Lack of effect of melatonin on the pituitary response to LH-RH in the ewe

A. M. Symons and Josephine Arendt

Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

Summary. Administration of 100 µg LH-RH to cyclic ewes gave peak values of circulating LH similar to those found at the time of oestrus although the duration of elevated LH levels was less. There was also considerable individual variation in response. Pretreatment or simultaneous administration with melatonin (500 µg or 2 mg) did not alter the individual response to LH-RH.

Introduction

Numerous studies in mammals have implicated a role for the pineal hormone melatonin in the control of reproductive function (see review by Johnson & Reiter, 1978). Although early work in this field is difficult to interpret because of experimental differences in species, age and lighting cycles, a general trend of an inhibitory role of the pineal in mammalian reproduction is suggested (Reiter, Vaughan, Vaughan, Sorrentino & Donofrio, 1975), although evidence of a progonadotrophic effect also exists (Turek, Desjardins & Menaker, 1975).

The pineal appears to be a neuroendocrine transducer which responds to changes in light intensity. Secretion of the pineal hormone melatonin, is inhibited by light and stimulated by dark in all species studied to date (Axelrod, 1978). Pineal activity is therefore one mechanism through which environmental lighting could influence reproduction, a phenomenon well known in seasonally breeding mammals such as the sheep (Yeates, 1949).

Although pinealectomy appears not to influence seasonal and oestrous cycles in sheep (Roche, Karsch, Foster, Takagi & Dziuk, 1970), Lincoln (1979) has demonstrated that denervation of the pineal in Soay rams leads to an inability to respond appropriately to changes in photoperiod. Furthermore, intra-cerebrally administered melatonin is effective in abolishing the preovulatory LH surge in the ewe (Domanski, Przekop, Skubiszewski & Wolinska, 1975). Arendt, Symons & Laud (1981) reported that the pattern and plasma concentration of melatonin in the ewe may be important for the transmission of information relating to daylength and hence season of the year. Target organs for released melatonin may be the CNS, the pituitary and/or the gonads. We have therefore used synthetic LH-RH to stimulate ovine pituitary LH release and examined the effect of melatonin pretreatment on the LH response.

Materials and Methods

Six 9-month-old Suffolk–Cross ewes were used. They were kept in natural lighting conditions and allowed a diet of 2 kg hay and 1 kg ewe nuts (B.P. Nutrition Ltd, Witham, Essex, U.K.)

per ewe per day. Melatonin (Sigma Chemical Co. Ltd, U.K.) and synthetic LH-RH (Hoechst Ltd, Milton Keynes, U.K.) were administered intravenously in 1–2 ml 0.9% (w/v) NaCl. The dose of LH-RH used (100 µg) was that which has been shown to release LH in quantities similar to the peak values found normally in sheep before ovulation (Symons, Cunningham & Saba, 1974). Initially, control ewes were injected i.v. with 100 µg LH-RH at 03:00 h and 15:00 h to establish the individual responses and ascertain whether the time of administration affected the response.

In other experiments, using the same animals, melatonin was administered i.v. at a dose of (1) 500 µg or 2 mg 3 h before injection of LH-RH at 15:00 h, or (2) 2 mg simultaneously with LH-RH at 15:00 h. Kennaway & Seamark (1980) have shown that oral doses of 2 mg melatonin proved adequate to raise normal daytime plasma melatonin in ewes to night-time levels. Blood was sampled from the jugular vein using heparinized Vacutainers (Becton/Dickinson Ltd) before LH-RH injection and at 15, 30, 60, 90, 120 and 180 min afterwards. Experiments during the night were performed under dim red light. Blood samples were centrifuged, the plasma was stored at –18°C before LH assays were performed using a modification of the method of Martensz, Baird, Scaramuzzi & Van Look (1976). Ovine LH for iodination and standard ovine LH (LH-NIH-S17) were kindly provided by the National Institutes of Health, U.S.A. Antiserum raised against ovine LH in rabbits (R2/1F) was kindly provided by Dr H. M. Fraser (M.R.C. Reproductive Biology Unit, Edinburgh). The antiserum was used at a final dilution of 1:60 000 and a 24-h preincubation period at 20°C with standards, blanks and samples was employed before the addition of ¹²⁵I-labelled LH (20 000 c.p.m.). A further 24-h incubation period at 20°C was followed by the addition of the second antibody (donkey anti-rabbit; Guildhay Antisera, Guildford) at a dilution of 1:20. After 20 min incubation at 20°C polyethylene glycol was added and incubation was continued for a further 1 h before centrifugation and aspiration of supernatants for gamma counting. The minimum detectable level of LH was 0.5 ng/ml plasma (2 standard deviations from zero standard binding) and cross-reactivity with FSH was less than 0.3%. Interassay coefficients of variation were 20.0% at 1.4 ng/ml plasma and 15.2% at 81.3 ng/ml plasma.

All experiments were performed between mid-January and early March, i.e. during the late breeding season. Blood samples were taken from all ewes twice weekly for progesterone determinations (Symons, 1973) from the previous October to confirm that the ewes had regular 17–18 day ovulatory cycles. The antiserum used in the progesterone assay was HP/S/53 (Guildhay Antisera, Guildford) at a final dilution of 1:10 000. The minimum detectable level of progesterone was 0.38 ng/ml plasma (2 standard deviations from zero standard binding) and the interassay coefficient of variation was 12.1% at 2 ng/ml plasma. Cross-reactivity with 11-deoxycorticosterone, corticosterone and 17-hydroxyprogesterone was <0.9%. Experiments were performed avoiding the days immediately before and after the natural preovulatory surge of LH because of evidence that the response to exogenous LH-RH at this time is different from that at other stages of the cycle (Reeves, Arimura & Schally, 1971).

Results and Discussion

The results for each of the 6 ewes are shown in Text-fig. 1. In Ewes 1, 2 and 3 peak levels of LH occurred 60 or 90 min after injection and ranged from 20 to 128 ng/ml plasma. In Ewes 4, 5 and 6 peak levels were found between 60 and 120 min after injection and ranged from 30 to 62 ng/ml plasma. There were therefore no obvious differences between the two groups. In a subsequent cycle pretreatment with melatonin 3 h before 100 µg LH-RH produced no significant alterations from control LH release patterns in individual animals. The individual responses of the 6 ewes to LH-RH on the three separate occasions, were consistent regardless of the melatonin treatment. We therefore conclude that the LH response to

LH-RH does not differ significantly during the day or night, when endogenous levels of melatonin are vastly different, and that melatonin administered during the day does not affect the LH-RH response. Moguilevsky, Scacchi, Deis & Siseles (1976) reported that neither pretreatment of adult male rats with melatonin (1 mg/day s.c. for 6 days) nor its simultaneous i.v. administration (500 µg) with 75 ng LH-RH modified the LH release in response to the hypothalamic hormone. However, melatonin was able to suppress the stimulatory effect of clomiphene, which probably stimulates LH release via a hypothalamic site of action. Martin, McKellar & Klein (1980) also reported that melatonin had no effect on LH-RH-stimulated LH release in adult male rats *in vivo* but significantly suppressed the response in neonatal male and female rats. This finding may indicate a role for melatonin in controlling pubertal development.

Text-fig. 1. The plasma LH response in individual Suffolk-Cross ewes injected i.v. with 100 µg LH-RH at zero time, with and without melatonin treatment.

Weinberg, Weitzman, Fukushima, Cancel & Rosenfeld (1980) used LH measurements in healthy mature men as an index of LH-RH response and found that supraphysiological doses of melatonin (7.2 mg infused over a 4-h period) had no effect on the LH response to 150 µg LH-RH given mid-way through the melatonin infusion. In contrast, Yamashita, Mieno, Shimizu & Yamashita (1978) and Mieno, Yamashita, Iimori & Yamashita (1978) demonstrated

that in immature and mature dogs administration of 10 or 100 µg melatonin/kg body weight 3 h before an intracarotid injection of 5 µg LH-RH/kg body weight inhibited the release of 17-oxosteroids by the testis into the spermatic vein. Pituitary gonadotrophins were not measured in these experiments and the possibility that LH-RH may directly affect the gonads was not considered.

Clearly, the dose, the time and the lighting regimen may be critical in determining a response of the hypothalamo-pituitary-gonadal axis to melatonin, as has been shown for the hamster (Turek *et al.*, 1975; Tamarkin, Westrom, Hamill & Goldman, 1976) although no time and/or photoperiod dependence of an inhibitory effect of melatonin at the pituitary level has yet been shown. Lincoln (1979) has also demonstrated that, in the ram, LH 'episodes' alter in their frequency from one stage of the year to another. It is possible that melatonin may be involved in the control of this component of the system and not the preovulatory LH surge mechanism.

Should a melatonin effect be demonstrable *in vitro* on the ovine pituitary, then further investigations *in vivo*, under a range of experimental conditions, may be justified in spite of these preliminary negative results. Such studies are underway in our laboratory.

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