Effects of long-term treatment with melatonin or melatonin plus ectopic pituitary transplants on testicular LH/hCG and prolactin receptors in juvenile Syrian hamsters (*Mesocricetus auratus*)

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Summary. Juvenile hamsters were injected daily with melatonin and some were also given transplants of 2 pituitaries under the kidney capsule. Weights of the testes and the accessory reproductive glands were reduced after 8 and after 12 weeks of melatonin treatment, but remained unaltered in animals treated with ectopic pituitary transplants. Levels of testicular LH/hCG receptors were significantly reduced by daily melatonin injections for 8 and 12 weeks. The presence of pituitary transplants in melatonin-injected hamsters prevented these reductions, and increased LH/hCG receptors above control levels. These changes in testicular LH/hCG receptors were closely related to alterations in serum prolactin concentration induced by melatonin and pituitary transplants. After 8, but not after 12 weeks of treatment, testicular prolactin receptor levels were reduced by melatonin and maintained by the presence of pituitary transplants. We conclude that: (a) juvenile male hamsters become sensitive to the effects of daily melatonin injections when they reach maturity; (b) daily melatonin injections can reduce the levels of testicular LH/hCG and prolactin receptors; and (c) the effects of melatonin on LH/hCG and prolactin receptors are probably due to suppression of endogenous prolactin release.

Introduction

Under conditions of natural photoperiod, reproductive activity in the Syrian (golden) hamster is restricted to spring and summer. Gonadal involution takes place in early fall. Artificial maintenance of long photoperiods in the laboratory allows reproductive functions to continue throughout the year, but gonadal atrophy can be readily induced by transfer to short photoperiod, blinding or appropriately timed injections of a pineal product, melatonin (Gaston & Menaker, 1967; Seibel & Schweisthal, 1969; Reiter, 1973, 1980; Tamarkin *et al.*, 1976). Testicular regression induced by short photoperiods is accompanied by a precipitous decline in the number of testicular LH/hCG and prolactin receptors (Bex & Bartke, 1977; Klemcke *et al.*, 1983). These changes are thought to be due to suppression of plasma prolactin concentrations in short photoperiods because they can be mimicked by treatment with an inhibitor of prolactin release, bromocriptine, and reversed by

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injections of prolactin or by treatment with prolactin-secreting ectopic pituitary transplants (Bex & Bartke, 1977; Klemcke *et al.*, 1984). There is some evidence that endocrine changes induced by treatment with melatonin may not be completely equivalent to those produced by exposure to short photoperiods. We have demonstrated that the decrease in the weight of the testes and the accessory reproductive glands induced by daily melatonin injections can be completely prevented by transplantation of two homologous pituitaries under the renal capsule (Richardson *et al.*, 1982). This procedure is only partly effective in preventing regression of the male reproductive system in animals subjected to short photoperiods or blinding (Matthews *et al.*, 1978; Bartke *et al.*, 1980a).

In the present study, we examined the time course of changes in testicular LH/hCG receptors in juvenile Syrian hamsters during a 12-week period of daily melatonin injections with and without concomitant treatment with ectopic pituitary transplants. We also measured testicular prolactin receptors after 8 and after 12 weeks of these treatments. Weight of the testes and the accessory reproductive glands, serum and pituitary concentrations of prolactin, LH and FSH and serum concentrations of testosterone were monitored throughout the study.

Materials and Methods

Juvenile Syrian hamsters (~45 days old) were purchased from Engle (Farmersburg, IN). They were maintained in groups of 4–5 animals per clear plastic cage in a windowless and environmentally controlled room ($22 \pm 2^\circ\text{C}$). The animals were exposed to light (fluorescent; Sylvania Super Saver 35; 150–300 foot candles; 1615–3230 lx) daily from 06:00 to 20:00 h (14L:10D). All hamsters received Wayne Lab Blox and tap water *ad libitum*. Some of the hamsters received two homologous anterior pituitary grafts beneath the capsule of the left kidney. Pituitary transplants were from male hamsters of the same age. Surgery was carried out with the animals under ether anaesthesia. Melatonin was obtained from Sigma Chemical Co. (St Louis, MO) and was prepared in ethanolic saline (1:90, v/v) every day before its injection at 16:00 h. The injections were subcutaneous at the back of the neck region in a volume of 0.1 ml and the daily dose was 25 µg melatonin/animal.

Eight hamsters were killed before the start of treatments and the remaining animals were divided into the following experimental groups: (1) controls, (2) daily melatonin injections and (3) daily melatonin injections and two anterior pituitary homografts. Controls received daily injections of ethanolic saline. Animals were treated for 1, 2, 3, 6, 8 or 12 weeks, at which time they were killed by decapitation. Trunk blood was collected into heparinized tubes. Testicular, accessory sex organ (seminal vesicles plus coagulating glands) and anterior pituitary weights were recorded. The testes were decapsulated, placed in polypropylene tubes, rapidly frozen in a solid CO₂/acetone mixture, and stored at -70°C until assayed for LH/hCG and prolactin receptors. Serum samples and pituitaries were stored at -20°C until assayed for hormone concentrations.

Measurement of LH/hCG and prolactin receptors was done by radioreceptor assay as reported previously (Klemcke *et al.*, 1981, 1984). Specific activities of ¹²⁵I-labelled prolactin (NIH-P-S-13) and ¹²⁵I-labelled hCG (CR 121; NIH) were 15 and 68 µCi/µg, respectively, with respective maximal binding abilities of 63 and 45%. Protein content of each testis sample was determined using a previously validated (Klemcke *et al.*, 1981) modification of the Lowry procedure (Markwell *et al.*, 1978) and BSA as a standard.

Immunoreactive LH and FSH concentrations were determined by RIA utilizing reagents supplied by the National Hormone and Pituitary Program, NIH. Results were expressed in terms of NIAMDD purified rat hormone preparations. Prolactin concentrations were also measured using materials supplied by the NIH. Because of the lack of parallelism between rat and hamster inhibition curves, the prolactin results were expressed with reference to a pool of standard hamster anterior pituitaries (SHAP; Donofrio *et al.*, 1973/74). Serum testosterone concentrations were measured using a kit from Diagnostic Products (Los Angeles, CA). Samples were assayed at the end of the experiment in a single assay. The average values of sensitivity and intra-assay coefficients

of variation were: LH—1.1 ng/ml, 7.6%; FSH—13.8 ng/ml, 12.8%; prolactin—150 ng SHAP/ml, 7.6%; and testosterone—0.2 ng/ml, 5%, respectively.

Data were analysed by analysis of variance followed by the Student–Newman–Keuls test for differences amongst multiple means. Data were examined for homogeneity of variance using Bartlett's test and for normality of distribution using the Kolmogorov–Smirnov test. Mathematical transformations were made when necessary (Sokal & Rohlf, 1981; Scheffler, 1984).

Results

Testicular LH/hCG and prolactin receptor levels

Both the concentration and total content of LH/hCG receptors in the testes were significantly reduced after 8 and after 12 weeks of daily melatonin injections (Group 2) as compared to vehicle-injected controls (Group 1). However, values in Group 2 hamsters did not differ from those of pre-treatment controls (Fig. 1a, b). In animals in Group 3 (pituitary transplants and melatonin), the content and concentration of testicular LH/hCG receptors were significantly higher than those in Group 2 animals at all times between 2 and 12 weeks. LH/hCG receptor levels in animals in Group 3 were also significantly higher than those in Group 1 animals throughout most of the study period. In addition, after the first week of treatment, the concentration of testicular LH/hCG receptors was significantly lower in animals in Group 2 than in those in the remaining two groups.

Concentration and total content of testicular prolactin receptors were reduced significantly in Group 2 animals after 8 weeks of treatment, and this reduction was completely reversed by the presence of pituitary transplants (Group 3, Table 1). After 12 weeks of treatment, melatonin had no effect on levels of prolactin receptors. However, pituitary transplants (Group 3) increased total content to quantities above those in Group 1 or 2 ($P < 0.05$).

Hormone concentrations

Throughout the period of treatment, serum prolactin concentrations were about 25% lower in Group 2 than in Group 1 hamsters (Fig. 1c). Although this difference was consistently observed, the apparent decrease was not significant at any time, and melatonin did not reduce serum prolactin values as compared with those of pre-treatment controls ($P < 0.05$). Transplantation of two pituitary glands under the renal capsule in melatonin-treated hamsters (Group 3) elevated serum prolactin concentrations at all times ($P < 0.05$).

Serum concentrations of testosterone in control hamsters fluctuated widely throughout the experimental period (Fig. 1d) but, except for the increase at 1 week ($P < 0.05$), did not significantly differ ($P < 0.05$) from pre-treatment values. Throughout most of the treatment period there was a trend towards a decrease in serum testosterone in Group 2 hamsters, but none of these apparent differences was statistically significant. Serum concentration of LH and FSH exhibited no significant treatment effects except for an increase ($P < 0.05$) in serum FSH values in Group 3 hamsters after 12 weeks (data not shown).

Pituitary prolactin content was reduced ($P < 0.05$) and pituitary FSH content increased ($P < 0.05$) after 12 weeks of treatment with melatonin (Group 2). Pituitary transplants appeared to cause an increase in pituitary gonadotrophin content after 6, 8 and 12 weeks, but only the effect on LH was statistically significant (data not shown).

Body and organ weights

Absolute testicular weight was significantly lower in Group 2 than in Group 1 hamsters, starting after 6 weeks of treatment (Fig. 1e). Relative (to body weight) testicular weight declined gradually, but significant differences with controls appeared only after 8 weeks of treatment (data not

Table 1. Effects of treatment with daily melatonin injections (Group 2) or melatonin plus two ectopic pituitary homografts (Group 3) on the concentration and total content of prolactin receptors in the testes of juvenile hamsters

Treatment	No. of hamsters	Prolactin receptors	
		fmol/mg protein	fmol/testes
8 weeks			
Group 1 (controls)	7	28.5 ± 4.4 ^b	1258 ± 146 ^{a,b}
Group 2	9	14.4 ± 2.6 ^a	626 ± 127 ^c
Group 3	8	27.4 ± 3.2 ^b	1631 ± 138 ^a
12 weeks			
Group 1 (controls)	8	23.1 ± 2.8 ^b	1050 ± 136 ^b
Group 2	8	23.0 ± 4.7 ^b	897 ± 317 ^{b,c}
Group 3	8	30.1 ± 2.8 ^b	1529 ± 140 ^a

Values are mean ± s.e.m.

Values with different superscripts are significantly different, $P < 0.05$.

shown). Absolute and relative weights of accessory sex glands were decreased in Group 2 hamsters after 12 and 8 weeks of treatment, respectively (Fig. 1f). Concomitant treatment with pituitary transplants (Group 3) prevented these effects of melatonin treatment (Fig. 1e, f). After 12 weeks of treatment anterior pituitary weight and body weight were significantly greater in melatonin-treated animals (Group 2) than in controls (Group 1: $P < 0.05$; data not shown).

Discussion

The main findings of the present study were that daily melatonin injections caused significant reduction in the number of LH/hCG receptors in the testes of Syrian hamsters and that ectopic pituitary transplants increased testicular content of LH/hCG receptors in melatonin-treated animals.

A decrease in the concentration and total content of prolactin receptors in the testes was detected after 8 weeks of daily treatment with melatonin and an increase in testicular prolactin receptors after treatment with both melatonin and pituitary transplants. The relative magnitude of treatment effects on testicular LH/hCG and prolactin receptors agrees with previous data (Klemcke *et al.*, 1984; Amador *et al.*, 1985) which indicate that testicular LH/hCG receptors are much more responsive to modification of serum prolactin than are prolactin receptors. These observations probably reflect the contribution of an as-yet-unidentified hormone(s) to the regulation of testicular prolactin receptors. We have no explanation for the normal levels of prolactin receptors after 12 weeks of melatonin treatment in spite of continued suppression of serum prolactin concentrations. Perhaps prolactin receptors can eventually develop in the presence of modestly, although significantly, reduced peripheral prolactin concentration.

Fig. 1. Testicular LH/hCG receptor concentration (a) and content (b), concentrations of prolactin (c) and testosterone (d) and weights of testes (e) and accessory sex glands (seminal vesicles plus coagulating glands) (f) as a function of duration of treatment with melatonin (Group 2) or melatonin plus two pituitary transplants (Group 3) in juvenile Syrian hamsters. Values are mean ± s.e.m., for the number of hamsters indicated. Values with a letter in common are not significantly different from each other ($P > 0.05$).

The possibility of a direct effect of melatonin either *in vivo* on the receptors or *in vitro* on receptor measurement is an unlikely explanation for these observations. Identical doses of melatonin were administered to animals given melatonin alone and to those given melatonin plus pituitary transplants, yet receptor levels were reduced in the former group and increased in the latter. Furthermore, *in-vitro* incubation of Syrian hamster testes in the presence of melatonin or other indoleamines did not alter LH/hCG receptor levels, or hCG-stimulated steroidogenesis (Cutty *et al.*, 1981; Jarriage *et al.*, 1984; A. G. Amador & A. Bartke, unpublished data). Ellis (1972) reported that melatonin and serotonin inhibited the *in-vitro* biotransformation of progesterone (17 α -progesterone), and androstenedione to testosterone in testicular minced and microsomal preparations. However, those studies were done in rats at non-physiological temperatures (37°C) and using phosphate buffer, whereas our studies were performed in hamsters at physiological temperatures (32°C) and using Krebs–Ringer. Also, they do not analyse the effect of melatonin on gonadotrophin-stimulated steroidogenesis. Although, there is evidence for testicular melatonin receptors in rodents (Lang *et al.*, 1981), this and the previously mentioned report (Ellis, 1972) do not imply that melatonin has to affect LH/hCG receptors. Therefore, the direct effects of melatonin on testicular function would be at LH/hCG receptor-independent sites.

In the present study, we did not attempt to identify the mechanisms responsible for depletion of testicular LH/hCG and prolactin receptors by melatonin. However, our data strongly suggest that these effects of melatonin were due, at least in part, to inhibition of endogenous prolactin release. Prolactin-secreting pituitary transplants completely reversed the melatonin effects. Moreover, changes in serum prolactin concentrations in animals treated with melatonin or melatonin plus pituitary transplants correlated very closely with changes in the concentration and content of LH/hCG receptors in the testes throughout the study. In further support of the role of prolactin in mediating the effects of injected melatonin on testicular hormone binding, treatment with melatonin caused no consistent changes in the concentrations of LH and FSH, adenohipophysial hormones capable of regulating testicular LH/hCG receptors. This hypothesis is also consistent with our demonstration that suppression of endogenous prolactin release in the hamster by treatment with bromocriptine is associated with loss of LH/hCG and prolactin receptors from the testes and that these changes can be completely prevented by concurrent treatment with ovine prolactin (Klemcke *et al.*, 1984).

On the basis of an earlier study (Richardson *et al.*, 1982), it could be anticipated that treatment with melatonin injections would cause a precipitous fall in serum testosterone values and major reductions in testicular and accessory sex gland weights. However, the effects of melatonin on these measurements in the present study were modest. Sisk & Turek (1983a, b) showed that, in Syrian hamsters raised in short photoperiod since birth, testicular regression does not occur until after the animals have undergone sexual maturation and achieved a testicular size identical to that observed in hamsters raised in long photoperiod. Only after this increase in testicular weight does photoperiod-related regression of the testes in these animals start (around 10 weeks of age). In bank voles exposed to a short photoperiod after weaning, the puberty-related increase in testes weight and LH/hCG receptor number is inhibited (Tähkä & Rajaniemi, 1985). Afterwards, full testicular regression follows. The present results demonstrate that melatonin-induced changes in testicular function, like those induced by photoperiod, can occur only after the Syrian hamster has reached sexual maturity. Furthermore, in Syrian hamsters injected for 17 days with bromocriptine, starting at Day 14 of age, testicular LH/hCG receptor concentration was unchanged, although the total content of LH/hCG receptors was decreased because of the reduced testicular weight (Klemcke *et al.*, 1984). The present results therefore represent a description of melatonin effects during early stages of the development of responsiveness of the pituitary–testicular axis to exogenous melatonin. Importantly, there was no hint of even the slightest reduction of testicular and accessory sex gland weights in animals given ectopic pituitary transplants in addition to melatonin.

On the basis of these observations, we suggest that suppression of endogenous prolactin release leading to the loss of testicular LH/hCG and prolactin receptors represents one of the mechanisms

by which melatonin treatment can suppress testicular activity in Syrian hamsters. The reduction in LH/hCG receptor levels in the testis is particularly significant in that it provides a possible explanation for the previously demonstrated ability of melatonin to suppress serum testosterone concentrations without altering serum LH concentration (Richardson *et al.*, 1982).

The evidence that loss of testicular hormonal receptors represents one of the earliest detectable responses to both short photoperiod (Bartke *et al.*, 1980b; Klemcke *et al.*, 1983) and exogenous melatonin treatment gives a further, although indirect, indication that changes in testicular receptors for adenohipophysial hormones may be one of the mechanisms responsible for seasonal loss and recovery of gonadal activity.

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References

- Amador, A., Klemcke, H.G., Bartke, A., Soares, M.J., Siler-Khodr, T.M. & Talamantes, F. (1985) Effects of different numbers of ectopic pituitary transplants on regulation of testicular LH/hCG and prolactin receptors in the hamster (*Mesocricetus auratus*). *J. Reprod. Fert.* **73**, 483–489.
- Bartke, A., Goldman, B.D., Bex, F.J., Kelch, R.P., Smith, M.S., Dalterio, S. & Doherty, P.C. (1980a) Effects of prolactin on testicular regression and recrudescence in the golden hamster. *Endocrinology* **106**, 167–172.
- Bartke, A., Goldman, B.D., Klemcke, H.G., Bex, F.J. & Amador, A.G. (1980b) Effects of photoperiod on pituitary and testicular function in seasonally breeding species. In *Functional Correlates of Hormone Receptors in Reproduction*, pp. 171–185. Eds V. B. Mahesh, T. G. Muldoon, B. B. Saxena & W. A. Sadler. Elsevier/North Holland, New York.
- Bex, F.J. & Bartke, A. (1977) Testicular LH binding in the hamster: modification by photoperiod and prolactin. *Endocrinology* **100**, 1223–1226.
- Cutty, G.B., Goldman, B.D., Doherty, P. & Bartke, A. (1981) Melatonin prevents decrease in plasma PRL and LH levels in male hamsters exposed to a short photoperiod. *Int. J. Androl.* **4**, 281–290.
- Donofrio, R.J., Reiter, R.J., Sorrentino, S., Jr, Blask, D.E. & Talbot, J.E. (1973/74) A method for measurement of prolactin in the hamster by means of radioimmunoassay. *Neuroendocrinology* **13**, 79–92.
- Ellis, L.C. (1972) Inhibition of rat testicular androgen synthesis *in vitro* by melatonin and serotonin. *Endocrinology* **90**, 17–28.
- Gaston, S. & Menaker, M. (1967) Photoperiodic control of hamster testis. *Science, N.Y.* **158**, 925–928.
- Jarrige, J.-F., Thieblot, P. & Boucher, O. (1984) Lack of melatonin action on testosterone production by superfused rat interstitial cells. *Acta endocr., Copenh.* **107**, 117–124.
- Klemcke, H.G., Bartke, A. & Goldman, B.D. (1981) Plasma prolactin concentrations and testicular human chorionic gonadotropin binding sites during short photoperiod-induced testicular regression and recrudescence in the golden hamster. *Biol. Reprod.* **25**, 536–548.
- Klemcke, H.G., Bartke, A. & Borer, K.T. (1983) Testicular prolactin receptors and serum growth hormone in golden hamsters: effects of photoperiod and time of day. *Biol. Reprod.* **29**, 605–614.
- Klemcke, H.G., Bartke, A. & Borer, K.T. (1984) Regulation of testicular prolactin and luteinizing hormone receptors in golden hamsters. *Endocrinology* **114**, 594–604.
- Lang, U., Aubert, M.L. & Sizonenko, P.C. (1981) Tissue distribution and subcellular location of melatonin receptors in the male rat. *Experientia* **37**, 613.
- Markwell, M.K., Haas, S.M., Bieber, L.L. & Tolbert, N.E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analyt. Biochem.* **87**, 206–210.
- Matthews, M.J., Benson, B. & Richardson, D.L. (1978) Partial maintenance of testes and accessory organs in blinded hamsters by homoplastic anterior pituitary grafts or exogenous prolactin. *Life Sci.* **23**, 1131–1137.
- Reiter, R.J. (1973) Comparative physiology: pineal gland. *Ann. Rev. Physiol.* **35**, 305–328.
- Reiter, R.J. (1980) The pineal and its hormones in the control of reproduction in mammals. *Endocr. Rev.* **1**, 109–131.
- Richardson, B.A., Petterborg, L.J., Vaughan, M.K., King, T.S. & Reiter, R.J. (1982) The effect of twice daily gonadotropin-releasing hormone (GnRH) administration and/or renal pituitary homografts on melatonin-induced gonadal atrophy in male Syrian hamsters. In *The Pineal and its Hormones*, pp. 129–142. Ed. R. J. Reiter, Alan R. Liss, Inc., New York.
- Scheffer, W.C. (1984) *Statistics for Health Professionals*. Addison-Wesley Publishing Co., Reading.
- Seibel, H.R. & Schweithal, M.R. (1969) Relationships between the pineal gland and the gonads of single and

- parabiosed golden hamsters. *J. Reprod. Fert.* **18**, 179, abstr.
- Sisk, C.L. & Turek, F.W.** (1983a) Developmental time course of pubertal and photoperiodic changes in testosterone negative feedback on gonadotropin secretion in the Golden hamster. *Endocrinology* **112**, 1208–1216.
- Sisk, C.L. & Turek, F.W.** (1983b) Gonadal growth and gonadal hormones do not participate in the development of responsiveness to photoperiod in the Golden hamster. *Biol. Reprod.* **29**, 439–445.
- Sokal, R.R. & Rohlf, F.J.** (1981) *Biometry*, 2nd edn. W. H. Freeman and Co., San Francisco.
- Tähkä, K. M. & Rajaniemi, H.** (1985) Photoperiodic modulation of testicular LH receptors in the bank vole (*Clethrionomys glareolus*). *J. Reprod. Fert.* **75**, 513–519.
- Tamarkin, L., Westrom, W.K., Hamill, A.I. & Goldman, B.D.** (1976) Effect of melatonin on the reproductive systems of male and female hamsters: a diurnal rhythm in sensitivity to melatonin. *Endocrinology* **99**, 1534–1541.

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