Antler growth in male red deer (Cervus elaphus) after active immunization against LH-RH

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Summary. Four sexually mature male red deer were actively immunized against LHRH and this caused 3 of the animals to cast their antlers prematurely in the autumn instead of the spring. Development of new antlers was initiated after casting, but the effects on the antler cycle were variable and correlated with the antibody titre, only the animal with the highest titre developed antlers that resembled those of a castrate and remained ‘in velvet’ for more than 6 months. In October, when all the immunized deer had peak circulating levels of LHRH antibodies, the testes were reduced in size compared to the maximum values of the controls. The blood levels of testosterone were reduced in the immunized animals, and there was a minimal increase in the circulating levels of testosterone in response to an i.v. injection of 100 μg ovine LH. The immunized stags showed no rutting behaviour in the autumn. The changes in the testes confirm that the immunizations were effective in blocking the secretion of the gonadotrophic hormones. The re-development of antlers in these animals indicates that gonadotrophins are not directly involved in stimulating antler growth.

Introduction

Antlers are grown by the males of all species of deer living in temperate climates. They are cast and regrown each year, and the cycle is regulated by seasonal changes in testicular activity (Wislocki, Aub & Waldo, 1947; Goss, 1963; Lincoln, Youngson & Short, 1970). When the testes decline in size and activity after the rutting season, the dead bony antlers are shed, and new ones grow out from the wound on the surface of the living pedicles. During growth, the antlers are covered in a richly vascularized skin bearing fine hairs which resembles velvet. The new antlers become fully developed by the time the testes redevelop for the next rutting season. At this stage the ‘velvet’ skin dies and peels off leaving the exposed bony antler, which is retained for use in the rut (Geist, 1966; Lincoln, 1972; Clutton-Brock & Albon, 1980).

The seasonal change in the secretion of testosterone by the testes appears to be the major hormonal influence controlling the cycle of casting and regrowth of the antlers. For example, administration of testosterone to sika or red deer in summer, during the period of antler growth, results in premature calcification of the antlers with cleaning of the velvet (Goss, 1968; Lincoln et al., 1970; Lincoln, Guinness & Short, 1972). In contrast, if the stags are castrated at this stage, the cleaning of the velvet does not occur and the animals permanently retain their living antlers. If these castrates are given an implant of testosterone, the velvet is shed within a few weeks, and the dead bony antlers remain firmly attached to the pedicles as long as the circulating levels of testosterone remain increased (Lincoln et al., 1970, 1972).

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While the role of testosterone in the cleaning and casting process is well established, there is some doubt as to whether the regrowth of the new antlers is merely a consequence of the withdrawal of testosterone, or stimulated by a separate hormone. The existence of an antler-stimulating hormone was first proposed by Wislocki et al. (1947) to account for the situation occurring in white-tailed deer in which regrowth of the antlers is initiated in the spring several months after the casting of the old antlers which occurs soon after the rut. One possibility is that the gonadotrophic hormones play such a role by acting directly on the antler pedicles to stimulate growth (Tachezy, 1956). In support of this, West & Nordan (1976) showed that suppression of secretion of the gonadotrophins in black-tailed deer by methallibure results in suppression of antler growth in some animals.

In the present study we have investigated the effects of immunizing red deer stags against luteinizing hormone-releasing hormone (LHRH). The secretion of this decapeptide by the hypothalamus is believed to control the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary gland, and the neutralization of LHRH by immunization can produce a physiological castrate with low circulating levels of the gonadotrophins, as described for a number of other species (rabbit: Fraser & Gunn, 1973; rat: Fraser, Gunn, Jeffcoate & Holland, 1974a; bull: Robertson, Wilson, Rowland & Fraser, 1981).

**Materials and Methods**

*Animals.* The observations were made on 4 yearling red deer stags living out-of-doors at Reede Hill Deer Farm in Fife, Scotland (56°N). The animals grazed in large paddocks with other red deer, and received supplementary food of hay and potatoes from October to May. They were fitted with numbered collars, and inspected every 1–3 days from January 1981 to April 1982 to record any changes in the antlers. A group of 20 yearling stags living in the same fields acted as controls.

In January 1981, the 4 experimental stags were immobilized by an i.m. injection of etorphine (1-0–1-5 ml Immobilon for large animals; Reckitt & Coleman, Hull) administered by a projectile dart. After capture, a blood sample was collected from the jugular vein, and measurements were made of the diameter of the testes, girth of the neck, length of the neck mane and size of the antlers. The animals were then immunized against LHRH (see below), given an i.m. injection of antidote to etorphine (1-0–1-5 ml Revivon; Reckitt & Coleman) and allowed to recover.

This procedure was repeated in April, July and September 1981, resulting in a total of 4 immunizations. In October 1981, the experimental animals and 4 non-immunized controls were captured and given an i.v. injection of 100 µg ovine LH (NIH-LH-S19) while still immobilized with etorphine. Blood samples were collected from the jugular vein every 15 or 30 min for 3 h. These samples were heparinized, and the plasma was separated within 30 min and stored at −20°C until required for analysis.

On 2 November 1981 the experimental animals were observed for a period of 2 h while grazing in their normal paddocks with other deer, including hinds. A record was made of their behaviour, particularly of the frequency of roaring. In February 1982, the immunized deer were captured for final measurements.

*Immunizations.* The stags were actively immunized against LHRH by a procedure similar to that described previously for immunization of rats, rabbits and sheep (Fraser & Gunn, 1973; Fraser et al., 1974a; Clarke, Fraser & McNeilly, 1978). For the first 3 immunizations, 4 mg LHRH (Hoechst, Frankfurt, Germany) were conjugated to 4 mg human serum albumin (HSA) (Sigma, London) by carbodiimide (Fraser, Gunn, Jeffcoate & Holland, 1974b). After dialysis against distilled water and saline (9 g NaCl/l), the conjugate was emulsified with Freund’s adjuvant and 4 ml emulsion were injected s.c. into each animal at 4 separate sites on the neck. The primary immunization (January) was performed using complete adjuvant, and the second and third (April and July) performed using incomplete adjuvant. For the fourth immunization (September), the
conjugation was carried out using 12 mg porcine thyroglobulin (Sigma) as carrier in place of HSA, and the emulsion was made with Freund's complete adjuvant.

Antibody titre to LHRH was assessed by incubating doubling dilutions of plasma from 1:100 to 1:12,800 with $^{125}$I-labelled LHRH (10,000 c.p.m.) overnight at 4°C in a final volume of 300 μl 0.1 M-phosphate buffer pH 7.4 containing 0.1% BSA. The precipitate was counted after separating free from bound hormone by addition of 1.5 ml ice-cold ethanol. The LHRH was labelled with $^{125}$I using lactoperoxidase (Miyachi, Chrambach, Mecklenburg & Lipsett, 1973), and purified on a 40 × 1 cm column of Sephadex G25 fine (Pharmacia) to enable the collection of mono-$^{125}$I-labelled LHRH. LHRH antibody titre was expressed as the highest initial dilution that bound 33% of tracer.

Radioimmunassays. The levels of testosterone in the plasma were determined by a specific radioimmunooassay (Corker & Davidson, 1978) which had a limit of detection of 0.10 ng/ml and an intra-assay coefficient of variation of 11.5%.

Results

The sequence of 4 immunizations between January and September was effective in inducing the production of antibodies capable of binding LHRH. The antibody titres were relatively low until August, but increased markedly in the autumn after the 3rd and 4th immunization (Text-fig. 1). All four stags responded to the treatment although the titres were never very high, and there was considerable individual variation. The animals were ranked according to their LHRH antibody titre in October 1981 and given a number 1–4 for highest to lowest.

Text-fig. 1. Summary of the changes in the antlers of control stags (mean + range) and LHRH immunized stags (individual cases) from January 1981 to April 1982. The timing of the 4 immunizations (arrows) and the antibody titre (plasma dilution binding 33% tracer) at different stages during the study are shown. The shape of the antlers at the end of treatment is also illustrated.
The effects of the immunizations on the antler cycle are summarized in Text-fig. 1 and Plate 1. There were differences between the individual animals correlated with the antibody titre. The animal with the highest titre (No. 1) cast its antlers in early October at a totally unexpected time of year (Pl. 1, Fig. 1). This animal developed a new set of antlers by January and these remained in velvet until the end of the study in April. The antlers consisted of simple curved beams with no branches except for a small brow tine on each side. In February this animal had a body coat resembling that of a hind with only a short neck mane. The two stags with the next highest titres (Nos 2 & 3), both cast their antlers in November or December (Pl. 1, Fig. 2). One cast in mid-November and produced a complete set of antlers by February (Pl. 1, Fig. 3) which became cleaned of velvet in March. The other animal cast one antler in late November and the second about 2 weeks later: there was only slight regrowth of antler tissue and within 4 weeks the new tissue had become calcified, forming a thin layer of bone over the surface of the antler pedicles (Pl. 1, Fig. 4). The immunized stag that had the lowest antibody titre (No. 4) retained its hard antlers throughout the autumn and winter, as did the non-immunized controls (Text-fig. 1).

The effects of the immunizations on testicular activity are summarized in Text-fig. 2 and Table 1. In October 1981 the immunized stags had smaller testes, reduced blood levels of testosterone and

Text-fig. 2. Changes in the concentration of testosterone in the blood plasma of 4 control stags (mean ± s.e.m.) and 4 LHRH-immunized stags (individual values) after i.v. injection of 100 μg ovine LH given on 23 October 1981 while the animals were sedated with etorphine.

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**Plate 1**

**Fig. 1.** LHRH-immunized stag (No. 1, with highest antibody titre) photographed on 2 November 1981 when the control stags had dead bony antlers and were showing rutting behaviour. The antlers are growing in velvet and the testes are inconspicuous.

**Fig. 2.** LHRH-immunized stag (No. 3, with third highest antibody titre), photographed on 23 October 1981, showing a ring of antler growth at the base of the old antlers; the old antlers had been cut off for the sake of safety.

**Fig. 3.** LHRH-immunized stag (No. 2, with second highest antibody titre), photographed on 28 February 1982, showing the appearance of the antlers produced during the winter; they are relatively normal in form except that there is no branching of the top tines. This animal shed the velvet from its antlers in March and thus produced two complete sets of antlers within 1 year.

**Fig. 4.** LHRH-immunized stag (No. 3), photographed on 28 February 1982, showing the new antlers that became calcified shortly after the casting of the old antlers thus inhibiting further growth.
were clearly sexually retarded compared to the controls. The injection of 100 μg ovine LH stimulated little or no increase in testosterone secretion in the immunized animals. There was a correlation between the LHRH antibody titre and the degree of testicular atrophy; the testes of the animal with the highest titre (No. 1) were small, flaccid and retracted into the inguinal canal (Pl. 1, Fig. 1).

Observations of the stags in the autumn of 1981 confirmed that the immunized animals were not showing rutting behaviour. During a period of 2 h on 2 November the animals were never heard to roar, while a total of 61 roars was recorded from the 4 control stags. The animal that had already cast its antlers at this time (No. 1) spent most of its time lying or grazing alone.

**Discussion**

Immunization against LHRH caused 3 of the 4 stags to cast their antlers prematurely in the autumn. For the animals developing the highest antibody titres, this effect was similar to that induced by castration (Lincoln et al., 1970, 1972). The antlers developed by the immunized deer also resembled those of castrates due to their small number of branches (Pl. 1, Fig. 3; Lincoln, 1975). These results, together with the plasma testosterone values, the size of the testes and various other reproductive parameters, indicate that the immunizations caused a reduction in the release of gonadotrophic hormones, presumably due to the neutralization of LHRH produced from the hypothalamus. Changes in the release of gonadotrophins and the activity of the testes have been recorded in detail for several other species (Fraser, Sharpe, Lincoln & Harmer, 1982).

The effects of immunization on testicular activity and the antler cycle in the deer were quite variable between animals, and this was apparently related to differences in the production of LHRH antibodies. Stag 1 which produced the highest antibody titres showed an almost complete regression of the testes and a marked effect on the antler cycle, while the Stag 4 which produced the lowest titres showed only partial regression of the testes, and no effect on the antlers although rutting behaviour was blocked. The recovery to normality after the immunizations were stopped was also variable between animals and correlated with the rate of decline in the titre of LHRH antibodies. Only Stag 1 was still showing a castration effect 6 months after the end of treatment. The two animals with an intermediate titre (Nos 2 & 3) had already begun to recover from the treatment by February, as judged by the presence of detectable levels of testosterone in the blood. One of these stags cleaned the velvet from its developing antler at a very early stage of growth and the other cleaned its antlers in March; both effects were attributable to a recovery in the secretion of testosterone (Text-fig. 1).
While there was individual variation in the antlers, all 3 stags which cast their old antlers in response to the immunizations showed resumption of antler growth. This was apparent slightly before casting as a swelling at the top of the antler pedicles (Pl. 1, Fig. 2), and resumption of growth appeared similar to that which occurs naturally in the spring in control animals. The special feature of the stags immunized against LHRH is that they had low blood levels of gonadotrophin due to immunological blockade of LHRH. Since these animals resumed antler growth it is concluded that this process is not dependent on direct stimulation by the gonadotrophic hormones.

References

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