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Prolactin regulation of testicular development and sexual behavior in yearling Suffolk rams

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Abstract

The aim of this study was to determine how the yearly prolactin rhythm might affect the sexual development of Suffolk rams (latitude 50°N). Five rams were injected daily with bromocriptine (35–45 μg kg^{-1} body weight) for 1 year, beginning in January (early winter) when rams were 11 months of age. Five control rams each received daily injections of the vehicle. In the controls, blood prolactin was <7.5 ng ml^{-1} in winter, increased ($P < 0.01$) to a peak of 172.6 ± 11.9 ng ml^{-1} after the spring equinox, and remained high during summer before declining ($P < 0.01$) to 29.6 ± 6.6 ng ml^{-1} at the autumn equinox. Suppression of the seasonal rise in prolactin secretion with bromocriptine slowed testicular growth (50%; $P < 0.05$) in April and May (spring), thus delaying the time of peak testis size and sperm production by 1 month. Serum testosterone level was lower (50%; $P < 0.01$) in the treated rams than the controls in June and July (early summer), due mainly to reduced stimulation of the testes by smaller ($P < 0.01$) LH pulse releases or to smaller ($P < 0.01$) testosterone responses to LH releases, respectively. Suppression of prolactin also seemed to disrupt the central activation of gonadotropin secretion in that seasonal increases in serum FSH level and LH pulse amplitude and frequency were unusually slow ($P < 0.05$). These anomalies did not affect testis growth, which was normal from June until development was complete. Rams were sexually inexperienced when libido was first tested in July (non-breeding season). Both groups were equally capable of learning and expressing sexual behavior (i.e. normal mounting and ejaculation frequencies), which was more intense in September (breeding season; $P < 0.05$). Results support the hypothesis (based on the location of prolactin receptors) that the spring increase in prolactin secretion could target both the testes and the hypothalamic–pituitary system and be involved in the seasonal regulation of sexual function in the young adult Suffolk ram.

Keywords: Prolactin; Suffolk rams; Testes growth; Testosterone; Sperm output; Libido

1. Introduction

Prolactin is a multifaceted pituitary hormone with over 300 known biological activities in vertebrates (Bole-Feysot et al., 1998). It acts at several levels of male reproduction in many mammals, playing a role in steroidogenesis and gametogenesis in the testis and influencing the reproductive tract and sexual behavior (Bartke, 2004). Prolactin secretion in the ram is positively related to day length, with annual maximum

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blood concentrations in the spring increasing as rams age (Ravault, 1976; Lincoln, 1998). Age-related increases in prolactin secretion together with those in FSH are thought to cause the progressive enlargement of the testes seen in mouflon rams during their first years of life (Lincoln, 1998). The recent discovery of prolactin receptors in the hypothalamus and pituitary (Tortonese et al., 1996, 1998) and in the testis (Lincoln et al., 2001) means that the elevation in blood prolactin concentration during long days could affect the reproductive processes of rams in several ways. Prolactin presumably plays a minor role in regulating seasonal breeding since rams with diverse annual reproductive patterns often have similar rhythms in circulating prolactin (Lincoln, 1990; Santiago-Moreno et al., 2005).

Prolactin seems to influence the growth phase of the natural testicular cycle in adult rams, at least in some breeds. Short-term suppression of prolactin secretion in Romanov rams in early summer with bromocriptine delays the start of testes growth by 2 weeks, but does not impair spermatogenesis (Barenton and Pelletier, 1980; Barenton et al., 1982). In contrast, testis size is not affected in Prealp rams administered bromocriptine during winter and early spring (Ravault et al., 1982). Unlike the testes of Prealps, those of Dorset rams decrease in size when prolactin is suppressed in late winter (Regisford and Katz, 1993). However, testis size is not affected when Dorset rams are treated with bromocriptine in late summer, although testosterone secretion is reduced. The number of sperm in the ejaculate does not change in either season. Studies to date offer little insight into how prolactin may regulate testicular growth in that reported changes in gonadotropin secretion and receptors are often contradictory (Barenton et al., 1982; Regisford and Katz, 1993, 1994a). It is also unclear whether prolactin hinders or facilitates the expression of sexual behavior in rams (Howles et al., 1980; Regisford and Katz, 1994b).

The aim of the present study was to suppress prolactin secretion in Suffolk rams during a complete annual reproductive cycle, enabling us to address: (i) whether the testes develop at a slower rate when blood prolactin level does not increase in the spring, (ii) whether impaired testicular growth means that the testes are less able to produce testosterone and (or) spermatozoa, and how this may affect the rest of the testicular cycle, (iii) whether unusual seasonal changes in LH and FSH secretion in the absence of prolactin reflect a disturbance in central regulation or in testicular endocrine function, and (iv) whether chronic prolactin deficiency alters the capability of young Suffolk rams to learn and display adult patterns of sexual behavior.

2. Materials and methods

2.1. Animals and management

Ten Suffolk ram lambs 8 months of age and weighing on average 42 ± 2 kg were selected from the sheep flock at the University of Manitoba for this investigation. Rams were kept together in an outside pen with access to an open-front barn. In southern Manitoba (latitude 50°N), day length (dawn to dusk) ranges from 8.1 to 16.4 h. Daily mean ambient temperature ranges from −20.6 °C in late January (winter) to 20.6 °C in mid-July (summer). Rams were fed a diet of hay consisting mainly of timothy grass and clover, supplemented with grain in the winter and spring. Drinking water and mineralized salt were available *ad libitum*. Care and treatment of rams were as prescribed by the Canadian Council on Animal Care.

2.2. Experimental protocol

Beginning in mid-October (early autumn) and continuing for 14 months, 5 rams received daily s.c. injections of bromocriptine (2-bromo-α-ergocryptine, Sandoz, Ltd.). Initially the rams received 2 mg day^{-1} in 1.0 ml of ethanol:0.9% saline (60:40, v/v). The daily dose was increased in a stepwise fashion to 4 mg in 1.0 ml of vehicle to compensate for increasing body weight. The 5 control rams received daily s.c. injections of the vehicle. Sites of injection were the areas free of wool in the vicinity of the fore and hind limbs. Rams were routinely injected at 09:30 h, but injection was delayed until 17:00 h on days when serial blood samples were taken. The experiment began after rams had been subjected to the drug for several weeks in the autumn.

A number of reproductive traits were examined between mid-January (early winter) and mid-December (late autumn) to determine how the absence of the spring increase in prolactin secretion might affect the rams' second, yearly sexual cycle. Body weight, testicular size and blood concentrations of prolactin, FSH, LH, testosterone and estrogen were monitored, as were patterns of episodic LH and testosterone secretion. Spermatogenic activity in the testes was assessed during and after testicular development by counting spermatozoa voided in urine or present in semen obtained by natural ejaculation. Sexual behavior of rams was evaluated in the non-breeding season (high prolactin) and in the breeding season (low prolactin), which for Suffolk sheep in this locale begins in mid-September and ends in late December.

2.3. Parameter recording and blood sampling

Body weight and scrotal circumference were recorded monthly, usually during the third and fourth weeks, respectively. Paired testes weight was estimated from scrotal circumference and a regression equation reported by Knight (1977) for Romney rams. Estimates of testes weight were used primarily to calculate testicular growth rates. Serial blood sampling was done monthly, usually during the fourth week of