Regulation of testicular LH/hCG receptors in golden hamsters (*Mesocricetus auratus*) during development*

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Summary. During prepubertal development in the golden hamster, there are major age-related changes in the number of testicular LH/hCG receptors. Between 22 and 35 days of age, there was > 10-fold increase in testicular LH/hCG receptors, followed by a decrease at Day 37. Concomitant with, but preceding slightly, the changes in receptors, were increases in plasma LH and FSH and most noticeably prolactin concentrations, between Days 10 and 20 of age. Inhibition of the increases in plasma levels of prolactin by daily injections of bromocriptine, between 14 and 31 days of age, resulted in suppressed testicular and seminal vesicle weights, and decreased content and concentration of testicular LH/hCG receptors. Similarly, the premature increase in plasma prolactin concentrations in prepubertal hamsters between 6 and 20 days of age, by means of ectopic pituitary transplants, resulted in increased testicular and seminal vesicle weights, as well as an increase in the concentration of testicular LH/hCG receptors. These results strongly suggest that increases in plasma prolactin values during development are important in enhancement of the development of testicular LH/hCG receptors.

Introduction

Changes in testicular luteinizing hormone/human chorionic gonadotrophin (LH/hCG) receptors during postnatal development have been studied in several species, including rats (Ketelslegers, Hetzel, Sherins & Catt, 1978; Geithövel, Brabant, Wickings & Nieschlag, 1981; Lefevre, Saez & Finaz, 1983), mice (Amador & Bartke, 1982), boars and bulls (Sundby, Torjensen & Hansson, 1981). Other studies have described changes in testicular LH receptors during fetal development in rabbits (Catt, Dufau, Neaves, Walsh & Wilson, 1975), humans (Huhtaniemi, Korenbrot & Jaffe, 1977) and rats (Gangnerau, Funkenstein & Picon, 1982). In general, there is an increase in total testicular content of LH receptors during development. There are, however, marked differences in the temporal pattern of both plasma hormones and LH receptors between species. It is therefore difficult to assess which hormone(s) are responsible for inducing the increase in concentration of these testicular receptors. It has been proposed that prolactin and/or follicle stimulating hormone (FSH) may be instrumental in inducing increases in the number of testicular LH receptors during development (Huhtaniemi, 1983).

The present studies were undertaken (a) to describe the changes in testicular LH/hCG receptors in male hamsters during postnatal development and (b) to determine the role of prolactin in inducing developmental changes in testicular LH/hCG receptors. We have therefore examined the

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concentration of testicular LH/hCG receptors in animals in which plasma prolactin concentrations were suppressed with bromocriptine, and in animals in which plasma prolactin concentrations were elevated by transplantation of homologous pituitaries under the kidney capsule.

Materials and Methods

Animals and sample collection. Golden hamsters (*Mesocricetus auratus*) (Lak:LVG (SYR)) were bred in our animal colony from animals purchased from Charles River Breeding Laboratories and were maintained from birth in a long photoperiod, 14 h light:10 h darkness (lights on at 07:00 h). Room temperature was maintained at $22 \pm 2^\circ\text{C}$, and the animals had free access to food (Wayne Breeder Blox) and water. Hamsters were killed by decapitation at 10:00 h. Plasma was saved for determinations of FSH, LH, prolactin and testosterone concentrations. Body weight and weights of the testes and seminal vesicles were recorded.

Experiment I. Hamsters were allocated to 10 groups and killed between the ages of 23 and 90 days. Plasma was saved for hormone analysis and testicular tissue was saved for subsequent assay of LH/hCG receptors.

Experiment II. These studies were done to determine more precisely the temporal pattern of plasma concentrations of hormones which precede the changes in testicular LH/hCG receptors seen in Exp. I. Animals maintained in a long photoperiod from birth (14L: 10D) were divided into 3 groups and killed at 10, 20, and 30 days of age. Plasma was saved for hormone analysis.

Experiment III. This experiment was designed to determine whether inhibition of the increases in plasma prolactin concentrations during development could prevent the prepubertal increase in testicular LH/hCG receptors detected in Exp. I. Begging at 14 days of age, male hamsters were injected with oil or bromocriptine (2-bromo- α -ergocriptine, kindly provided by Dr R. I. Elton, Sandoz, Inc., East Hanover, New Jersey). The dosage of bromocriptine used was that which has been shown to cause chronic suppression of plasma prolactin in adult hamsters ($39.3 \mu\text{g}/10 \text{ g}$ body weight) (Bartke, Hogan & Cutty, 1980). All males were killed at 32 days of age, by which time control animals had presumably been exposed to large increases in plasma prolactin values. Plasma was saved for hormone analysis and testicular tissue was frozen for assessment of testicular LH/hCG receptors. Plasma glucose concentrations were also determined using dextrose strips, since previous studies have shown that bromocriptine can block glucose-induced insulin increase (El-Denshary, Ismail, Gagerman, Sehlin & Täljedal, 1982).

Experiment IV. This experiment was conducted to determine whether premature elevation of plasma prolactin concentrations could enhance the development of testicular LH/hCG receptors. On Day 6 of age, males were sham operated or received a transplant of one-half or one anterior pituitary under the right kidney capsule. Adult female hamsters served as pituitary donors. All animals were killed at the age of 20 days, when plasma concentrations of prolactin were still low in age-matched, oil-injected controls. Plasma samples were saved for hormone analysis and testicular tissue was frozen for determination of testicular LH/hCG receptors.

Measurement of testicular LH/hCG receptors. Testicular LH/hCG binding sites were measured by radioreceptor assay using ^{125}I -labelled hCG, as previously described (Bartke, Klemcke & Amador, 1981; Klemcke, Bartke & Goldman, 1981). The ^{125}I -labelled hCG (CR 121, NIH) had an average maximum binding of 47% and an average specific activity of $52 \mu\text{Ci}/\mu\text{g}$. The concentration of protein in testicular membrane preparations was determined by a modification of the Lowry method (Markwell, Haas, Bieber & Tolbert, 1978) using bovine serum albumin as the standard.

Radioimmunoassays. Plasma FSH was quantified by a radioimmunoassay procedure using iodinated rat FSH (NIH I-3), and rat FSH standard (NIH RP-1), as previously described (Bartke

et al., 1981). Plasma LH was determined using the ovine:ovine LH RIA kits as previously described for the hamster (Bex, Bartke, Goldman & Dalterio, 1978). The inter-assay and intra-assay coefficients of variation for FSH were 5.2% and 7.7% respectively, and for LH were 5.7% and 6.2% respectively. Plasma prolactin was measured using an homologous radioimmunoassay (Soares, Colosi & Talamantes, 1983) using iodinated hamster prolactin and a specific antiserum against hamster prolactin. The intra-assay coefficient of variation was 6%.

Statistics. The data were analysed using a one-way ANOVA, Student-Newman-Keuls, Student's *t* test, or the Pearson correlation test. The data were tested for homogeneity of variance by Bartlett's test and for normality of distribution by the Kolmogorov-Smirnov test. Log or square root transformations were done as needed (Sokal & Rohlf, 1981).

Results

Experiment I: developmental changes in testicular LH/hCG receptors

Between 23 and 35 days of age, there was a greater than 10-fold increase in LH/hCG receptor concentration, which was followed by an immediate decrease between 35 and 38 days of age (Text-fig. 1a). The concentration of LH/hCG receptors continued to decline gradually until 53 days of age, when the concentration of receptors was comparable to that observed in mature 90-day-old hamsters. Changes in the total content of testicular LH/hCG receptors followed a similar pattern with a marked increase between 23 and 35 days of age, followed by a decrease at Day 38. There was

Text-fig. 1. Testicular hCG binding and testes weight as a function of age in hamsters during development. Values are mean $\pm$ s.e.m. for the numbers of hamsters indicated. Different letters represent significant differences ($P < 0.05$) amongst groups using the Student-Newman-Keuls procedure of the multiple range test.

little difference in the total content of testicular LH/hCG receptors between the ages of 38 and 90 days (Text-fig. 1b). Plasma testosterone concentrations remained relatively unchanged between 23 and 38 days of age then gradually increased between 40 and 58 days of age (Text-fig. 1c). Testicular growth occurred in three phases: slow growth between 23 and 35 days of age, followed by rapid growth between 35 and 47 days of age, and a final phase of slow growth between 47 days of age and maturity (Day 90) (Text-fig. 1d).

Experiment II: developmental changes in plasma hormones

Between Days 10 and 30 of age there were gradual increases in plasma values of LH and FSH (Text-figs 2a & b). These changes were significant only for plasma concentrations of FSH which increased significantly by Days 20 and 30. However, the most dramatic change in plasma hormones was that of prolactin (Text-fig. 2c): there was a >30-fold change between Days 10 and 20 of age, followed by a significant decrease at Day 30. Increases in testicular weight in these animals (Text-fig. 2d) occurred on a schedule similar to that in Exp. I. Testicular weight increased slightly but significantly between Days 20 and 30, when there was an approximate 4-fold increase in testicular weight.

Text-fig. 2. Plasma hormone levels and testicular weight in hamsters as a function of age during development. Values are mean $\pm$ s.e.m. for the numbers of hamsters indicated. Different letters represent significant differences ($P < 0.05$) amongst groups using the Student–Newman–Keuls test of the multiple range test.

Experiment III: effects of inhibition of prolactin release on the development of testicular LH/hCG receptors

In the immature male hamster, treatment with bromocriptine between 14 and 31 days of age caused a reduction of more than 90% in plasma prolactin concentrations measured 24 h after administration of the last dose of bromocriptine. Suppression of plasma prolactin values during this period resulted in a significant decrease in the concentration and total content of testicular LH receptors as compared to that seen in control animals that underwent normal prepubertal development (Text-figs 3a & 3b). Similarly, testicular weight and seminal vesicle weight were also significantly reduced compared to values in control animals (Text figs 3d & 3e). Plasma gonadotrophin

Text-fig. 3. Measurements taken in sesame oil- or bromocriptine-injected immature hamsters killed at 32 days of age. Values are mean $\pm$ s.e.m. for the numbers of hamsters indicated. Statistical analysis was by Student's *t* test.

Table 1. Correlation between testicular and seminal vesicle weight, plasma prolactin, FSH and LH concentrations, and testicular LH receptor concentration and total content in hamsters injected with oil or bromocriptine and killed at 31 days of age

	Testes weight	Seminal vesicle weight	Plasma LH	Plasma FSH	Plasma prolactin	Receptor concentration
Receptor content	0.000	0.003	0.399	0.070	0.001	0.000
Receptor concentration	0.013	0.059	0.558	0.273	0.002	
Plasma prolactin	0.017	0.047	0.228	0.426		
Plasma FSH	0.006	0.164	0.248			
Plasma LH	0.460	0.063				
Seminal vesicle weight	0.000					

Values are for *P* after analysis by Pearson's correlation coefficients.

concentrations were not altered significantly by bromocriptine treatment (Text-figs 3f & 3g) although they appeared to be lower than those in control animals. Moreover, bromocriptine effects appeared to be specific inhibition of reproductive development rather than general developmental effects as supported by the observation that there were no effects on body weight (Text-fig. 3c) or blood glucose levels (data not shown). In this experiment the content of LH receptors/testes correlated significantly ($P < 0.05$) with testis weight, seminal vesicle weight, and plasma prolactin

concentrations, but did not correlate with plasma LH or FSH values (Table 1). Also, the concentration of LH receptors correlated ($P < 0.05$) with testis weight and plasma prolactin, but not with plasma LH or FSH concentrations.

Experiment IV: effects of elevation of plasma prolactin on the development of testicular LH/hCG receptors

Pituitary grafts, of either 1/2 or one pituitary, resulted in a stepwise increase in plasma prolactin concentrations and for both types of graft, plasma prolactin values were significantly greater than those in control animals at this time (Text-fig. 4c). This premature significant elevation of plasma prolactin concentrations in prepubertal hamsters between Days 6 and 20 of age appeared to advance testicular growth significantly (Text-fig. 4b) and to increase the concentration of testicular LH/hCG receptors (Text-fig. 4a). Animals receiving a whole pituitary demonstrated an approximately 2-fold increase in the concentration of testicular LH/hCG receptors. The receptor concentrations between groups were not significantly different by a Student-Newman-Keuls test, possibly due to high variations in receptor concentrations within groups. There was a significant direct correlation between plasma prolactin values and testicular LH/hCG receptor concentration across all groups ($r = 0.88$, $P < 0.01$).

Text-fig. 4. Concentration of LH/hCG receptors, weight of testes, and plasma prolactin concentrations as a function of the number of pituitaries grafted in 20-day-old hamsters. Values are mean $\pm$ s.e.m. for the numbers of hamsters indicated. Different letters represent significant differences ($P < 0.05$) amongst groups using the Student-Newman-Keuls test of the multiple range test.

Discussion

Comparison of the temporal pattern of changes in testicular LH receptors, testicular weight, and plasma testosterone concentrations during development in the golden hamster reveals that (a) there was a dramatic 10-fold increase in the concentration of testicular LH receptors between 23 and 35 days of age; (b) these receptor changes preceded and therefore probably are instrumental in initiating testicular growth which occurred most noticeably between 35 and 47 days of age; and (c) these receptor changes may be involved in increasing the sensitivity of the testis to LH, as suggested by

the increases in plasma testosterone concentrations that occurred between Days 40 and 58 of age. The developmental changes in concentration of LH receptors in the golden hamster, described here, are similar to receptor changes observed in the bull and the rat (Geisthövel *et al.*, 1981; Sundby *et al.*, 1981; Lefevre *et al.*, 1983). In contrast to this, in the pig, concentration of testicular LH receptors is high 3 weeks after birth and decreases steadily thereafter until it reaches values observed in mature boars (Sundby *et al.*, 1981, 1983; Vandalem & Hennen, 1981).

There has been much speculation about which hormone(s), LH, FSH or prolactin, is(are) a major regulator of testicular LH receptors during development. In the hamster, LH is probably not involved, since plasma LH concentrations did not increase significantly before the increases in LH receptor concentrations. However, it is possible that FSH could be a major regulator of LH receptors, because plasma FSH concentrations increased significantly on Days 20 and 30 of age just before increases in LH receptor concentrations. Similar results have been reported for the rat (Greenstein, 1978; Ketelslegers *et al.*, 1978; Hodson, Robertson & de Kretser, 1983). Also, treatment of immature hypophysectomized rats with FSH caused an increase in LH receptor content (Chen, Payne & Kelch, 1976). However, in hamsters, ovine FSH had little effect on testicular LH binding in hypophysectomized hamsters or those with regressed gonads (Bex *et al.*, 1978; Bartke, Klemcke, Amador & Van Sickle, 1982).

The present results suggest prolactin as the principal hormone involved in the developmental regulation of testicular LH receptors in the golden hamster. The increase in LH receptor concentration and content observed during development was preceded by dramatic increases in plasma prolactin concentrations at Day 20, as found by Vomachka & Greenwald (1979). The role of prolactin in controlling the development of LH receptors is strongly supported by the observations that inhibition of prolactin release in bromocriptine-treated animals suppressed development of testicular LH receptors. The concentration and content of testicular LH/hCG receptors in these animals were directly correlated with plasma prolactin concentrations but not with plasma LH or FSH concentrations. The inhibitory effect of suppressed prolactin concentrations on testicular development appears not to be a general effect on development or a non-specific metabolic effect, since both body weight and glucose levels remained unaffected by bromocriptine treatment. Similarly, premature elevation of plasma prolactin values by pituitary grafts enhanced the development of testicular LH receptors. In this study there was also a direct correlation between plasma prolactin values and the concentration of testicular LH/hCG receptors. Together, therefore, these results strongly suggest that prolactin is a major regulator of testicular LH/hCG receptors. It does not eliminate the possibility that FSH is also involved in regulating receptor concentrations.

This conclusion concerning prolactin effects on LH/hCG receptors in hamsters is in agreement with studies of rats (Morris & Saxena, 1980; Huhtaniemi & Catt, 1981; Hodson *et al.*, 1983). Additionally, prolactin has previously been shown to increase the number of testicular LH receptors and stimulate testicular function in dwarf mice (Bohnet & Friesen, 1976; Bartke, Goldman, Bex & Dalterio, 1977; Amador & Bartke, 1982), in hamsters with photoperiod-induced testicular atrophy (Bex *et al.*, 1978), and in hypophysectomized rats (Hafiez, Lloyd & Bartke, 1972; Zipf, Payne & Kelch, 1978). Results obtained in hypophysectomized animals suggested that prolactin can act directly on the Leydig cells to induce formation of LH receptors and potentiate the effects of LH on testosterone production (Hafiez *et al.*, 1972; Zipf *et al.*, 1978; Bartke *et al.*, 1982). Our data do not permit evaluation of the possibility that prolactin might increase the number of testicular LH receptors by altering the relative proportions of different types of Leydig cells which, in the rat, were reported to contain different numbers of LH receptors (Chase & Payne, 1983).

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