Regulation of gonadotrophin secretion in rams from birth to sexual maturity

II. Response of the pituitary-testicular axis to LH-RH infusion

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Summary. The response of the pituitary–testicular axis to LH-RH infusion was investigated in rams from birth to sexual maturity. During early postnatal life ram lambs were responsive to LH-RH stimulation and heightened pituitary sensitivity was observed in rams aged 2–3 months. It is suggested that a change in sensitivity of the pituitary–testicular axis occurs at this time and perhaps represents the time of initiation of the pubertal process.

Introduction

The mechanisms involved in the control of the onset of puberty in mammals remain poorly understood. Several hypotheses have been proposed: these include a change in the sensitivity of the hypothalamus to gonadal steroids (Ramirez & McCann, 1965) and/or a change in pituitary sensitivity to hypothalamic releasing factors (Debeljuk, Arimura & Schally, 1972; Miyachi, Nieschlag & Lipsett, 1973). The pineal gland and the amygdaloid region of the brain have also been implicated as playing some role in the pubertal process (Relkin, 1971). Changes in the peripheral levels of LH, FSH and testosterone in rams have been studied from birth to sexual maturity (Lee *et al.*, 1976) and the present study represents an attempt to investigate whether changes in the sensitivity of the pituitary to LH-RH occur during sexual maturation.

Materials and Methods

Male crossbred Merino/Corriedale lambs born in the spring of 1972 (September–October) at the State Research Farm, Werribee, were used. Each animal in this study was surgically prepared with a carotid loop after birth. Angiocaths (Deseret Pharmaceutical Co., Utah, U.S.A.) were placed in the carotid artery via the carotid loop (18-gauge needle) and in the jugular vein (16-gauge needle) 2–3 hr before treatment with LH-RH. Immediately after catheterization the animals were restrained in cages and silastic tubing infusion lines were connected from a glass syringe containing LH-RH to the carotid artery. The dose of LH-RH (Hoechst) infused into the carotid artery was 5 µg over a period of 60 min in all the rams studied. Venous blood samples were collected at 40, 20 min and immediately before infusion and at 10, 20, 30, 60, 90 and 120 min after the start of infusion.

Plasma LH, FSH and testosterone levels were measured by specific radioimmunoassays, the characteristics of which have been described (Lee *et al.*, 1976).

Text-fig. 1. Concentrations of LH, FSH, and testosterone in the plasma of rams of different ages after an intracarotid infusion (between vertical lines) of 5 µg LH-RH (10 µg in E) over a period of 60 min (120 min in E). A, Rams 78 (●), 50 (○) and 82 (□) at Day 1 and Rams 268 (▲) and 56 (△) at Day 2; B, Rams 272 (●) and 44 (□) at Day 6 and Rams 277 (○) and 34 (△) on Day 7; C, Rams 952 (○) and 951 (△) at 2 weeks and

Rams 480 (●) and 952 (○) at 4 weeks; D, Ram 951 (●) at 6 weeks and Rams 951 (●) and 952 (○) at 8 weeks; E, Rams 539 (●) and 905 (○) at 9 weeks (2 months); F, Rams 632 (○) and 911 (Δ) at 3 months and Ram 101 (●) at 4 months; G, Rams 632 (○) and 985 (●) at 5 months, Rams 800 (Δ) and 567 (▲) at 7 months, and Rams 1000 (□) and 999 (■) at 12 months.

Results

LH

In all the animals studied (Text-fig. 1), LH levels were elevated significantly at 10 min after the start of LH-RH infusion. The levels remained elevated during the course of infusion and declined once the infusion had finished. Animals at 4, 6 or 7 months of age showed sustained elevations of LH levels even after the completion of infusion. A significant LH response to LH-RH was seen in all animals after birth (Days 1 and 2, Text-fig. 1A), basal values of ≤ 1.0 ng/ml increasing to peak levels of ≥ 20 ng/ml by 60 min. During the next 3–4 weeks, smaller LH responses were observed. At 2–3 months (Text-figs 1D and 1F) of age, when the animals were almost twice as heavy as those at 2–4 weeks (12 kg compared to 7 kg), an increase in LH response was seen. A comparison of the areas under the curves, assessed by planimeter, of the LH responses shown in Text-fig. 1C versus those in Text-figs 1D and 1F revealed a significance of $P < 0.025$ using an unpaired *t* test.

FSH

The response of FSH to LH-RH treatment did not always follow that seen for LH. Basal FSH levels in animals aged 1 or 2 days were < 30 ng/ml (Text-fig. 1A). Two out of five animals in this group exhibited significant two- to three-fold increases in FSH levels after LH-RH infusion. No significant increases were seen in the animals at other ages except at 6 and 8 weeks (Text-fig. 1D) and at 2 months (Text-fig. 1E) when concentrations were ≥ 2 –4 times the basal values. The FSH levels fell when the LH-RH infusion was ceased.

Testosterone

A significant rise in the levels of testosterone was observed 60 min after the initiation of LH-RH infusion in all the animals studied. Basal testosterone concentrations were < 120 ng/100 ml and increased to 200 ng/100 ml or more after 60 min of infusion. These levels remained elevated for the next hour and were invariably higher than the levels observed at the end of the LH-RH infusion.

Discussion

These results clearly demonstrate that the pituitary-testicular axis in rams is responsive to LH-RH stimulation very early in neonatal life. Our findings support those of Foster, Cruz, Jackson, Cook & Nalbandov (1972) who found that fetuses *in utero* and neonates were able to respond to intracarotid injections of crude ovine hypothalamic extract or purified porcine gonadotrophin-releasing factor. Furthermore, these studies provide evidence that the sensitivity of the pituitary to LH-RH appears to increase at 6–8 weeks of age. We have previously shown (Lee *et al.*, 1976) that gonadotrophin levels in the blood of developing rams reached peak levels at approximately 5 weeks of age. It is reasonable therefore to conclude that a change in sensitivity of the hypothalamic-pituitary-testicular axis occurs at this time, perhaps representing the time of initiation of the pubertal process. This conclusion is supported by the study of Crim & Geschwind (1972) in which rams castrated at the age of 60 days exhibited greater LH elevations following the operation than at other ages. In addition, the work of Skinner, Booth, Rowson & Karg (1968) on the changes of pituitary gonadotrophin content during the postnatal development of the Suffolk ram showed clearly that a rapid rise in pituitary gonadotrophin content occurred in rams from 42 days of age.

In this study, rapid increases in testosterone concentrations were observed following LH-RH infusion; a significant rise occurred within 60 min of the start of the infusion. This differs from observations made in men (Roth, Grumbach & Kaplan, 1973; de Kretser, Burger, Hudson & Keogh, 1975), in whom the magnitude of the response was poor and a longer time (8–12 hr) was required before significant testosterone elevations were observed. Galloway, Cotta, Pelletier & Terqui (1974) gave a single intravenous injection of LH-RH (100 μ g and 800 μ g) to intact rams and showed elevations of testosterone concentration in the blood which occurred simultaneously with LH rises. Although the dose of LH-RH used in our study was considerably less ($< 1/20$) than that used by Galloway *et al.* (1974) and was given by a different method (intracarotid infusion), the results of the two studies are in accord.

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