THE OCCURRENCE OF HYDROXYINDOLE-O-METHYLTRANSFERASE ACTIVITY IN FETAL SHEEP PINEAL TISSUE AND ITS RELATIONSHIP TO PREPARTURIENT ENDOCRINE CHANGES

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The key role of the fetus in the endocrine changes preceding parturition in the sheep is now well established. Liggins *et al.* (1974) have shown that the complex series of events which result in parturition are initiated by the fetal hypothalamus. As yet, however, the factors which activate the hypothalamus itself are not known. In the adult, the pineal gland has been shown by many workers to exert an important modifying influence on the function of the hypothalamo-pituitary system (see Reiter, 1973) and the possible involvement of the pineal at parturition must be considered.

Among the substances which mediate the endocrine functions of the pineal gland are the methoxyindoles melatonin and 5-methoxytryptophol. Present methods for measurement of these hormones in body tissues and fluids are, however, unsatisfactory and most assessments of pineal function are based on the determination of tissue activity of hydroxyindole-O-methyltransferase (HIOMT, EC 2.1.1.4), a key enzyme in their biosynthesis (Urry *et al.*, 1972; Hyypä *et al.*, 1973).

As part of a study of the role of the fetal pineal, the HIOMT activity in pineal glands of fetal sheep has been determined throughout gestation and the first few days of neonatal life.

Pineal glands from fetal and adult sheep of mixed breeds (mainly Merino cross) were obtained from the local metropolitan abattoirs (SAMCOR) or from our own flocks. The gestation length in these sheep is 147-150 days. Tissues from animals slaughtered between 09.00 and 13.00 hours only were used. Fetuses were obtained 20-40 min after maternal death. After weighing of the fetus, a 20-ml blood sample was collected by cardiac puncture, and the pineal gland and other organs removed. The pineal glands were immediately frozen on solid CO₂ and assayed within 24 hr. In those animals for which the day of mating was unknown, the gestational age was estimated from crown-rump length, body weight, and weights of the other organs such as kidneys and testis, from nomograms based on data accumulated in this laboratory.

Activity of HIOMT was assayed by the method of Axelrod *et al.* (1965). Melatonin was confirmed as the radioactive product by thin-layer chromatography in several solvent systems (Ebels *et al.*, 1972). Under these assay con-

Table 1. Mean ($\pm$ S.E.M.) values for pineal gland weight, HIOMT activity, adrenal weight and plasma cortisol in the fetal lamb

Gestational age (days)	Pineal wt (mg)	HIOMT activity		Adrenal wt (mg)	Plasma cortisol (ng/ml)
		Units/mg tissue	Units/gland		
130	7.2 $\pm$ 0.9 (8)	3.3 $\pm$ 0.6 (8)	24.9 $\pm$ 5 (8)	238 $\pm$ 4 (7)	16 $\pm$ 5.7 (4)
132	6.1 $\pm$ 0.4 (11)	2.4 $\pm$ 1.2 (7)	15.5 $\pm$ 7.5 (7)	—	10.4 $\pm$ 4.5 (4)
134	8.8 $\pm$ 2.4 (4)	1.5 $\pm$ 0.8 (4)	20.9 $\pm$ 14 (4)	281 $\pm$ 21 (3)	9.3 $\pm$ 0.2 (4)
136	8.6 $\pm$ 1.0 (5)	3.3 $\pm$ 0.7 (3)	24.4 $\pm$ 5.9 (3)	—	19.4 $\pm$ 6.0 (3)
138	9.6 $\pm$ 1.2 (8)	11.3 $\pm$ 4.0 (6)	136 $\pm$ 70 (3)	341 $\pm$ 16 (6)	18.8 $\pm$ 1.8 (5)
140	12.2 $\pm$ 2.0 (7)	6.5 $\pm$ 2.2 (3)	71.7 $\pm$ 31 (3)	361 $\pm$ 26 (2)	—
142	11.1 $\pm$ 0.5 (7)	7.0 $\pm$ 1.2 (6)	75.5 $\pm$ 13 (6)	380 $\pm$ 25 (4)	11.9 $\pm$ 1.0 (4)
144	10.4 $\pm$ 1.1 (9)	8.0 $\pm$ 2.2 (6)	88.4 $\pm$ 28.7 (9)	424 $\pm$ 49 (6)	18 $\pm$ 3.3 (4)
146	12.1 $\pm$ 1.6 (8)	8.1 $\pm$ 2.3 (6)	120 $\pm$ 40 (6)	452 $\pm$ 27 (5)	31.8 $\pm$ 9.5 (4)
148	15.4 $\pm$ 1.2 (7)	*23.7 $\pm$ 4.0 (5)	*419 $\pm$ 85 (18)	480 $\pm$ 25 (5)	43 $\pm$ 5 (5)
150	15 $\pm$ 2.0 (9)	33 $\pm$ 6.2 (9)	496 $\pm$ 92 (9)	*611 $\pm$ 21 (9)	62 $\pm$ 8 (9)
1-3-day-old lambs	13.7 $\pm$ 1.5 (12)	20.6 $\pm$ 3.6 (11)	*246 $\pm$ 31 (11)	655 $\pm$ 52 (11)	82 $\pm$ 9 (8)
Adults	69.3 $\pm$ 7.6 (18)	74.9 $\pm$ 7.8 (18)	5190 $\pm$ 630 (18)	—	—

The numbers of animals are shown in parentheses.

* Significance difference ($P < 0.05$) from preceding group.

ditions, it was found that production of melatonin was linearly related to substrate concentration, tissue concentration and time up to 60 min. The within-assay coefficient of variation was 9.8% and the between-assay variation was 7.8%. The HIOMT activity was expressed as units for which 1 unit = 1 pmol [^{14}C]melatonin formed in 60 min at 37°C. Plasma cortisol was measured by the competitive protein-binding method of Bassett & Hinks (1969) and expressed as ng/ml plasma. Student's *t* test was used to evaluate the data.

Table 1 shows the ontogeny of HIOMT activity in relation to pineal and fetal adrenal weight and plasma cortisol. The weight of the pineal gland in the fetal sheep increased linearly with age from approximately 1 mg at 100 days gestation to about 20% adult size at term. There was no obvious sex difference in the pineal weight. Some pigmentation of the gland was observed but this was not related to size or HIOMT activity, and did not appear to decline with increased gestational age as suggested by Jordan (1911). The sudden rise in HIOMT activity in the last 4–5 days of pregnancy coincides with the period of rapid endocrine change associated with parturition (Comline & Silver, 1961; Liggins *et al.*, 1974). The change in plasma cortisol seen relates to these changes. In view of the recent evidence concerning the role of the pineal gland in hypothalamic regulation, it is interesting to speculate that changes in fetal pineal–hypothalamic–pituitary–adrenal interactions may induce parturition in sheep. Further work is however required to determine the exact temporal relationship between events and to discover if the increase in pineal HIOMT and presumptive release of methoxyindoles is obligatory in the parturition process.

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