Concentration of melatonin in plasma of goats treated with Regulin

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ABSTRACT

The duration of melatonin release from Regulin implants and the diurnal variation in plasma melatonin concentration was investigated in 18 cashmere wethers, divided into 3 treatments: C -control; T - treated with melatonin 18 mg Regulin implant on 10.11.88; PT - previously treated with melatonin every 6 weeks for 10 months before receiving an implant on 10.11.88. Mean concentration of plasma melatonin in T remained high 52 pg/ml for up to 42 days after implant, then declined to 8.5 pg/ml at 50 days remaining relatively low for the rest of the sampling period. Goats from PT maintained much higher concentration of plasma melatonin 226 pg/ml for 42 days after implant. Control goats showed very low mean concentration at 5 pg/ml. Between animal variation in plasma melatonin concentration was high coefficients of variation were between 100% and 200% within all treatments.

At 8 days post-implant plasma melatonin concentration of goats in C showed a nocturnal increase averaging 12.5 pg/ml, between sunset and sunrise. The same diurnal fluctuations were evident at 29 days post-implant. Goats in treatment T also showed a nocturnal rise in plasma melatonin concentration with average increases of 15 and 24 pg/ml at 8 and 29 days post implant between sunset and sunrise.

It can be concluded that the melatonin implants used in this trial lasted for 42 days after administration and a nocturnal rise in melatonin concentration was evident in control and treated goats.

Keywords Melatonin, goats, blood levels, plasma.

INTRODUCTION

The effect of photoperiod and the hormone melatonin on seasonal cycles of reproduction (Lincoln and Short, 1980; Karsch *et al.*, 1984) and wool growth (Hutchinson, 1965) is well documented.

Efforts to advance the breeding season of deer, sheep and goats and increase annual fibre production in some mammals, have resulted in the development of a slow release implant of melatonin (Regulin) by an Australian company, Regulin Ltd. Recent research using Regulin in goats has shown a conclusive link between melatonin and cashmere growth (Moore and Bigham 1989; Litherland *et al.*, 1990.) Potential use of Regulin as a commercial tool to increase annual cashmere production prompted the following experiment which aimed to determine the duration of melatonin release by Regulin implants, in goats, and to investigate diurnal variation in melatonin concentration in treated and untreated goats.

MATERIALS AND METHODS

Eighteen 2 to 4 year old cashmere wethers were randomly divided into 3 treatment groups. On 10 November 1988, Regulin was implanted subcutaneously at the base of the left ear. Implants contained 18mg of melatonin Regulin Ltd. Melbourne. The treatments were: T - treated with 1 Regulin implant; PT - treated with 1 Regulin implant but having been previously treated with one Regulin implant every 6 weeks from 22nd January 1988, receiving their seventh implant on 10.11.88; C -untreated control.

To determine the duration of melatonin release from the implant, weekly blood samples were taken at 10.00am from groups T and PT, commencing 8 days after implant. Group T were bled at weekly intervals from 8 to 64 days following implant, then fortnightly until 106 days post implant. Group PT were bled weekly from 8 until 42 days following implant at which time an eighth implant was administered as part of

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another trial. Control goats (C) were not bled at weekly intervals as daytime melatonin concentration is expected to be low (Maeda *et al.*, 1984) and natural daytime melatonin concentration was estimated from samples taken at 10.00am on day 8 and 29 only. Diurnal variation in plasma melatonin was determined from blood samples collected over 24 hours from groups T and C at 8 and 29 days post-implant. Blood collection began at 10.00am and further samples were taken at 4, 7, 8, 9, 12, 16, 19, 20 and 21 hours after this time. Blood was collected from the right jugular vein, contralateral to the melatonin implant (Howse *et al.*, 1987) using 10ml heparinised vacutainers. Whole blood samples were stored at 4°C and plasma separated within 1 hour of collection. Plasma samples were frozen within 24 hours after collection. Determination of melatonin concentration in plasma was carried out using radio-immuno assay (MAF Technology, Ruakura).

Analysis of results involved 2 parts according to the experimental objectives. Mean weekly levels of plasma melatonin in groups T and PT were compared using Cochran's T-test for unequal variances. A comparison of daytime and nighttime concentration of melatonin in groups T and C used a paired-comparison T-test. For this analysis, data was grouped into "day" and "night" periods for individual animals, so that the mean difference between day and night levels within treatments were analysed. "Day" was defined as being after sunrise and before sunset, as was "night" the reverse (see Table 1).

RESULTS

Duration of Melatonin Release

Mean concentration of plasma melatonin of goats in T remained high for up to 42 days after implant Figure 1. Between 42 and 50 days post-implant, mean melatonin concentration in this group dropped from (mean ± S.E.M) 45.6 ± 28.5 pg/ml to 8.5 ± 5.7 pg/ml, and continued to remain between 7.8 ± 5.81 pg/ml and 19.6 ± 9.6 pg/ml for the rest of the sampling time. Goats in PT maintained a high concentration of plasma melatonin until 42 days following implant, after which time they were not sampled. Mean plasma melatonin concentration for PT at 42 days was 185.2 ± 92.3 pg/ml (Figure 1). A comparison between groups T and PT showed no significant difference in mean melatonin concentration at any sampling time. Control goats showed very low daytime levels of plasma melatonin with means of 5.3 ± 2.25 pg/ml and 4.5 ± 2.1 pg/ml at 8 and 29 days post-implant.

There was marked variation between individual animals in all groups and high coefficients of variation (CV) generally between 100% and 200% reflected this.

TABLE 1 Plasma melatonin concentration (pg/ml) for periods of day and night for treated (T) and control (C) goats. At 8-9 days post-implant, sunset=8.18pm and sunrise=5.53am. At 29-30 days post-implant sunset=8.39pm and sunrise=5.45am. * = P<0.05

	Days Post-Implant			
	8		29	
	Day	Night	Day	Night
Group C				
1	1.9	4.1	1.9	6.0
2	1.8	10.6	2.5	6.5
3	2.8	25.0	2.5	27.7
4	16.8	19.9	15.5	15.0
5	3.3	9.1	8.1	19.4
6	1.3	6.7	2.3	4.1
Mean	4.65	12.57	5.47	13.12
± s.e.m	2.45	3.32	2.22	3.79
Mean Diff. between day and night	7.9 *		7.7	
± s.e.d	3.0		3.9	
Group T				
7	76.4	74.0	236.0	247.3
8	136.5	201.7	31.2	105.9
9	107.4	117.7	2.8	5.7
10	24.0	18.1	27.0	23.7
11	57.5	60.5	51.4	69.0
12	47.9	67.7	40.9	81.1
Mean	74.95	89.95	64.88	88.78
± s.e.m	16.81	25.84	34.86	35.12
Mean Diff. between day and night	15.0		23.9	
± s.e.d	10.72		11.9	

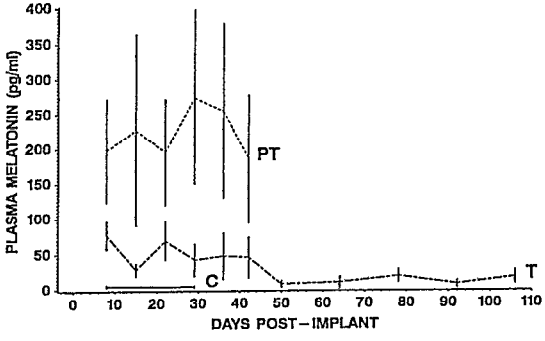

FIG 1 Mean concentration (pg/ml) of plasma melatonin in treated (T and PT) goats implants with Regulin on 10.11.88 and control (C) goats. Samples were taken at weekly intervals beginning at 8 days post-implant. Standard error bars are displayed at each point.

Diurnal Variation

Diurnal fluctuations in plasma melatonin concentration were evident in both groups C and T. At 8 days post-implant there was a significant rise ($P<0.05$) in mean nighttime melatonin levels, in group C but not T Table 1. At 29 days post-implant, nighttime melatonin levels were slightly higher ($0.05<P<0.10$) than daytime levels in both treatments.

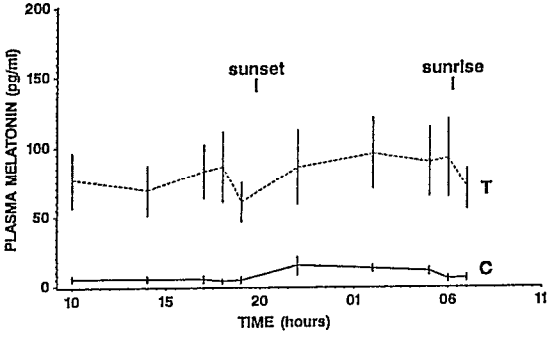

FIG 2 Mean diurnal concentration (pg/ml) of plasma melatonin in trated (T) and control (C) goats. Sampling begin at 10am on the 8th days following implant and continued overnight to 7am the next day. Standard error bars are displayed at each point.

At 8 days post-implant (Figure 2) plasma melatonin concentration in group C increased by an average of 7.9 ± 3.0 pg/ml from sunset to sunrise and similarly at 29 days post-implant (Figure 3) mean nocturnal increase in plasma melatonin was 7.7±3.9 pg/ml. Mean concentration of melatonin in group T showed mean nocturnal increases (15 ± 10.7 pg/ml and 23.9 ± 11.9 pg/ml) at 8 and 29 days post-implant Figures 2a and b.

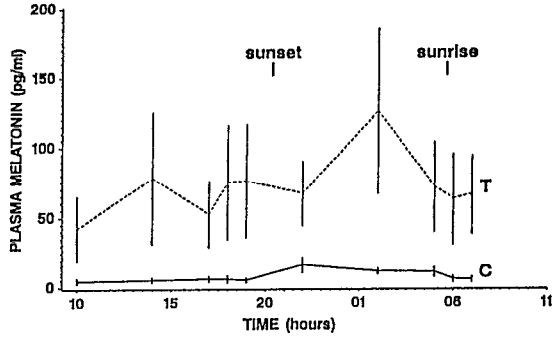

FIG 3 Mean diurnal concentration (pg/ml) of plasma melatonin in trated (T) and control (C) goats. Sampling begin at 10am on the 29th days following implant and continued overnight to 7am the next day. Standard error bars are displayed at each point.

DISCUSSION

The results show that Regulin melatonin implants, elevated daytime concentrations of plasma melatonin well above those normally found in untreated goats, for 42 days after administration. This duration of release is shorter than the 70 days reported for sheep using similar Regulin implants (Howse *et al.*, 1987). During the period from 42 to 106 days post-implant the mean concentration of melatonin in group T was above mean daytime levels seen in most control animals at 8 and 29 days. This result suggests that melatonin was still being released from the implants but at a lower rate than between 8 and 42 days.

Previous treatment with melatonin altered the response of goats to melatonin implant, as revealed in consistently higher melatonin levels in group PT which at 42 days post-implant were approximately 4 times higher than goats in group T. The failure of such large differences to achieve statistical significance was due to the high between animal variation.

High variation in plasma melatonin between individuals in groups T and PT was also evident in group C. This suggests that melatonin implants exaggerated but did not cause variation between animals in plasma melatonin concentration. Between animal variation in plasma melatonin using Regulin implants is poorly documented though standard errors reported for sheep (Howse, *et al.*, 1987) suggest that there is less individual variation for sheep than goats. However, the range of plasma melatonin levels reported for cattle (Sutherland and Entwistle, 1988) indicates considerable variation between animals in that species. There is need for further research on the circulatory response of goats to Regulin implants.

The nocturnal increase in plasma melatonin in control goats meets the expectation of a natural diurnal fluctuation in melatonin secretion as reported for goats (Maeda, *et al.*, 1984) and other mammals (Axelrod, 1974; Brainard, *et al.*, 1981). Four of the six goats in T also showed an increase in nighttime levels of plasma melatonin though the mean nighttime increase for this group was not significant. Two of the six goats in T showed no nocturnal increase in plasma melatonin at 8 or 29 days post implant and it is possible that the melatonin treatment may have masked the natural response to photoperiod in these 2 goats.

Natural daytime levels of plasma melatonin reported for goats used in other studies (Kennaway and Seamark 1980; Maeda *et al.*, 1984) are similar to those found in this experiment. Nighttime levels in control goats in this study are within the range found by Maeda, *et al.*, (1984) who report normal nighttime levels of between 20 and 120 pg/ml for Saanen and Shiba goats.

There is a need to further explain the different responses of individual goats under different melatonin treatment regimes and compare the information in this study with that from trials undertaken at different times of the year.

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