Intraruminal soluble glass boluses containing melatonin can induce early onset of ovarian activity in ewes

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Summary. Soluble phosphate glass boluses (preparations A, B, and C) have been developed to release melatonin into the reticulo-rumen of ewes at relatively different (fast, medium or slow) rates. A fourth type (preparation D) containing no melatonin was used as a sham control. Four groups of seasonally anoestrous Suffolk-cross ewes were respectively dosed with preparations A, B, C or D on 4 July. Plasma samples were collected twice weekly for melatonin and progesterone assay.

In Groups A, B and C, elevated daytime plasma concentrations of melatonin could be detected for about 5 weeks after bolus administration. However, the pattern of hormone release was variable between groups, with Group C animals maintaining higher plasma melatonin concentrations for a longer period. The control animals had undetectable daytime melatonin levels. The onset of cyclic ovarian activity in the animals treated with the 'slow' release bolus (Group C) was significantly ($P < 0.05$) advanced compared to the control group. The 'fast' and 'medium' release treatments (Groups A and B) did not significantly alter the onset of ovarian activity.

The results indicate the potential of a novel and convenient method of melatonin delivery for induction of early breeding activity in ewes.

Introduction

Secretion of the pineal hormone melatonin mediates the effect of changing photoperiod upon seasonal reproductive function in the ewe (see reviews by Bittman, 1984; Kennaway, 1984). Timed administration of supplementary melatonin by feeding (Kennaway *et al.*, 1982; Arendt *et al.*, 1983), injection (Nett & Niswender, 1982) or infusion (Bittman & Karsch, 1984) can stimulate an early onset of breeding activity by pharmacologically mimicking the onset of short photoperiodic conditions. Continuous melatonin administration via subcutaneous (English *et al.*, 1986) or vaginal implants (Nowak & Rodway, 1985) is probably perceived as a 'very short-day' response (Lincoln & Ebling, 1985) and can also induce early breeding activity.

In breeds such as the Suffolk-cross and Mule, both timed and continuous melatonin treatments are only effective around mid-summer, probably following exposure to a critical interval of long photoperiod (Nowak & Rodway, 1985; English *et al.*, 1986). In the less seasonal Merino cross-breeds, melatonin treatment can be effective before the summer solstice (Kennaway *et al.*, 1982) and can induce a relative increase in ovulation rate (Kennaway *et al.*, 1984).

It is clear that any convenient and inexpensive method of melatonin administration not only provides a useful research tool but would be of practical benefit to the commercial sheep industry.

The ruminal stomach provides an ideal site for controlled delivery preparations (Telfer, 1984; Cardinal, 1985) which, by virtue of their size, shape and density are normally retained in the reticulum. This study describes performance data of a biodegradable soluble glass/melatonin device developed for oral administration in the ewe. Containing the same amount of melatonin, 3 types of

bolus designed to release the hormone within the rumen at different rates, were administered to seasonally anoestrous Suffolk-cross ewes in mid-summer. Daytime plasma melatonin concentrations provided an index of the release pattern of melatonin from the ruminal boluses whilst cyclic ovarian activity was indicated by plasma progesterone profiles.

Materials and Methods

Melatonin/soluble glass boluses. Polymer-bonded soluble phosphate glass boluses incorporating powdered melatonin (150 mg) (Sigma Chemical Corporation, Poole, Dorset, U.K.) were prepared at the Research and Development Laboratories, Pilkington Brothers p.l.c. in collaboration with the Department of Biochemistry, University of Surrey. Each bolus was cylindrical in shape (diameter 1.8 cm, length 3.0 cm) with an average mass of 23 g, resulting in an average specific gravity of 3.0. Variation in the polymer-bonded soluble glass formulation alters the aqueous solubility of the bolus which in turn determines the release-rate of melatonin. Preliminary in-vitro studies (data not published) indicated 3 formulations (preparations A, B and C) that would dissolve within the rumen at relatively different (fast, medium and slow) rates. A fourth type of bolus (preparation D) containing no melatonin was developed as a sham-control treatment.

Experimental animals. Sixteen maiden Suffolk-cross ewes, born in spring 1984, were maintained under natural photoperiod at the MAFF Central Veterinary Laboratories (CVL), Weybridge, Surrey (latitude 51°41'N). The animals were penned together in a large open shed within an area measuring 10 × 5 metres and were fed a ration of sheep rearer nuts (Southern Counties Agricultural Trading Society, Godalming, Surrey, U.K.) at 230 g/ewe/day and allowed free access to hay and water.

The ewes were randomly divided into four groups of four and on 4 July 1985 (Day 0) were respectively dosed (oesophageal balling gun no. V687A, Arnolds Veterinary Products, Reading, Berkshire, U.K.) with preparations A, B, C or D. The animals were weighed 2 days before and 116 days after treatment.

Blood sampling. From 28 June to 31 October 1985, blood samples (10 ml) were collected between 09:00 and 11:00 h twice weekly by jugular venepuncture using heparin-coated vacutainers (Becton Dickinson Ltd, Cowley, Oxfordshire, U.K.) and the plasma stored at -20°C until hormone assay.

Melatonin assay. Plasma melatonin was estimated by a direct radioimmunoassay (RIA) (Fraser *et al.*, 1983) as described previously (Poulton *et al.*, 1986). Plasma samples of 25, 100 and 478 pg melatonin/ml had inter-assay coefficients of variation of 21, 19 and 13% respectively and intra-assay coefficients of variation of 12, 12 and 7% respectively. The limit of assay sensitivity, as expressed as the value of two standard deviations below maximum (zero melatonin standard) binding, was 4 pg/ml for 500 µl plasma assayed.

Progesterone assay. Plasma progesterone was estimated by a direct enzyme-linked immunoabsorbent assay (ELISA) (Sauer *et al.*, 1986) as described previously (English *et al.*, 1986). Plasma samples of 0.2 and 1.5 ng progesterone/ml had inter-assay coefficients of variation of 23 and 13% respectively and intra-assay coefficients of variation of 3 and 4% respectively. The limit of assay sensitivity, expressed as the value of two standard deviations below maximum (zero progesterone standard) binding, was 0.1 ng/ml for 20 µl plasma assayed.

Reproductive activity. To distinguish between 'normal' and 'short luteal phase' oestrous cycles, the former were defined as a series of at least 3 plasma samples with detectable progesterone concentrations, providing at least one of these samples had a progesterone concentration > 1.0 ng/ml. The onset of cyclic ovarian (breeding) activity was defined as the occurrence of at least 2 consecutive 'normal' cycles.

Statistical analysis. Values are given as mean ± s.e.m. Daytime plasma melatonin concentrations in Groups A, B and C, during the period 1–33 days after bolus administration, were analysed using two-way analysis of variance and tested at each blood sampling episode using one-way analysis of variance. Onset of ovarian activity between all groups was assessed by one-way analysis of variance. Differences between treatment groups were tested using Duncan's new multiple range test at 2 levels of significance ($P < 0.05$ and $P < 0.01$).

Results

Pooled plasma melatonin and individual plasma progesterone concentrations are presented in Fig. 1.

In Groups A, B and C, daytime plasma melatonin concentrations > 10 pg/ml could be detected until 33–40 days after treatment. Group D ewes had undetectable levels throughout the study. During Days 1–33 (Day 0 = day of treatment) there was no difference in overall melatonin concentrations between Groups A, B and C although there was variation over time ($P < 0.002$).

Fig. 1. Twice-weekly plasma melatonin (●) concentrations (means $\pm$ s.e.m., $n = 4$) in Suffolk-cross ewes ($N = 4$) orally dosed with soluble glass/melatonin boluses (preparations A, B, C or D) on 4 July 1985 (* $P < 0.05$, ** $P < 0.01$, Groups A and B vs Group C). Occurrence of cyclic ovarian (breeding) activity is indicated by consecutive oestrous cycles as defined by individual plasma progesterone profiles.

The plasma melatonin profile was similar within groups and biphasic in nature. In Groups A and B, melatonin peaks of about 300 and 150 pg/ml occurred at Days 1 and 7. In Group C, peaks of about 180 and 200 pg/ml occurred at Days 1 and 18. At Days 14, 18 and 21, plasma melatonin concentrations were significantly greater in Group C (Fig. 1).

There was a significant group effect in the onset of cyclic ovarian activity ($P < 0.03$) with the mean date of onset in Group C (9 August, 36 ± 3 days) significantly advanced compared to that of the control group (14 September, 72 ± 3 days) ($P < 0.05$). The start of ovarian cyclicity was not advanced in Groups A (3 September, 61 ± 11 days) or B (21 August, 48 ± 10 days). There were no other significant interactions.

All groups displayed a similar weight increase over the 4-month experimental period. Weights (overall mean $\pm$ s.e.m. values) of the ewes, 2 days before and 116 days after treatment, were 55 ± 2 and 61 ± 2 kg, respectively.

Discussion

All animals in Groups A, B and C displayed elevated daytime plasma concentrations of melatonin for approximately 5 weeks, indicating that, while dissolving in the rumen, these preparations released (immunologically active) hormone which was taken up into the peripheral blood system over this period. As endogenous melatonin is mainly secreted nocturnally, it was assumed that daytime plasma levels could be attributed to the treatments.

It would appear that no boluses were regurgitated, indicating that the bolus mass was sufficient to be retained in the ruminal system and the consistent plasma melatonin profile within each treatment group suggests that the boluses were uniformly and not prematurely degraded. All animals gained weight over the experimental period suggesting an absence of any adverse treatment effects.

Plasma concentrations in Group C ewes were markedly elevated (> 50 pg/ml) 11–25 days after bolus administration compared to those in the other groups. It would appear that this extended period of elevated plasma hormone concentration has contributed to the consistent early onset of ovarian activity in these animals. The critical (daytime) plasma melatonin concentration and the duration of such treatment required to mimic effectively a short photoperiodic response in ewes remain to be accurately defined. This study suggests that at least nocturnal concentrations (i.e. 30–200 pg/ml) for approximately 5 weeks are needed, although supraphysiological levels over a longer period are also effective (Hsieh & Chien, 1985).

The sham-control ewes exhibited the start of ovarian activity earlier than expected of Suffolk-cross ewes in this locality (Arendt *et al.*, 1983; English *et al.*, 1986). Within an enclosed area, ewes displaying early cyclic activity may (via pheromonal cues?) stimulate a similar but premature response in other (untreated) animals. In an 'early breeding' flock, this is actually a desirable phenomenon but may confound experimental treatment versus control comparisons. This issue should be considered in further studies of this nature.

Caution should also be exercised in drawing conclusions from a study that was limited in group size and restricted to one breed. However, these preliminary results provide a positive indication of the efficacy of this technique. Field trials to establish the performance of the soluble glass/melatonin bolus with respect to induction of early oestrus and subsequent lambing rates are currently underway. Similar trials have been extended to the goat and deer.

A biodegradable intraruminal device capable of sustaining elevated plasma melatonin concentrations would provide a novel and inexpensive method of inducing those seasonal responses in ruminants associated with short photoperiod.

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