, therefore, pretreatment with hCG caused an increase in the content of LH/hCG receptors and in testosterone response to the second dose of hCG. A similar mechanism may be involved in the normal response of animals with regressed gonads to hormonal cues that initiate gonadal redevelopment in the spring. The influence of testicular LH/hCG receptor content on the steroidogenic response may not be limited to situations in which the content of receptors is reduced. In hamsters kept in long photoperiods and with pituitary grafts, LH/hCG receptor values are elevated and the increase in plasma testosterone concentrations after hCG administration is greater than that observed in sham-operated control males (A. G. Amador, A. Bartke & M. P. Hogan, unpublished observations).

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