Preliminary studies of the effects of bromocriptine on testicular regression and the spring moult in a seasonal breeder, the male blue fox (*Alopex lagopus*)

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Summary. Bromocriptine administration in the form of slow-release injections to male blue foxes during March–May abolished the normal spring rise in plasma prolactin concentrations seen in May and June. The spring moult was prevented and the treated animals retained a winter coat of varied quality and maturity until the end of the study in August.

Plasma testosterone concentrations fell normally from March until August. Testicular regression was, however, delayed, although there were individual variations in response. Estimation by DNA flow cytometry in early July of the relative numbers of haploid, diploid and tetraploid cells in the testis showed that, in the treated animals, 74–80% of the cells were haploid (maturing germinal cells), 4–6% tetraploid (mainly primary spermatocytes) and the rest diploid cells (somatic cells and the remaining germinal cell types). In the control males, however, no haploid cells were detected and the majority of cells were diploid (93–99%). At castration in August, histological examination revealed various stages of testicular regression in the treated and control animals.

Introduction

The male blue fox (*Alopex lagopus*) is a seasonal breeder, with 5-fold annual changes in testicular size and associated seasonal changes in blood concentrations of a range of hormones (Smith *et al.*, 1984, 1985, 1986). These variations are probably influenced by seasonal changes in environmental cues such as daylength and temperature, but little is known about the neuroendocrine mechanisms that mediate these cues. Males of other species exhibit variations in plasma concentrations of prolactin that appear to be well correlated to photoperiod, with highest values during long days, both in the Northern hemisphere (e.g. ram: Ravault, 1976; goat: Buttle, 1974; white-tailed deer: Mirarchi *et al.*, 1978) and Southern hemisphere (e.g. ram: Kennaway *et al.*, 1981). The development of a specific radioimmunoassay for prolactin in the blue fox revealed a similar correlation in this species (Smith *et al.*, 1985).

The role of prolactin in the control of male reproductive function is still poorly understood (Bartke, 1980). The effects of prolactin undoubtedly vary depending on the species concerned, the age of the animal and, under experimental conditions, dosage and time of administration. In the golden hamster at least, alterations in prolactin release provide one of the mechanisms that mediate the effect of photoperiod on the testis (for review see Bartke *et al.*, 1985).

Control of the moulting cycle, on the other hand, and the roles of prolactin and melatonin secretion in the mediation of photoperiodic cues, are more fully understood. We have previously shown that melatonin administration depresses plasma prolactin concentrations in the male blue fox and delays the spring moult (Smith *et al.*, 1987a). A similar situation exists in mink (Allain *et al.*, 1981); in addition, prolactin injections induce an early spring moult (Martinet *et al.*, 1981). Likewise, experimental work in mink suggests that a decline in plasma prolactin concentrations, whether produced by administration of melatonin (Allain *et al.*, 1981), maintenance of the animals under short days (Rose *et al.*, 1985), or bromocriptine treatment (Martinet *et al.*, 1984; Rose *et al.*, 1987) plays a central role in the control of the autumn moult. It is likely that melatonin influences the release of LHRH, dopamine and other factors from the hypothalamus which in turn regulate the secretion of LH, FSH and prolactin from the anterior pituitary (Cardinali, 1981), thereby influencing gonadal activity and fur growth.

Bromocriptine inhibits prolactin release, both by binding to dopamine receptors on anterior pituitary cells (Coronin *et al.*, 1978) and by decreasing dopamine turnover (Fuxe *et al.*, 1978). Bromocriptine has previously been shown to depress plasma prolactin concentrations in, among other species, the male blue fox (Mondain-Monval *et al.*, 1985), male golden hamster (Stroud *et al.*, 1985) and ram (Barenton *et al.*, 1982).

The purpose of the present study was to test, by using bromocriptine, the suspected involvement of prolactin in mediating the effects of melatonin on testicular regression and the spring moult by depressing the physiological rise in plasma prolactin as daylength increases.

Materials and Methods

Animals. The study took place from March to August 1985 and involved 8 male blue foxes. The animals were approximately 9 months old at the beginning of the study and had shown normal testicular development before the mating season. They were housed individually under natural conditions of daylength and temperature at the Research Farm for Furbearing Animals, Heggedal, Norway (latitude: 59° 47'N; longitude: 10° 27'E). The males were within visual and olfactory contact of vixens on the farm but none was allowed to mate during the study period. The mating season during the study was defined as the period between the first and last matings on the farm.

Bromocriptine treatment. A slow-release preparation of bromocriptine adsorbed to microcapsules (CB-154, Sandoz, Basle, Switzerland, providing 25 mg bromocriptine/ml, with a reported release period of 21–28 days) was injected intramuscularly in 4 foxes on 4 occasions (6–8 March, 2–3 April, 29 April and 24 May 1985). All 4 animals were given 4 ml (100 mg bromocriptine), injected in 1-ml volumes at 4 different sites, on each date. Four males served as controls.

One of the foxes in the group receiving bromocriptine had to be killed on 14 June because of an injury unrelated to the study.

Blood collection. Blood samples (10 ml) were collected 1–3 times weekly into heparinized tubes without anaesthesia between 09:00 and 11:00 h, using light manual restraint. Plasma was separated by centrifugation and stored at –20°C until assay.

Hormone assays. Blood samples were analysed for prolactin and testosterone using specific radioimmunoassays previously described and validated for use in the blue fox (prolactin: Mondain-Monval *et al.*, 1985; testosterone: Møller *et al.*, 1984). For the prolactin assay, the sensitivity was 2 ng/ml and the intra- and inter-assay coefficients of variation were 8.1% and 13.9% respectively. The corresponding values for the testosterone assay were 20 pg/ml, 4.9% and 11.7%, respectively. Plasma concentrations of FSH were measured by a heterologous radioimmunoassay (unpublished). This assay utilized an anti-human FSH antiserum (M91/1) donated by A. S. McNeilly, a purified canine FSH preparation (LER-1685-3A) as reference standard and a highly purified ovine FSH (LER-1976-A2) for iodination as tracer, provided by L. E. Reichert. The mean detection limit was 20 ng/ml and the intra- and inter-assay coefficients of variation were 6.0% and 10.6%, respectively.

All serial samples from the same animal were measured in duplicate in one assay.

Measurement of testicular volume. The length (which included the head of the epididymis) and width of the testes of all males were measured through the scrotum by using calipers, with no allowance for scrotal skin thickness, at approximately 10-day intervals during the study period. Values for length (2a) and width (2b) were converted to volumes (V) by means of the formula for a prolate spheroid, $V = 1.33\pi ab^2$ (Wildt *et al.*, 1982).

DNA flow cytometry. On 3 July 1985 testicular biopsies were taken from 6 of the 7 males still alive, as described previously (Smith *et al.*, 1987a). The aspirated material was stored in 70% ethanol until preparation for DNA flow cytometry (Smith *et al.*, 1987a), which enables classification of testicular cells into 3 categories (haploid, diploid or

tetraploid) depending upon the DNA content of the cell nuclei. The haploid population consists of spermatids and spermatozoa, the tetraploid population mainly of primary spermatocytes, and diploid cells include the remaining germinal cell types and all testicular somatic cells (Smith *et al.*, 1984).

Histological examination of testicular material. All the males were castrated on 14–15 August 1985, and midline sections of each testis fixed for 24 h in Bouin's solution. The material was then stored in 70% ethanol before preparation for routine sectioning at 4 µm and staining with haematoxylin and eosin.

Sections of testis were examined by light microscopy and 200 cross-sections of tubuli per fox were classified into four categories according to the stage of spermatogenesis. The categories were the presence of (1) spermatogonia only, (2) spermatogonia and primary spermatocytes, (3) spermatogonia, spermatocytes and round spermatids and (4) spermatogonia, spermatocytes and elongated spermatids, with or without round spermatids. The normal seasonal variations in the occurrence of these categories in both immature and adult males have been described elsewhere (Smith *et al.*, 1986, 1987b).

Examination of fur. The males were killed on 14–15 August 1985 and the skins sent to Oslo Fur Auctions where they were assessed for pigmentation, maturation of the fur, hair quality, coat density and texture.

Results

Plasma prolactin

As shown in Fig. 1(a) a normal rise in prolactin concentrations occurred in the controls, from 2.7–9.7 ng/ml in March (mean 4.9 ng/ml, $n = 12$) to 7.3–20.7 ng/ml in May and June (mean 12.4 ng/ml, $n = 28$). This rise was totally abolished in the treatment group, in which plasma prolactin concentrations remained low throughout the study period (2.1–6.0 ng/ml).

Plasma FSH

Plasma FSH concentrations (Fig. 1b) remained low throughout the study period in the control and treatment group (controls: non-detectable–100 ng/ml; treated: non-detectable–44 ng/ml).

Plasma testosterone

Plasma concentrations of testosterone fell gradually in a similar way in the control and treated animals (Fig. 1c) from March (controls: 0.51–9.36 ng/ml, mean 5.1 ng/ml, $n = 12$; treated: 0.78–4.35 ng/ml, mean 2.1 ng/ml, $n = 8$) to July and August (controls: 0.03–2.59 ng/ml, mean 0.4 ng/ml, $n = 24$; treated: 0.02–1.06 ng/ml, mean 0.4 ng/ml, $n = 18$).

Testicular regression

Testicular regression, as judged by testicular volume, appeared to be delayed in the treated animals compared with the controls (Fig. 1d). Comparison of testicular weights at castration on 14–15 August, however, indicated that only 1 of the treated males (110E) had heavier testes than the other animals by this time.

DNA flow cytometry

Table 1 depicts the relative numbers (%) of haploid, diploid and tetraploid cells in testicular biopsies taken on 3 July 1985 from 6 of the 7 males still alive. By this time, the testes of the control animals contained predominantly diploid cells (93–99%), the remaining cells being tetraploid. In contrast, both of the treated animals biopsied had large haploid cell populations (spermatids or spermatozoa) amounting to 74–80% of the testicular cells. The tetraploid cell populations were similar in relative size to the controls (4–10%) and the diploid populations were consequently smaller (12–14%).

Fig. 1. Variations in (a) plasma prolactin, (b) plasma FSH, (c) plasma testosterone and (d) testicular volume in a group of 4 foxes treated 4 times at 25–27-day intervals with a slow-release preparation of bromocriptine and 4 untreated controls, from March until May 1985. The arrows in (a) depict the dates of administration of bromocriptine. The horizontal bar under the figure indicates the duration of the mating season on the farm during the study. The symbols at the bottom right of the figure depict the mean testicular weights of the individual males at castration on 14–15 August 1985. Values are means $\pm$ s.e.m. for all the animals in each group. One of the animals receiving bromocriptine was killed on 14 June because of an injury unrelated to the study.

Histological examination of the testes

The relative proportions of 4 tubular categories in the testes of the males at castration in August 1985 are shown in Table 2. In the control group, virtually all tubular sections (92–99%) contained spermatogonia as the most advanced germinal cells, the remainder (1–8%) containing also primary

Table 1. Relative numbers (%) of haploid, diploid and tetraploid cells in testicular biopsies taken on 3 July 1985 from 6 of the 8 animals

	Haploid	Diploid	Tetraploid
Controls			
167E	0	93.3	6.7
168E	0	98.1	1.9
298E	0	98.1	1.9
312E	0	98.6	1.4
Bromocriptine-treated			
110E	74.4	11.9	3.7
306E	80.1	14.0	5.9

Table 2. The relative numbers (%) of 4 categories of seminiferous tubules in the testis at castration on 14–15 August 1985

	Tubular category*			
	1	2	3	4
Controls				
167E	96	4	0	0
168E	92	8	0	0
298E	99	1	0	0
312E	92	8	0	0
Bromocriptine-treated				
110E	0	6	43	51
306E	70	30	0	0
103E	95	5	0	0

*1, Spermatogonia only; 2, spermatogonia and primary spermatocytes; 3, spermatogonia, spermatocytes and round spermatids; 4, spermatogonia, spermatocytes and elongated spermatids, with or without round spermatids.

spermatocytes. The treatment group showed great differences between individuals. In one of the 3 animals, the tubular sections resembled those seen in the control group (103E: category 1, 95%; category 2, 5%). Another male (306E) showed a shift towards more mature germinal cell types (70% of tubules containing spermatogonia only and 30% also containing primary spermatocytes), but the primary spermatocytes were clearly degenerate. The third male (110E), in contrast, had no tubules with only spermatogonia, and only 6% with primary spermatocytes as the most advanced germinal cells. In this male, 43% of tubular sections contained cells up to the round spermatid stage, and 51% of tubules contained elongated spermatids. However, in this fox the tubular lumen was absent, indicating that spermiation had not taken place.

Moulting cycle

The control animals underwent a normal moult during May and June. None of the treated males, however (including the fox which was killed on 14 June), lost their winter coat. The typical appearance in July 1985 of males in the control group and of those receiving bromocriptine is illustrated in Fig. 2. All 3 of the foxes given bromocriptine had mature winter pelts, with normal

Fig. 2. Appearance in July 1985 of (a) control animals and (b) males treated with bromocriptine from March to May 1985.

hair quality, texture and density. Two of the pelts gave the impression of having undergone a change of hair pigment, especially in the guard hairs, to a uniform beige/yellow colour. The third was discoloured by urine.

Discussion

The present study shows that bromocriptine treatment initiated in March suppresses the annual rise in plasma prolactin concentrations that normally occurs during increasing daylength (May–June) and prevents the spring moult. Previous (unpublished) studies have shown the same effect even when bromocriptine treatment was begun as late as May, after plasma prolactin concentrations have started to increase; these males retained the winter coat through the following winter and moulted normally in the spring.

Bromocriptine treatment appeared also to influence testicular regression, despite the fact that testosterone concentrations remained unchanged. The mechanism of this effect is unclear: bromocriptine treatment has been reported to stimulate FSH release in sheep (Ravault *et al.*, 1982), but no such effect was seen in the present study (Fig. 1b). High doses of bromocriptine may also interfere with other hormones, in particular LH (Shaban & Terranova, 1986), growth hormone (Flückiger *et al.*, 1976) and MSH (Penny *et al.*, 1979).

Prolactin is believed to modify the sensitivity of the hypothalamic–pituitary axis to testosterone

feedback during testicular regression of the golden hamster (Matt *et al.*, 1984). There is, however, no evidence for this in the blue fox; suppression of the spring rise in plasma prolactin concentrations by melatonin does not appear to alter LH release despite the fact that testosterone production is prolonged (Smith *et al.*, 1987a). A decrease in testosterone secretion does not appear to be the primary stimulus for the spring moult since this moult and the simultaneous rise in plasma prolactin concentrations continue normally after castration (Smith *et al.*, 1985).

Other studies also indicate that there is no fixed relationship between the rise in plasma prolactin concentrations and the breeding season. Carr & Land (1982) observed simultaneous prolactin rises in castrated rams of 3 breeds of sheep with different seasonality of reproduction. Likewise, while some species of deer have high blood concentrations of gonadotrophins (e.g. roe deer: Schams & Barth, 1982), others (e.g. white-tailed deer: Mirarchi *et al.*, 1978) have low gonadotrophin concentrations when prolactin values are high. This is in agreement with our own (unpublished) studies of seasonally-breeding canid furbearers with different mating seasons.

It is still unclear whether the rise in prolactin is triggered by increasing daylength or temperature. The effects of these 2 variables are impossible to differentiate under natural conditions since both show marked and parallel annual changes at this latitude (Smith *et al.*, 1985). Plasma prolactin concentrations in golden and Djungarian hamsters, maintained in constant temperature, change readily in response to photoperiod (Bex *et al.*, 1978; Duncan & Goldman, 1984). Studies of other species, however, have demonstrated a rise in prolactin values in response to increasing temperature (cattle: Tucker & Wettemann, 1976; rat: Mueller *et al.*, 1974).

Studies of mink indicate that both the furring cycle and testicular development may also be influenced by α -MSH secretion (Ellis *et al.*, 1982). Work on the Djungarian hamster (Duncan & Goldman, 1984), however, suggests that the effects of bromocriptine on the pelage colour cycle are mediated by prolactin suppression alone, without any suppression of MSH. Burchill & Thody (1986) suggest direct effects of dopaminergic agents on hair follicles. Further studies are clearly warranted before the changes seen in the present study after bromocriptine treatment can be ascribed to the reduction in plasma prolactin concentrations.

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