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The role of the pineal gland in seasonality

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Summary. The life time reproductive performance of 2 flocks of Merino crossbred ewes pinealectomized at 7–60 days of age, and maintained in South Australia did not differ from that of sham-operated control animals kept in the same flocks. The pineal gland is therefore not a major determinant of reproductive success, but a role for the pineal in adjusting breeding activity to season is not excluded. It was confirmed that pineal denervation of adult ewes by cranial sympathectomy could have disruptive effects and evidence of a seasonal change in pineal function was obtained from studies of plasma levels of melatonin, a putative pineal hormone, and a pineal peptide with gonadotrophin releasing activity.

That melatonin stems mainly, but not exclusively, from the pineal gland was confirmed by studies of urinary excretion values. Adult Merino ewes produced 39–90 μg melatonin/day, mostly (80%) during the dark period, whereas pinealectomized animals produced less than one-fifth this amount.

A link was provided between the pineal gland and diet. Melatonin and other 5-methoxyindoles influenced the activity of α-chymotrypsin and may regulate the availability of precursor substances required for the synthesis of neurotransmitters important in reproduction.

Introduction

The characteristic seasonality in reproductive activity found in most mammals is a major factor ensuring that their young are born at the most propitious times of the year. Regulation is achieved through a complex interaction between endogenous rhythms and environmental determinants, the most conspicuous of which is the seasonal change in photoperiod. For many species it is probable that these and other light-influenced changes involve the pineal gland (Reiter, 1980). However, the evidence available for the domestic ruminants remains far from conclusive.

Studies involving pinealectomy

Physiologists classically study a gland's function by removal and monitoring the aftermath. The effect of pinealectomy on ruminant reproduction was first reported by Roche, Karsch, Foster, Takagi & Dziuk (1970). In that study, 10 mature ewes (Suffolk–Hampshire crossbred) were pinealectomized and the effects on the incidence of oestrus, ovulation and the length of the

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oestrous cycle during the ensuing year were noted. When comparisons were made with sham-operated and intact control ewes, no differences were seen between groups. There also appeared to be no effect of pinealectomy on levels of serum luteinizing hormone (LH) during the non-breeding and breeding season. The authors' conclusion that the pineal was not a predominant mediator of the effect of light on seasonal breeding activity in ewes therefore appeared justified. However, studies with various laboratory mammals, for example, the ferret (Herbert, 1972), have shown that some effects of pinealectomy are not seen in the current reproductive season following the operation but show a delay before being expressed.

To examine whether there were delayed effects of pinealectomy in sheep, 10 Merino–Dorset crossbred ewe lambs (7–60 days of age), were pinealectomized (Group P) between November 1974 and November 1975 and maintained with a fertile ram under field conditions (Mortlock Experimental Station, Clare, South Australia). An equal number of ewe lambs sham-operated at the same ages as those in Group P were run as controls (Group C). The ram was fitted with a marking harness and daily observations on mating activity were made. The mean age at first recorded matings in both groups was similar, being 13·5 months (8–14 months) in Group P and 12·5 months (7–13·2 months) in Group C. The 21 pregnancies recorded for the Group P ewes yielded 29 lambs (140%) and the 14 pregnancies of the Group C ewes produced 18 lambs (128%) during the 2-year period studied. There were no differences between the groups in the length of gestation (Group P, 147–150 days; Group C, 146–149 days) or the course of parturition or in the duration of post-partum anoestrus (Group P, 3·7–5·2 months; Group C, 3·0–6·0 months). Pinealectomy therefore had little effect on the onset of breeding activity or fecundity of these ewes over a 2-year period.

In November 1977, the fertile ram was replaced with a vasectomized ram and the daily observations of oestrus were continued. There was no marked alteration in the timing of the characteristically long breeding season, even 4 years after the operation. Concurrent observations (not shown) on the lifelong reproductive performance of a flock of 28 pinealectomized Saxon–Merino ewes maintained under field conditions at Struan in south-eastern South Australia confirmed this result. Similarly, the reproductive capacity of 9 Saxon–Merino rams pinealectomized at the same time as the ewes (summer 1974) was not significantly different from that of the sham-operated controls. However, detailed endocrine studies on these rams in 1975–76 (Kennaway, Obst, Dunstan & Friesen, 1981) have indicated that pinealectomy may have compromised the ability of the rams to respond to changes in environmental stimuli because pinealectomy resulted in apparently seasonally inappropriate secretion of prolactin and LH. A similar interpretation may also be placed on the data of Barrell & Lapwood (1978, 1979a).

Information concerning the photoperiod reaches the non-photoreceptive mammalian pineal gland through a pathway involving the eyes, the accessory optic tract and the sympathetic innervation from the superior cervical ganglia. Interruption of this tract by removing the superior cervical ganglia effectively denervates the pineal gland and isolates it from photic influences. Superior cervical ganglionectomy (cranial sympathectomy) forms the basis of much experimental work relating the pineal to season and has recently been extended to the larger domesticated species. Buttle (1977) reported that, when castrated male goats were ganglionectomized in winter, the normal spring rise in plasma prolactin was advanced but the operation had no apparent effects if carried out in summer. More dramatic effects are recorded for sheep by Lincoln (1979) and Barrell & Lapwood (1979b). Following ganglionectomy Lincoln (1979) found that the Soay ram with its strict breeding season failed to respond normally to changes in daylength with the pronounced seasonal differences in testis size, sexual flushing, aggressive behaviour and endocrine changes that normally occur. The results of Lincoln (1979) and Barrell & Lapwood (1979b) showed that long-term variations in the reproductive characteristics persisted after ganglionectomy. These were minor compared with the cyclic changes described for the normal animal, and their timing was inappropriate for the season.

Our own experiments have shown that cranially sympathectomized ewes are similarly affected. In a study of 9 Merino crossbred ewes which were ganglionectomized when in anoestrus (October 1978), there was considerable delay (11–19 months) in their return to breeding activity in comparison with the 5 sham-operated animals prepared at the same time who all showed oestrous activity within 2 months of the operation.

The more dramatic effects of ganglionectomy compared with pinealectomy in the Merino crossbred are presently being investigated. The problem is complex because, as noted by Lincoln (1979), apart from any effects on the pineal, ganglionectomy may affect vasomotor control of blood vessels in the brain and influence other neural functions involved in the response to photoperiod. Another consideration is that sympathetic nerves from the superior cervical ganglion also innervate the salivary glands which are involved in normal sexual responses of some species (Reed, Melrose & Patterson, 1974).

Seasonal correlates of pineal gland function

Extensive studies have identified several active principles which may mediate pineal gland function. Presently our most useful index of pineal function is the measurement of melatonin in blood or urine although other putative pineal hormones are now receiving attention (Leone *et al.*, 1979).

Man is the only species for which data on the actual amounts of melatonin produced each day are available (Fellenberg, Phillipou & Seamark, 1980a). These data were obtained by administering (i.v.) deuterium-labelled melatonin and following the specific incorporation of the deuterium into a unique urinary metabolite, 6-sulphatoxymelatonin. Our recent measurements of production rates of melatonin in sheep using this approach indicate that an adult Merino ewe produces 39–90 μg melatonin/day (A. J. Fellenberg, C. D. Matthews, G. Phillipou & R. F. Seamark, unpublished observations). After pinealectomy the urinary excretion rate of 6-sulphatoxymelatonin is only about one-fifth of the value measured in sham-operated animals which is consistent with melatonin being primarily but not exclusively (Kennaway, Frith, Phillipou, Matthews & Seamark, 1977) a pineal product (Table 1). Furthermore, in the intact ewe or ram, over 80% of the melatonin production occurred during the dark period. Consequential on these low production rates and the high plasma clearance rates (Rollag, Morgan & Niswender, 1977) daytime plasma melatonin levels are low (Rollag & Niswender, 1976; Kennaway *et al.*, 1977). Assessment of seasonal changes of pineal function based on plasma melatonin measurements is further complicated by the episodic nature of melatonin secretion, and frequent, if not continuous, sampling techniques must be used (Kennaway, Porter & Seamark, 1978).

Table 1. Effects of pinealectomy or sham-operation (1974–75) on urinary excretion of 6-sulphatoxymelatonin (1979) by sheep

	Conc. of 6-sulphatoxymelatonin (μg/24 h)			
	Female	Male	Castrate	Mean
Sham	4·6, 8·9, 11·3, 14·0, 15·0	13·1	3·0	10·7
Pinealectomized	2·4, 2·3	2·3	1·9	2·2*

* $P < 0{\cdot}008$ (Mann–Whitney test) compared with sham operated animals.
† Determined by the procedure of Fellenberg *et al.* (1980b).

Until recently the most complete investigation of seasonal change in circulating melatonin in normal intact ewes was provided by the work of Rollag, O'Callaghan & Niswender (1978). Serum samples were collected from 8 ewes at 2-h intervals over a 96-h period at 4 times of the year—during the breeding season, anoestrus, and during the transition from the breeding season to anoestrus and from anoestrus to the breeding season. In addition ewes were sampled during the breeding season at different stages of the oestrous cycle. The distinct circadian rhythm in serum melatonin was evident in all sampling periods, with the mean (±s.e.m.) night-time concentrations (297 ± 46·5 pg/ml) being 2–3 times greater ($P < 0{\cdot}05$) than daytime levels (140 ± 16·8 pg/ml). No seasonal differences in these levels were found but it was noted that in each season circulating melatonin remained at high levels throughout the dark period. The ewes were therefore being subjected to different periods of exposure to melatonin depending on the seasonal change in the dark period. On the basis of these and other data, Rollag *et al.* (1978) have proposed a hypothetical model of the way in which the pineal gland may be operating in the photoperiodic control of reproduction. The underlying postulate is that the hypothalamo–hypophysial axis exhibits a varying sensitivity to melatonin which is determined by two endogenous rhythms, one circadian and the other circannual. In their scheme the critical factor is not the duration of the period when plasma melatonin is elevated, but the time of day at which the levels are elevated. We (D. J. Kennaway, L. M. Sanford and H. G. Friesen, unpublished observations) have re-investigated these seasonal changes and extended the observations to include ewes subjected to artificial changes in daylength. In contrast to the earlier findings, clear evidence of seasonal change in melatonin secretion was obtained, the night-time circulating levels of melatonin being reduced just before the onset of the breeding season (September) and increased before anoestrus (May).

A reduction in pineal activity is also indicated as heralding the onset of puberty in man (Silman, Leone, Hooper & Preece, 1979); further studies using other indices of pineal function are warranted.

The arguments that melatonin is the major pineal hormone are compelling but not conclusive. The pineal contains other biologically active indoleamines and peptides (Benson & Ebels, 1978). Of these the nonapeptide arginine vasotocin is presently most favoured. Pavel (1978) has shown that arginine vasotocin can mimic all the endocrine effects of melatonin and, because melatonin injected intravenously or intraventricularly both induces the release of arginine vasotocin into the cerebrospinal fluid and significantly decreases the pineal content of this substance, it has been suggested that melatonin serves as a releasing hormone for arginine vasotocin from the pineal gland (Pavel & Goldstein, 1979). This hypothesis, though important in its own right, also serves to highlight the erstwhile minority view that the importance of melatonin and other indoleamines may not be in their peripheral actions as hormones (Minneman & Wurtman, 1975) but in their local actions as diffusion activators (Huxley, 1935) where they may serve to regulate other pineal functions (Reiter, Lukaszyk, Vaughan & Blask, 1976) or the function of neighbouring brain structures (Romijn, 1978).

Our present interest in these matters is centred on a pineal peptide which possesses both immunoreactive and biologically active gonadotrophin-releasing activity (GnRH) but which is distinguishable from the synthetic decapeptide GnRH on account of its greater molecular weight (P. Kotaras, J. E. A. McIntosh & R. F. Seamark, unpublished observations). Initial estimates of the size of this substance based on gel filtration studies indicated a single immunoreactive species with a relative molecular mass of about 60 000. However, subsequent studies (unpublished) carried out at monthly intervals over a 15-month period revealed that the apparent molecular mass of the immunoreactive species may vary with the month of the year, ranging from about 60 000 in mid-winter (May–June) to about 2000 in mid-summer (January–February). These studies, although still preliminary, suggest that the pineal gland may be an extra-hypothalamic source of GnRH that can be seasonally adjusted. Various proposals have been made as to the way pineal secretion may reach the regulatory centre in the hypothalamus and/or the pituitary gland, and the recent demonstration that GnRH-like analogues can act directly on the ovary (Ying & Guillemin, 1979) indicates that GnRH-like pineal peptides might also act in this way.

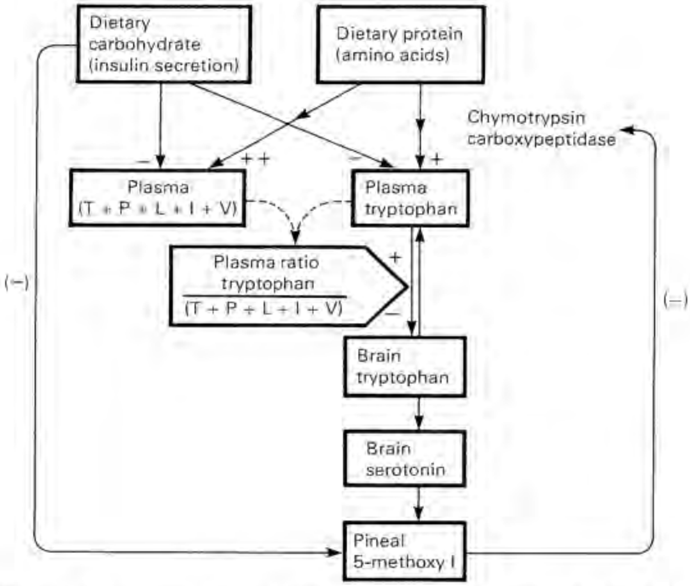
The change of molecular size of the pineal immunoreactive GnRH-like peptide is most likely to be regulated by the changing activity of specific proteases. Extensive studies on the 60 000 molecular weight substance have shown that the GnRH-like sequences are covalently bound and not readily dissociated. Treatment with trypsin enhanced the immunoreactivity 10-fold and at the same time reduced the mean molecular size to about 12 000. Other proteases are currently being investigated to discover whether they can effect further breakdown of the molecule (P. Kotaras, unpublished observations). One interesting feature emerging from these studies is the finding that the activity of certain proteases can be greatly affected (regulated?) by the indoleamines. For example, melatonin and certain other 5-methoxylated indoleamine derivatives are potent competitive inhibitors of the esterase activity of chymotrypsin when tested with the synthetic substrate *N*-acetyl-L-tyrosine ethyl ester (Table 2). The apparent seasonal change in size of the pineal GnRH-like material may thus reflect local influence of pineal indoleamines on protease activity.

Table 2. Inhibition of α-chymotrypsin- and trypsin-catalysed reactions by pineal indoleamines

	% Inhibition	
Indoleamine	Chymotrypsin	Trypsin
Melatonin	53	0
5-Methoxytryptophol	83	0
5-Methoxytryptamine	79	
5-Methoxy indole acetic acid	50	
6-Hydroxy melatonin	18	
Tryptophol	52	
Tryptamine	37	
Tryptophan	4	
Serotonin	6	0
N-Acetylserotonin	9	0

Enzyme activities were determined using the pH stat technique (Kaplan & Laidler, 1967). Reactions were carried out at 20°C in 6 ml equilibrated buffer (5 mM-Tris, 40 mM-NaCl, 20 mM-CaCl, pH 7·9) containing substrate (10 mM, *N*-acetyl-L-tyrosine ethyl ester for chymotrypsin or *p*-tosyl-L-argenine methyl ester for trypsin) plus specified indoleamine (5 mM). Reactions were started by addition of 0·2 ml enzyme solution containing 20 μg α-chymotrypsin (bovine, Sigma Chemical Co., St Louis, Missouri, U.S.A.) and the reaction rate was compared with that for incubation mixtures containing no added indoleamine.

Another possibility is that melatonin or other active pineal indoleamines may act on protease activity elsewhere in the body. If the influence extends to the gut region one may speculate that pineal hormones could help regulate the availability of neurotransmitter precursors and thus close what was previously thought of as an 'open loop' control of neuronal serotonin levels (Wurtman, 1976). The rate at which serotonin-containing brain neurones synthesize their neurotransmitter varies with the brain concentration of tryptophan (Wurtman, 1976). This is determined principally by dietary factors which alter the ratio of plasma tryptophan to other neutral amino acids (e.g. valine, isoleucine and leucine) which interfere with the uptake of tryptophan into the brain. As the action of chymotrypsin in the digestive process is to catalyse the hydrolysis of peptide bonds which are on the carboxyl terminal side of the aromatic amino acids tryptophan, tyrosine and phenylalanine, this enzyme together with carboxypeptidase are key factors in making available these important neurotransmitter precursor substances. While still very speculative, this hypothesis does allow a link between light-influenced seasonal changes and other environmental factors, principally diet, which determine seasonality (Text-fig. 1).



Text-fig. 1. Proposed sequence describing possible involvement of pineal indoleamines in modulating diet-induced changes in brain serotonin concentration. The ratio of tryptophan to the combined levels of tyrosine (T), phenylalanine (P), leucine (L), isoleucine (I) and valine (V) in the plasma is thought to control the tryptophan level in the brain. By inhibiting chymotrypsin activity, melatonin and other 5 methoxylated pineal indoleamines (5 methoxy I) may play a part in mediating the feedback control of neurotransmitter precursor availability.

Prospectives

Explanation of the relationship between pineal function and seasonality in domesticated ruminants is still very much in its preliminary stages. The paradoxical effect, or rather lack of effect, of pinealectomy in sheep subject to normal seasonal changes, remains an enigma which may be resolved when current experiments in which pinealectomized sheep are subjected to changing light regimens are concluded. The availability of reliable assays for melatonin and its metabolites, and the prospect of assays for other putative pineal hormones becoming available in the near future, should also considerably facilitate experimental investigation. Recent major discoveries on pineal function in smaller laboratory animals, particularly the hamster, provide every encouragement to pursue these investigations as they promise developments of possible agricultural benefit. The practical agricultural importance of being able to extend or adjust the breeding season of our domesticated ruminants needs no emphasis. One way this can be achieved in smaller laboratory animals is by adjusting the lighting regimen to which they are exposed. However, while this is an effective way of adjusting season in a laboratory situation, it presents considerable practical problems in application to domesticated ruminants under normal husbandry conditions (Dunstan, 1977). Tamarkin, Westrom, Hamill & Goldman (1976) have shown that administration of melatonin may mimic the effects of exposing an animal to a dark period and melatonin treatment could therefore replace housing animals in controlled lighting. This approach may be particularly well suited to ruminants because melatonin is orally active in sheep and goats, and doses as low as 2 mg will maintain plasma melatonin at high night-time levels in these species for periods of 7 h or more (Kennaway & Seamark, 1980). We are extending these observations to explore the effects of orally administered melatonin on reproductive activity in ewes.

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