Gonadotrophin secretion and pituitary responsiveness to LHRH in castrated and intact male rabbits exposed to different photoperiods

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Summary. Adult male wild rabbits were exposed to at least 16 weeks of 16L:8D before experiments began. Plasma LH and FSH concentrations increased significantly ($P < 0.001$) when rabbits were castrated in 16L:8D but declined when rabbits were transferred to 8L:16D. Concentrations had returned to normal for castrated rabbits in 16L:8D by 74 days after the start of the 8L:16D treatment. Treatment of intact male rabbits with an injection of LHRH before and after transfer to short daylengths caused a transient increase in plasma LH which lasted 50–80 min and this produced a concomitant rise in plasma testosterone. The daylength change had no effect on this response even though testicular size declined after the transfer to short daylengths. Rabbits moulted in response to exposure to 8L:16D. This suggests that hypothalamic activity responds to photoperiod and that changes in pituitary responsiveness to LHRH and steroid negative feedback are unimportant.

Introduction

Rabbits (*Oryctolagus cuniculus*) in the wild have an annual cycle of reproduction which is shown in males by significant seasonal changes in testicular size (Andersson *et al.*, 1979; Boyd, 1985). As for many other mammals (Sadleir, 1969; Lincoln & Short, 1980) this cycle is affected by seasonal changes in daylength (Boyd, 1985, 1986a). It has long been established for mammals that daylength is transduced to an endocrine signal via the pineal gland (Reiter, 1980) but it is still unclear exactly how the link between the pineal and the hypothalamo–hypophysial axis operates to stimulate or inhibit breeding. Some studies have suggested that the ultimate effect of changes in daylength is to cause changes in the sensitivity of this axis to negative feedback by gonadal steroids (Legan *et al.*, 1977; Lincoln, 1977; Goodman & Karsch, 1981). However, other studies have produced good evidence for gonadotrophin secretion being more directly controlled by photoperiod (Turek *et al.*, 1975; Pelletier & Ortavant, 1975; Ellis & Turek, 1980; Simpson *et al.*, 1982; Lincoln, 1984).

If negative feedback relies entirely on gonadal steroid hormones, then gonadectomy should release this inhibition on gonadotrophin release. Plasma concentrations of gonadotrophins should then increase and, if changing sensitivity to gonadal steroids is important in causing seasonal gonadal regression, then plasma gonadotrophin values would be expected to remain elevated even when animals are exposed to the short daylengths that would normally induce gonadal regression. Alternatively, a decline in plasma gonadotrophin concentrations after gonadectomy and then transfer to an inhibitory daylength would suggest that sensitivity to steroid negative feedback is less important than regulation of gonadotrophin release by other more direct mechanisms. Measuring the responsiveness of the anterior pituitary to luteinizing hormone-releasing hormone (LHRH) (Carlson & Perrin, 1979) will show whether changes in pituitary physiology are involved in this process. The present study investigates these hypotheses in wild-caught rabbits.

Materials and Methods

Study animals. Adult male wild rabbits were captured on farmland in Cambridgeshire, U.K., during October and November. Adults were distinguished from juveniles by the presence of descended testes and the degree of ossification of the proximal apophysis of the tibia (Thompson & Worden, 1956). Rabbits were caged individually in lightproof rooms where daylight was simulated with cold fluorescent lamps under the control of electrical time switches. Food, which was a standard diet of rabbit pellets, and water were freely available to each rabbit throughout the study. Temperature was not closely controlled but varied within the same limits for all rabbits. Rabbits were exposed to 16–20 weeks of 16 h light:8 h darkness (16L:8D) before experiments began.

Experiment 1. Twelve rabbits were assigned to 3 groups at random after the pretreatment period in long daylengths. Group 1 (4 rabbits) was maintained in 16L:8D throughout the experiment (88 days). Groups 2 and 3 (4 rabbits each) were anaesthetized with an injection (i.m.) of 0.4 ml Hypnorm, containing fentanyl (0.20 mg/ml) and fluanisone (10 mg/ml) (Janssen Pharmaceutical Ltd., Grove, Oxford OX12 0DQ, U.K.), and castrated on Day 1. Group 2 was kept in 16L:8D for the remainder of the experiment while Group 3 was kept in 16L:8D for a further 13 days and then transferred to 8L:16D for the remainder of the experiment. Blood samples (1–2 ml) were obtained from the ear vein at intervals throughout the experiment, the frequency of sampling being determined by expected rates of change in plasma hormones. Each rabbit was given an injection (i.m.) of 0.15 ml Hypnorm 5–10 min before sampling to encourage blood flow to the ears and to induce mild anaesthesia. All rabbits weighed between 1400 and 1600 g. Blood samples were centrifuged and the plasma stored at -20°C .

Experiment 2. Eleven rabbits were allocated at random to 2 groups after the pretreatment in long daylengths. Group 4 (3 rabbits) was maintained in 16L:8D throughout the experiment (23 weeks). Group 5 (8 rabbits) was transferred to short daylengths during Week 3. During Weeks 2, 6 and 12 all rabbits were treated (i.v.) with a single injection of 1 μg synthetic LHRH (acetate salt dissolved in autoclaved saline) (Sigma Chemical Company Ltd, Poole, Dorset BH17 7NH, U.K.). After the injection, blood (1 ml) was sampled from an ear vein at 10-min intervals for 90 min. Rabbits were kept lightly anaesthetized throughout this procedure as described for Exp. 1. Blood samples were centrifuged and stored at -20°C .

The testicular size of all rabbits was measured at weekly intervals. Testes were manipulated into the scrotum and the total length of the testis, including the head and tail segments of the epididymis, was measured to the nearest 0.1 mm. The maximum testicular width was also measured to the same accuracy and the presence or absence of moult was recorded.

Radioimmunoassays. Plasma LH, FSH and testosterone radioimmunoassays were carried out as described by Boyd (1986a). Samples from Exp. 1 were assayed for LH and FSH while those for the LHRH treatments in Exp. 2 were analysed for LH and testosterone. Both LH and FSH were measured in single assays which had sensitivities of 0.2 and 0.4 ng/ml plasma respectively and intra-assay coefficients of variation of 8.6% and 15.7% respectively. Highly purified rabbit LH and FSH were used as standards. The testosterone assay had a sensitivity of 0.07 ± 0.01 ng/ml plasma. The intra- and inter-assay coefficients of variation were 5.5 and 9.4% respectively.

Statistical procedures. Estimates of testicular weight were calculated from the measurements of length and width using the formula given by Boyd (1986a). Differences between groups were evaluated with two-tailed *t* tests. Analysis of variance by repeated measures (Sokal & Rohlf, 1981) was used to evaluate changes in testicular size and hormone concentration with time. A probability of <0.05 was assumed to be significant.

Results

Experiment 1

Plasma LH and FSH concentrations of Group 1 rabbits remained low throughout the experiment (Fig. 1), in contrast with Groups 2 and 3 in which plasma FSH increased by 20–30 times and LH by 2–4 times after castration (Days 1–16, $P < 0.001$ in each case). There was no significant difference in plasma LH and FSH concentrations between Groups 2 and 3 before the transfer to short daylengths.

After transfer to short daylengths, plasma LH declined in Group 3 until Day 47 when it began to increase (Days 15–88, $F(10,30) = 2.66$, $P < 0.05$) (Fig. 1a). This increase was sustained until the end of the experiment. In contrast, plasma LH values in Group 2, which was kept in constant long daylengths throughout, showed no significant variation over the same period ($F(10,30) = 0.61$, $P > 0.05$) (Fig. 1a). On Day 47 plasma LH concentrations in Group 3 were significantly lower than in Group 2 ($t = 3.741$, d.f. = 6, $P < 0.01$).

Plasma FSH concentrations also declined in Group 3 after the transfer to short daylengths and

Fig. 1. Changes in the mean ($\pm$ s.e.m.) plasma LH (a) and FSH (b) concentrations in intact ($\circ$, $N = 4$) and castrated ($\bullet$, $N = 4$) male rabbits in 16L:8D. Group 3 rabbits ($N = 4$) were transferred to 8L:16D on Day 14 ($\downarrow$). All rabbits were pretreated with at least 16 weeks of 16L:8D.

this was followed by a slow increase (Days 15–88, $F(10,30) = 2.37$, $P < 0.05$) (Fig. 1b). No significant change occurred in plasma FSH values in Group 2 over the same period ($F(10,30) = 1.78$, $P > 0.05$). However, unlike plasma LH, there were no significant differences between plasma FSH values in Groups 2 and 3 on any sampling date after Day 16.

Rabbits in Group 3 were in full moult on Day 60 while rabbits in the other two groups did not moult during the experiment.

Experiment 2

After transfer to short daylengths during Week 3, the testicular size of rabbits in Group 5 declined (Weeks 1–10, $F(9,63) = 29.57$, $P < 0.001$) (Fig. 2). This decline was followed by moult during Weeks 8–10 and a significant testicular regrowth (Weeks 10–23, $F(13,91) = 3.42$, $P < 0.01$) to a weight similar to that before the transfer to short daylengths. During Weeks 6–15 testicular weight in Group 5 was significantly lower than that for Group 4 ($P < 0.05$ in each week) kept in constant long daylengths (Fig. 2). However, testicular size in Group 4 declined during the study

Fig. 2. Changes in the mean ($\pm$ s.e.m.) estimated testicular weight of rabbits transferred from 16L:8D to 8L:16D during Week 3 (—■—) and kept in 16L:8D throughout (·····●·····). All rabbits were pretreated with at least 16 weeks of 16L:8D. The time of moult is shown by the open bar for Group 4 and by the closed bar for Group 5. The arrows on the time axis (A, B and C) show when rabbits were treated with an injection of LHRH (see text and Fig. 3).

($F(22,44) = 8.76$, $P < 0.01$) and this decline was particularly rapid between Weeks 14 and 15. Rabbits in Group 4 moulted during Weeks 20–23.

The general effect of the injection of LHRH was an immediate increase in plasma LH which reached a peak 10–40 min after the injection and had returned to basal levels 50–80 min later (Fig. 3). Plasma testosterone values began to increase 10–15 min after the injection and this increase coincided with the LH peak. Plasma testosterone reached an asymptote 30–40 min after the injection but remained elevated for the rest of the sampling period (90 min) (Fig. 3).

There was no significant difference between Groups 4 and 5 in their plasma LH profiles when tested at Weeks 2 and 6, but during Week 12 plasma LH concentration was significantly higher in Group 5 than in Group 4, 10 min after the injection ($t = 6.69$, d.f. = 9, $P < 0.001$). This was because, during Week 12, plasma LH was slower to respond to the LHRH injection in Group 4 compared with Group 5 (Fig. 3).

The profiles of plasma testosterone were not altered significantly by the stage of development of the testes (Figs 2 & 3) and did not differ between Groups 4 and 5. However, the increase in plasma testosterone during Week 12 appeared to be slower in Group 4 than Group 5.

Discussion

The reduced plasma concentrations of gonadotrophins resulting from exposure of castrated rabbits to short daylengths after a period in long daylengths coincided with testicular regression when

Fig. 3. Profiles of mean ($\pm$ s.e.m.) plasma LH ($\cdots\circ\cdots$) and testosterone ($\text{—}\bullet\text{—}$) concentrations in Groups 4 and 5 after a single injection of LHRH ($\blacktriangledown$) at times A, B and C corresponding to those shown on the time axis of Fig. 2 (A, B and C).

intact rabbits were exposed to a similar daylength regimen (Fig. 3) (Boyd, 1985, 1986a). This agrees with observations for other seasonally breeding mammals, such as hamsters (Simpson *et al.*, 1982; Urbanski & Simpson, 1982, 1983; Urbanski *et al.*, 1983), ground squirrels (Zucker & Licht, 1983) and sheep (Pelletier & Ortavant, 1985) in which, after gonadectomy, gonadotrophin concentrations declined during the inactive phase of the reproductive cycle. A similar response has also been found in some birds (Storey & Nicholls, 1981; Dawson & Goldsmith, 1984). Removal of gonadal steroids, therefore, did not nullify the response to photoperiod in rabbits and, since pituitary responsiveness to LHRH did not decline after transfer to short daylengths, daylength primarily affects a hypothalamic component of the reproductive system.

It took several weeks for plasma gonadotrophins to reach minimal values after the transfer to short daylengths, but this slow rate of change corresponds with a delay of 3–4 weeks before significant testicular regression occurs (Fig. 2). However, castration may have affected hypothalamo-pituitary physiology to reduce the decline in LH and FSH which would normally occur at this time (Urbanski & Simpson, 1983). These results, together with those from the LHRH injections, give no indication that changing sensitivity to gonadal steroid feedback is a major factor in the seasonal regression or regrowth of the gonads in male rabbits. However, gonadotrophin secretion was not fully suppressed after transfer from long to short daylengths and so it is still possible that some dependence on steroid negative feedback exists (Turek & Ellis, 1981).

The testes of wild rabbits can still produce testosterone in response to stimulation by LH, even when in the regressed state (Exp. 2). Boyd (1985) showed that rabbits in the laboratory with regressed testes had reduced spermatogenesis, indicating that the response of the reproductive system of male rabbits to seasonal changes in daylength may be manifest more as increased and

decreased fertility rather than an event which is switched on or off as in the starling (Dawson & Goldsmith, 1984).

Boyd (1986a) found low plasma LH and testosterone concentrations in anaesthetized intact male rabbits throughout periods of rapid gonadal development and regression. The same opioid anaesthetic was used in this study. Opioids are known to reduce pulsatile LH secretion in Soay rams through their effect on LHRH release (Ebling & Lincoln, 1985). Consequently, the release of LH may have been affected by the anaesthetic although, in this study, plasma LH and FSH concentrations still increased after castration. This suggests that gonadal steroids may have a role in sensitizing rabbits to the effects of opioids.

Exposure of rabbits to short daylengths after at least 16 weeks in long daylengths can induce testicular regression (Boyd, 1985, 1986a). However, prolonged exposure to long daylengths also caused testicular regression (Fig. 2) which agrees with the hypothesis that long daylengths are responsible for the development of a process of inhibition of reproductive activity which eventually leads to regression of the gonads (Robinson & Follett, 1982; Boyd, 1986b).

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