Effects of different numbers of ectopic pituitary transplants on regulation of testicular LH/hCG and prolactin receptors in the hamster (*Mesocricetus auratus*)*

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Summary. Adult male hamsters were given transplants of $\frac{1}{2}$, 1, 2, 3 or 4 pituitaries under the kidney capsule and were killed 4 weeks later. Pituitary transplants produced a significant, dose-related increase in plasma prolactin levels, no changes in plasma LH and an increase in plasma FSH. Concentration of LH/hCG receptors in the testes was significantly increased in animals with 2 or 3 transplants and concentration of testicular prolactin receptors was significantly increased in those given 2 transplants. The apparent stimulatory effects of $\frac{1}{2}$, 1 or 4 transplants on testicular LH/hCG and prolactin binding were not statistically significant. Some of the animals were injected with 0.3 i.u. hCG/g body weight 24 h before being killed. This produced a significant reduction in the levels of prolactin receptors and an apparent reduction in the levels of LH/hCG receptors in the testes. Elevation of plasma testosterone concentrations in response to hCG was significantly greater in animals given 3 or 4 pituitary transplants than in the remaining groups. These results provide further evidence that prolactin increases the number of LH/hCG and prolactin receptors in the hamster testis and suggest that changing the number of ectopic pituitary transplants may result in biphasic effects on the testis, with 2 or 3 transplants being maximally stimulatory.

Introduction

There is a considerable amount of evidence that prolactin can influence the number of luteinizing hormone/human chorionic gonadotrophin (LH/hCG) receptors and its own receptors in the testis (Bohnet & Friesen, 1976; Aragona, Bohnet & Friesen, 1977; Bex & Bartke, 1977; Barkey, Shani & Barzilai, 1979; Kelly, Seguin, Cusan & Labrie, 1980; Huhtaniemi & Catt, 1981; Klemcke, Bartke & Borer, 1984). The effects of prolactin on many of its target organs are biphasic with low doses being stimulatory and high doses being inhibitory or relatively ineffective (McNatty, Sawers & McNeilly, 1974; Morris & Saxena, 1980; Prins & Lee, 1983). We have therefore examined the effects of different amounts of prolactin on testicular LH/hCG and prolactin binding in the Syrian (golden) hamster. We have already demonstrated that the effects of hyperprolactinaemia in the hamster are markedly different from the well-documented inhibitory effects of hyperprolactinaemia on the pituitary–testicular axis in other species (Bartke, Steger, Klemcke, Siler-Khodr & Goldman, 1982b).

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Materials and Methods

Animals and sample collection

Syrian hamsters (Lak:LVG [SYR]) were purchased from Charles River Lakeview or raised in our animal colony from animals of the same strain, and maintained since birth in a long photoperiod (14 h light:10 h dark). Room temperature was maintained at $22 \pm 2^\circ\text{C}$, and the animals had free access to food (Wayne Breeder Blox) and water.

Adult (>8 weeks of age) male hamsters were anaesthetized with ether and had $\frac{1}{2}$, 1, 2, 3 or 4 pituitaries transplanted under the kidney capsule, using 8–10 animals per group. Pituitaries were obtained from adult female hamsters and no more than 2 pituitaries were grafted per kidney. Control animals either underwent the same surgical procedure without receiving a transplant (sham-operated; N = 10) or were untreated (N = 5). After 4 weeks, the animals were injected with 0.3 i.u. hCG (A.P.L.; Ayerst, New York)/g body weight or 0.9% (w/v) NaCl solution, 0.01 ml/g body weight. The animals were decapitated 24 h later. Trunk blood was collected and plasma was separated and stored for measurement of prolactin, LH, FSH and testosterone concentrations. The testes were immediately removed, decapsulated, weighed and stored for measurement of LH/hCG and prolactin receptors. Plasma testosterone, LH, FSH and prolactin concentrations were measured by radioimmunoassays validated for the hamster, as previously described (Bartke *et al.*, 1982b; Soares, Colosi & Talamantes, 1983). The intra- and interassay coefficients of variation and the average sensitivities of the assays were, respectively, as follows: testosterone, 4.7%, 15%, 128 pg/ml; LH, 2.1%, 15.1%, 5 ng/ml; FSH, 1.4%, 13.2%, 21 ng/ml; prolactin, 6%, 10%, 0.5–1.0 ng/ml.

Testicular hCG/LH binding was measured by radioreceptor assay following procedures previously described (Klemcke, Bartke & Goldman, 1981). The ^{125}I -labelled hCG (CR-121, NIH) used had an average specific activity of 52 $\mu\text{Ci}/\mu\text{g}$ with an average maximum binding of 46%. Testicular prolactin binding was measured by radioreceptor assay using procedures previously described (Klemcke *et al.*, 1983, 1984) which utilized iodinated ovine prolactin (NIAMDD-oPRL-15; 30.5 i.u./mg). The ^{125}I -labelled ovine prolactin had a maximum binding of 81% with a specific activity of 53 $\mu\text{Ci}/\mu\text{g}$. The concentration of protein in testicular membrane preparations used for measurement of hCG and prolactin binding was determined by a modification of Lowry's method (Markwell, Haas, Bieber & Tolbert, 1978), using bovine serum albumin as the standard.

Statistics

The data were assessed by one-way or two-way analysis of variance. All 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 of data were utilized as needed (Sokal & Rohlf, 1981).

Results

Transplantation of one-half of a pituitary under the renal capsule was sufficient to cause a significant increase in plasma prolactin concentrations (Text-fig. 1). Increasing the amount of ectopic adenohypophysial tissue to 3 pituitaries per recipient resulted in a steep dose-related increase in plasma prolactin concentrations, but transplantation of 4 pituitary glands failed to produce a further increase. Regardless of the number of pituitary transplants, injection of a large dose of hCG 24 h before killing the animals had no effect on plasma prolactin values (Text-fig. 1).

As expected from previous studies (Bartke, Siler-Khodr, Hogan & Roychoudhury, 1981; 1982b), ectopic pituitary transplants increased peripheral FSH concentrations without altering those of LH (data not shown). However, the increase in the concentration of FSH in the plasma of pituitary-grafted hamsters in the present study was significant only in animals bearing 4

Text-fig. 1. Effects of different numbers of ectopic pituitary transplants and hCG injections on plasma prolactin concentrations in male Syrian hamsters. Animals were killed 4 weeks after grafting, and 24 h after receiving a single subcutaneous injection of 0.3 i.u. hCG/g body weight or saline. Values are mean $\pm$ s.e.m. for 4–5 hamsters; those points marked with the same letter are not significantly different from each other ($P > 0.05$).

transplants (from 253 ± 33 to 490 ± 123 ng/ml; mean $\pm$ s.e.m.; $n = 5$ per group; $P < 0.05$). In hamsters injected with hCG, plasma LH and FSH concentrations were not significantly different from those in saline-injected animals (data not shown).

Effects of alterations in plasma prolactin values associated with increasing amounts of ectopic pituitary transplants on testicular receptors appeared to be dose-dependent (Text-fig. 2). In saline-injected animals, LH/hCG and prolactin receptor concentrations gradually increased coincident with increases in plasma prolactin. These elevations were significant ($P < 0.05$) with 2 pituitary transplants for prolactin receptors, and with 2 and 3 transplants for LH/hCG receptors. Testicular LH/hCG receptor concentrations showed an apparent decrease commensurate with reduced plasma prolactin values when pituitary transplants were increased from 3 to 4. Measured unoccupied prolactin receptor concentrations remained relatively constant in this range of treatment.

When compared with corresponding saline-injected controls, testicular prolactin receptor concentrations were significantly reduced after hCG injections in animals with 0, $\frac{1}{2}$, 2 or 4 pituitary transplants (Text-fig. 2b). Testicular LH/hCG receptor concentrations were decreased non-significantly after administration of hCG in animals given 3 pituitary transplants. When these results were expressed in terms of total content (fmol receptors/pair of testes; data not shown) similar treatment trends were noted. Total content of testicular prolactin receptors in animals given 4 pituitary transplants was significantly lower than in those given 2 such transplants (1206 ± 127 and 2000 ± 302 fmol/testes respectively; $P < 0.05$).

Plasma testosterone concentrations measured 24 h after a saline injection were not affected by ectopic pituitary transplants (Text-fig. 3). In hamsters injected with hCG, plasma testosterone concentrations were 2- to 9-fold higher than in the corresponding saline-injection controls. In hamsters bearing 3 or 4 pituitary transplants, plasma testosterone values measured 24 h after hCG administration were significantly higher than those measured in all other hCG-injected groups.

Discussion

The very pronounced and dose-related increase in peripheral prolactin concentrations 4 weeks after transplantation of different amounts of adenohypophysial tissue under the kidney capsule was not unexpected. Pituitaries transplanted to this site produce a significant and persistent elevation of plasma prolactin values in several rodent species, including the Syrian hamster (Bex, Bartke,

Text-fig. 2. Effects of different numbers of ectopic pituitary transplants and hCG injections on concentrations of testicular receptors for (a) LH/hCG and (b) prolactin in Syrian hamsters. Animals were killed 4 weeks after grafting, and 24 h after receiving a single subcutaneous injection of 0.3 i.u. hCG/g body weight or saline. Values are mean $\pm$ s.e.m. for 4–5 hamsters; those points marked with the same letter are not significantly different from each other ($P > 0.05$).

Goldman & Dalterio, 1978; Bartke *et al.*, 1982b), and a progressive increase in serum prolactin in rats treated with an increasing number of ectopic pituitary transplants was described previously (Chen, Amenomori, Lu, Voogt & Meites, 1970). It is, however, unclear why maximal plasma prolactin concentrations in the present study were detected in animals given 3 transplants and grafting of an additional pituitary caused no further increase. Peripheral prolactin concentrations measured in animals treated with pituitary transplants in the present study can be regarded as various degrees of hyperprolactinaemia because plasma prolactin values in adult male hamsters maintained in a long photoperiod are sufficiently high to allow the animals to remain continuously in breeding condition (Bex *et al.*, 1978; Bartke, Goldman, Klemcke, Bex & Amador, 1980).

Text-fig. 3. Effects of different numbers of ectopic pituitary transplants and hCG injections on plasma testosterone concentrations in male Syrian hamsters. Animals were killed 4 weeks after grafting, and 24 h after receiving a single subcutaneous injection of 0.3 i.u. hCG/g body weight or saline. Values are mean $\pm$ s.e.m. for 4–5 hamsters; those points marked with the same letter are not significantly different from each other ($P > 0.05$).

Parallel dose-related increases in plasma prolactin concentrations and in the concentration of LH/hCG receptors in the testes in animals given pituitary transplants are consistent with our previous observations that the number of testicular LH/hCG receptors in Syrian hamsters increases after treatment with ovine prolactin or with ectopic pituitary transplants (Bex & Bartke, 1977; Bex *et al.*, 1978; Klemcke *et al.*, 1984), and declines after pharmacological or short photoperiod-induced suppression of endogenous prolactin release (Bex & Bartke, 1977; Klemcke *et al.*, 1984). The apparent dose-dependency of this effect lends further support to the role of prolactin in maintaining LH receptors in rodent testes (Bohnet & Friesen, 1976; Aragona *et al.*, 1977; Bex & Bartke, 1977).

The increased concentration and total content of testicular prolactin receptors in pituitary-grafted hamsters in this study agree with our previous report that injections of ovine prolactin increase and of bromocriptine decrease the number of testicular prolactin receptors in this species (Klemcke *et al.*, 1984). Maximal elevation in testicular prolactin binding was observed in animals that had been given 2 ectopic pituitary transplants and in which plasma prolactin concentrations were only modestly elevated (Text-fig. 1). The substantial further increase in peripheral prolactin concentrations produced by transplantation of 3 or 4 pituitaries failed to increase testicular prolactin binding and, instead, led to a decrease in the total content of prolactin receptors in the testes ($P < 0.05$ in animals given 4 transplants). We have no evidence whether these findings are in any way related to occupancy of prolactin receptors. A very similar biphasic relationship between the number of ectopic pituitary transplants and their effects was observed in a study of the influence of prolactin on the growth of the lateral prostate of male rats (Prins & Lee, 1983). Although there is much precedent for the same aspect of gonadal function being stimulated by low and inhibited by high doses of prolactin (McNatty *et al.*, 1974; Morris & Saxena, 1980), the observed biphasic effect of prolactin on its own testicular receptors in the golden hamster was not anticipated. Our previous studies indicated that adult males of this species might be unique in responding to chronic hyperprolactinaemia with a significant increase in testicular weight without evidence of inhibition of any of the examined details of pituitary or testicular function (Bartke *et al.*, 1982b).

The substantial loss of testicular prolactin receptors 24 h after hCG administration observed in hamsters in the present study is reminiscent of the reported ability of LH and LHRH to reduce

testicular prolactin binding in the rat (Auclair, Kelly, Coy, Schally & Labrie, 1977; Katikineni, Davies & Catt, 1981). The existence of similar responses of testicular prolactin receptors to elevation of gonadotrophin concentrations in these two species contrasts with the divergent responses observed after prolactin administration, i.e. 'down-regulation' in the rat (Morris & Saxena, 1980) and 'up-regulation' in the hamster (Klemcke *et al.*, 1984). Chronic elevation of peripheral prolactin concentrations in pituitary-grafted hamsters had no effect on the ability of hCG to deplete prolactin receptors in the testis. The failure of hCG to cause a significant depletion of LH/hCG receptors in the present study was unexpected but is very unlikely to be due to measuring hCG binding before these changes may have taken place: at 48 and 72 h after administration of the same dose of hCG to adult hamsters, LH/hCG receptor levels in the testes were significantly increased (A. G. Amador, A. Bartke, H. G. Klemcke, T. M. Siler-Khodr & M. H. Stallings, unpublished observations).

In previous studies of adult hamsters housed in long photoperiods (Klemcke *et al.*, 1984), and of male rats (Chan, Katikineni, Davies & Catt, 1981), the response of testicular LH/hCG and prolactin receptors usually paralleled each other. However, in the current study there was a dissociation in the regulation of LH and prolactin receptors. Injections of hCG into hamsters with 2 or 4 pituitary transplants had no effect on testicular LH/hCG receptors, whereas prolactin receptor concentrations were significantly reduced.

Prolactin can markedly stimulate endogenous FSH release in hamsters (Bartke *et al.*, 1981; Bartke, Klemcke, Amador & Van Sickle, 1982a). Although prolactin stimulates testicular hCG binding in hypophysectomized hamsters (Bartke *et al.*, 1982b), a stimulatory effect of FSH on testicular LH binding has been reported for the rat (Chen, Payne & Kelch, 1976) and it is therefore possible that the effects of prolactin on testicular receptors may be mediated, in part, by an increase in endogenous FSH levels.

Our results further suggest that chronic elevation of peripheral prolactin values had a profound effect on the steroidogenic response of the testis to a large dose of hCG. By 24 h after hCG administration, plasma testosterone concentrations were almost 3-fold higher in hamsters with 3 or 4 pituitary transplants than in the remaining animals. This increase occurred in the absence of any measurable difference in the number of unoccupied LH/hCG or prolactin receptors between animals given 3 or 4, as compared to $\frac{1}{2}$ –2 pituitary transplants, and thus may reflect the ability of prolactin to potentiate the response of the testes to LH by mechanism(s) other than the well-documented effect on LH/hCG receptors, perhaps by affecting testicular cholesterol ester stores (Bartke, 1971), or enzymic activities (Hafez, Philpott & Bartke, 1971; Musto, Hafez & Bartke, 1972). However, loss of testicular LH/hCG receptors induced by prolonged exposure of adult hamsters to short photoperiods is associated with a marked reduction in the amount of testosterone produced *in vitro* in response to hCG stimulation (Bartke *et al.*, 1980; A. Bartke, M. P. Hogan & D. Gonzales, unpublished observations).

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