Changes in Plasma Tryptophan and Melatonin Content in Penned Sheep

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Abstract
The present study was undertaken to examine the relationship between the plasma levels of melatonin and its precursor tryptophan. No circadian changes in plasma total tryptophan content were evident which could be related to the marked night-time rise in plasma melatonin. An automated programmable blood sampling device suited to studies of circadian rhythms in blood constituents is described.

Introduction
Melatonin, the putative pineal hormone, is derived from tryptophan via a route which involves the important neurotransmitter substance serotonin as an intermediary. It has been recently demonstrated in the rat that the rate at which brain neurons synthesize serotonin is dependent upon the availability of tryptophan to the brain and this varies with food consumption (Wurtman 1976). Little is known, however, of the relationship, if any, between the availability of tryptophan and melatonin synthesis.

In this study we have measured plasma tryptophan and melatonin levels at hourly intervals throughout a 24-h period to search for possible interrelationships between the two compounds.

To facilitate these studies, a method was developed to automatically collect blood from the penned but otherwise unconstrained sheep at programmed intervals. The lack of disturbance caused by this approach was assessed by measurement of plasma cortisol.

Materials and Methods

Animals
The sheep used were six Merino crossbred ewes, 4–6 years of age, obtained from Mortlock Experimental Station, Mintaro, S.A. Prior to experiments they were housed in pens measuring 0·8 by 1·1 m, and acclimatized to the 14 h light : 10 h dark lighting regime for at least 16 days. Lucerne chaff, 800 g, was given at 1100 and 1600 h daily.

Blood Sampling Procedure
The procedure developed involved a device where venous blood is sampled and anticoagulant infused via a double lumen cannula constructed from vinyl tubing and attached to a double channel peristaltic pump. Sampling times were programmed by using an automatic switching device and a second peristaltic pump was used to back-flush the cannulae during periods when blood was not required.
A schematic diagram of the sampling device is shown in Fig. 1. The double cannula was constructed from 2 m of Portex size 5E vinyl tubing (4 mm o.d., 2.9 mm i.d.; Boots Co., Australia) by inserting Portex size 2 vinyl tubing (2 mm o.d., 1 mm i.d.) into the lumen through a small hole in the wall 8 cm from the end. The hole was then sealed with Silastic adhesive (Dow Corning Corp., Midland, Michigan, U.S.A.), and the adhesive allowed to cure for at least 24 h. For use, the 14G and 18G blunted needles which served as connections for the outer and inner cannulae were fitted and the cannulae sterilized by immersion in a chlorhexidine-cetrime alcohol solution for 10 min, and then flushed and kept filled with sterile saline.

![Schematic diagram of blood sampling device](image)

**Fig. 1.** Schematic diagram of blood sampling device. The schema represents the situation during periods when sample collection was not required and both peristaltic pumps were operative. For sample collection the single channel pump was switched off by the programmable timer during the collection period.

To insert the cannula, the sheep was anaesthetized with either sodium pentobarbitone (17 mg/kg; May and Baker Pty Ltd, West Footscray, Vic.) or xylazine (0.35 mg/kg; Bayer Aust.) accompanied by xylocaine infiltration (40 mg; Astra Chemicals Ltd, North Ryde, N.S.W.). The cannula was introduced into the jugular vein via a maxillary vein and inserted 4 cm towards the heart and then tied in place with black braided silk 2/0. The wound was sprayed with antibiotic and closed with size 0 Dexon thread (American Cyanamid Company, Pearl River, New York, U.S.A.). The sheep was then returned to its usual pen to recover, and the cannula suspended from above the animal by rubber bands to enable free movement without fouling of the tubing. The cannula was attached to the pumping system during the recovery period by connecting the outer tubing to one channel of a double channel pump which was arranged to continuously deliver sterile saline containing 250 i.u./ml heparin (Weddell Pharmaceuticals Ltd, Sydney, N.S.W.) at a rate of 6 ml/h; the inner tubing was connected to the second channel which delivered sterile saline at 12 ml/h. The connection of the inner tubing was made via a T-junction, one inlet of which was connected to a single channel peristaltic pump which was not operative during the recovery period. Particular attention was paid to maintaining the sterility of the cannulae.

After the 24-h recovery period the flow of the inner tubing was reversed and the pump tubing connected to a fraction collector (Paton Industries Pty Ltd, Stepney, S.A.). Blood was collected into clean glass tubes which were periodically removed, centrifuged at 4000 rev/min for 10 min and the plasma stored at $-20^\circ$C. Anticoagulants were not required in the tubes due to a small amount of heparin returning with the sampled blood.
The amount of heparin solution contaminating samples could be controlled by altering the rate of infusion of the heparinized saline and the sampling rate, and was readily monitored by measurement of changes in haematocrit or plasma protein, or by admixing a dyestuff such as Indocyanine Green (Aynson, Wescott and Dunning Inc., Baltimore, Maryland, U.S.A.) with the heparin. When blood was not required, a programmable timer switched on a second peristaltic pump resulting in a flow of sterile saline at 25 ml/h through the T-junction. As the double channel pump remained on during this period, 12 ml saline was pumped to the fraction collector and 13 ml into the sheep each hour, thus maintaining the patency of the cannulae.

**Analytical**

Total plasma tryptophan was assayed by the fluorometric method of Wapnir and Stevenson (1969). Indole was found not to contribute to fluorescence. Plasma melatonin was determined by the method of Kennaway et al. (1977). Plasma cortisol was determined by the method of Bassett and Hinks (1969).

**Results**

Fig. 2 shows the melatonin and total tryptophan content of plasma samples obtained at hourly intervals throughout a 24-h period. No consistent ultradian or light–dark related patterns in the level of tryptophan content were evident, nor was there any consistent rise in plasma levels which could be associated with feeding. By contrast, night-time levels of plasma melatonin were significantly \( P < 0.01 \) higher than day-time levels in five of the six ewes studied. Cortisol levels in these same samples were all below 6 ng/ml and there was no evidence of an ultradian or circadian rhythm.
Discussion

The levels of tryptophan found in sheep plasma are comparable to those previously reported by Schweigert et al. (1946) and the levels of melatonin with the characteristic dark-time rise are comparable with those reported by Rollag and Niswender (1976) and by Kennaway et al. (1977).

In the 24-h profiles determined, no changes were seen in the plasma tryptophan which could be related either to the nocturnal rise in melatonin or to feeding. It remains, however, to exclude any effects of periodic fluctuations in the plasma content of neutral amino acids as these are known to compete with tryptophan for entry into the brain (Fernstrom and Wurtman 1973).

The cannula and blood collecting system described has, we believe, wide application in physiological and nutritional studies. The length and flexibility of the cannula allows the animals to be housed comfortably in roomy pens without additional constraints. The programmable nature of the system serves to minimize blood loss and disturbance, and reliable sampling for periods of 3 weeks or more is readily achieved.

Clotting of the blood within the system was not a problem provided adequate concentrations of heparin were used and attention was paid to the cleanliness of the pump, tubing, T-junction and needles.

The lack of stress imposed by the sampling procedure was reflected in the low levels of cortisol measured which compare with those previously reported for unstressed animals (Bassett and Hinks 1969).

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References


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