Melatonin: Daily Cycle in Plasma and Cerebrospinal Fluid of Calves

Abstract. *Melatonin was measured by radioimmunoassay in jugular vein plasma and lateral ventricle cerebrospinal fluid collected from calves at 12 times of the day and night. Melatonin in cerebrospinal fluid increased 17-fold from an average (± standard error) of 38 ± 8 picograms per milliliter during the day to an average of 637 ± 133 picograms per milliliter during the night (P < .001). Plasma concentrations of melatonin increased sixfold from an average, per milliliter, of 19 ± 4 picograms during the day to 121 ± 24 picograms during the night (P < .001).*

Evidence suggests that the brain is an important target tissue for the pineal hormone melatonin (*N*-acetyl-5-methoxytryptamine). Melatonin readily penetrates into the rat and cat brain after systemic injection; the highest uptake occurs in the hypothalamus (*1, 2*). Exogenous melatonin also has numerous neurotropic effects. These include induction of sleep in domestic chicks, cats, and humans (*3, 4*); alteration of electroencephalogram activity in the cat and chicken (*4, 5*); elevation of serotonin and γ-aminobutyric acid levels in the hypothalamus (*6*); and alteration of the concentrations of plasma luteinizing hormone, follicle stimulating hormone, and prolactin, apparently by action on the hypothalamus and midbrain (*7*). Finally, in the domestic chicken melatonin has been found in several brain regions, the highest concentration being found in the hypothalamus (*8*). Melatonin has also been identified in lumbar cerebrospinal fluid (CSF) from humans (*9*). We show in this report that endogenous melatonin is present in calf CSF collected from the lateral brain ventricle, thus providing additional support for the hypothesized neurotropic function of melatonin.

In the domestic chicken, sheep, rat, and human, melatonin concentrations in the blood follow a daily cycle in which the highest concentrations occur during darkness (*8, 10, 11*). These observations suggest that potential target tissues, such as the brain, may be exposed to the highest melatonin concentrations at night. Pang *et al.* (*8*) found that melatonin levels in the chicken brain were much higher during the dark period than during the light period. We show in this report that in the calf, melatonin levels in brain ventricle CSF and jugular vein blood exhibit similar daily cycles (*12*).

Six 9-month-old Guernsey calves, each weighing about 114 kg, were used in this study. They were housed in a sheltered environment with an average temperature of 25°C (23° to 29°C) and 14 hours of light per day (*13*). A maintenance ration was fed twice daily, and water was provided ad libitum. The calves were surgically implanted with cannula guides positioned stereotaxically for sampling from the lateral brain ventricles (*14*). Cerebrospinal fluid samples were obtained by inserting into the guide cannula a sterile sampling cannula (18-gauge) 4.5 to 6.5 cm long with a 9-mm long beveled tip. The calves showed no signs of discomfort during sampling. Samples were collected over five consecutive weeks with 36 to 48 hours between sampling times. At each sampling time, about 3 ml of CSF and 6 ml of blood were obtained. The heparinized blood was centrifuged, and both the CSF and plasma were frozen and stored at − 15°C.

The concentration of melatonin was measured by a radioimmunoassay procedure described previously (*10, 15*). Portions of CSF and plasma (100 to 500 μl) were assayed in triplicate. The substance measured by the radioimmunoassay was chromatographed on Gelman silica gel G chromagrams, with a mixture of chloroform and methanol (9 : 1 by volume) being used for development. Two CSF and two plasma samples, each containing a high concentration of immunoreactivity, and one pooled plasma sample containing a low concentration of immunoreactivity were examined. The only material detected in these samples by radioimmunoassay comigrated with tritiated melatonin.

The melatonin content of plasma and CSF showed a daily rhythm with the highest concentrations occurring during the dark period (Fig. 1). Melatonin levels in CSF increased 17-fold from an average (± standard error) of 38 ± 8 pg/ml during the day to an average of 637 ± 133 pg/ml during the night ($P < .001$) (*16*). The greatest concentration changes were coincident with lighting transitions. Plasma melatonin levels increased only sixfold from an average level of 19 ± 4 pg/ml during the day to 121 ± 24 pg/ml during the night ($P < .001$). The average melatonin concentration in CSF was five times higher than the plasma concentration during the night while only twice as high during the day.

The presence of a relatively high concentration of melatonin in brain ventricle CSF is compatible with a neurotropic function for this hormone. Several studies (*2, 17*) show that melatonin injected

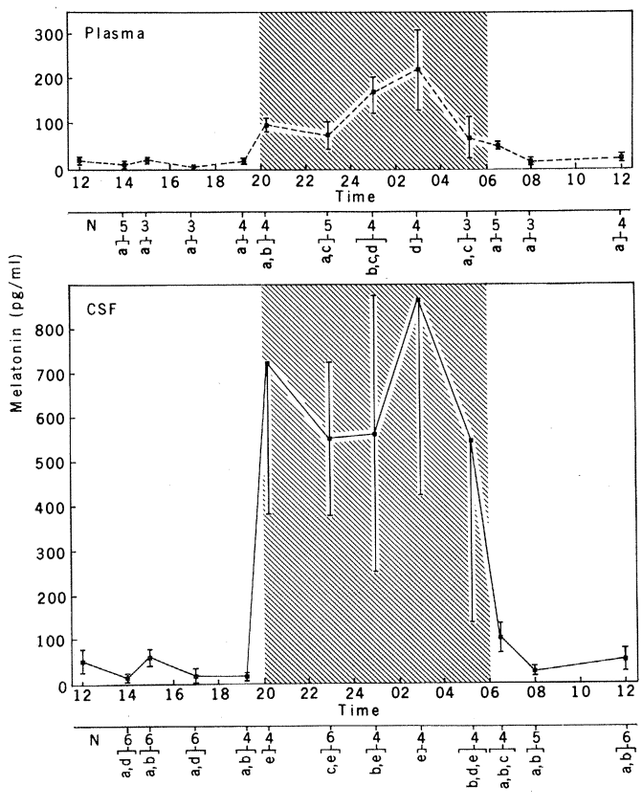

Fig. 1. Concentrations of melatonin in calf plasma and cerebrospinal fluid at selected times of the day (lights on from 0600 to 2000 hours). Data points are means ± 1 standard error of samples from *N* number of calves. Letters indicate that when two means do not have any letter in common they are significantly different ($P < .05$) [see (*16*)].

into the brain ventricular system rapidly penetrates into the brain parenchyma with uptake being highest in the hypothalamus. This indicates that melatonin in CSF has access to brain areas important for neuroendocrine control.

The reason that melatonin concentration in CSF is higher than that in plasma is not known. Melatonin could be secreted directly into the third ventricle (*18*); it could be actively transported into the CSF from the blood; or, it could be the result of retrograde flow in the superior sagittal sinus (*19*). Secretion directly into the third ventricle, although the most attractive hypothesis, is not supported by anatomical evidence. The bovine pineal gland has a relatively dense glial layer between the pineal parenchyma and the lumen of the third ventricle (*20*), and it does not exhibit the intimate contact with the suprapineal recess such as occurs in some lagomorphs and rodents (*18*).

There is a degree of uncertainty about the initial source of plasma and CSF melatonin in mammals, because retinae, Harderian glands, and pineal glands are all capable of melatonin synthesis (*21*). However, in chickens, removal of the pineal gland reduces melatonin to nondetectable levels in plasma, confirming that the pineal gland is the primary source of melatonin in this species (*8*). Although it seems likely that most of the melatonin found in the plasma of rats, sheep, humans, and calves, as well as the melatonin in calf and human CSF, originates from the pineal gland, critical experimental support is still lacking.

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13. Illumination by 40-watt fluorescent bulbs (GE F40 WW) located 3 m above the floor. Light intensity during the day was about 431 lux at the level of the calf's head, while at night the room was dimly lighted (much less than 22 lux) by two shaded fluorescent bulbs located about 10 m from the calf pens.
14. Cannula guides were 15-gauge syringe needles, 2 cm in length and fitted with a flange on the needle hub to facilitate anchoring to the skull. The cannula guides were placed on the frontal bone with the aid of a stereotaxic apparatus; we used coordinates of 0.9 to 1.2 cm lateral from the skull midline and 0.8 to 1.5 cm anterior from the ear bar. The cannula guides were secured to the skull with screws and dental acrylic cement. When not in use, the cannula guides were capped. The calves were prepared with these implants about 7 months before use in the present study.
15. Plasma and CSF samples, packed in Dry Ice, were shipped by air from Columbia to Fort Collins where all assays were performed.
16. From analysis of variance (probability of the F value), individual sample time means compared by least significant differences with a $P = .05$ (Student's t-test).
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Does "Blastocyst Estrogen" Initiate Implantation?

Abstract. *Fertilized eggs were incubated for 2 hours in a medium containing estradiol-17β and then transferred into the uteri of day 5 pseudopregnant rats. These eggs, but not estrogen-free control eggs, induced a local increase in capillary permeability. We suggest that the blastocyst factor which induces the local increase in capillary permeability during early pregnancy is estrogen synthesized by the blastocyst.*

In previous publications we introduced a new concept which states that the preimplantation embryo (PIE) has the capacity to synthesize steroid hormones, and that these PIE steroids are essential for preimplantation embryogenesis and for implantation of the blastocyst (*1–3*). In support of this concept, it has been shown that the PIE contains steroid hormones (*2, 4*) and can synthesize them (*2, 3, 5*). However, the definitive support for the concept will have to come from evidence demonstrating the function of PIE steroids. Accordingly, the purpose of the present study was to determine whether "blastocyst estrogen" plays a role in the initiation of implantation in the rat.

In the rat, and in a few other species which have been studied, the earliest macroscopically demonstrable reaction of the uterus to the presence of a blastocyst is a local increase in capillary permeability. This reaction can be demonstrated experimentally by injecting intravenously a macromolecular dye, such as Chicago Blue, and inspecting the uteri 15 minutes later: in the area where each blastocyst is located, a discrete blue band is seen across the uterus. Apparently, the dye can leave the circulation only in loci where an increase in capillary permeability has occurred. The increase in permeability is a necessary preliminary for the decidual reaction (*6*), and the decidual reaction, in turn, is a prerequisite for the implantation process. It seems obvious that the blastocyst provides the stimulus for the induction of increased capillary permeability, since the reaction occurs only in the immediate vicinity of the blastocyst. Nevertheless, we have not encountered in the literature any suggestion concerning the nature of the blastocyst's stimulus.

In castrated rats and rabbits (*7*) and in immature rats (*8*), systemic injection of estrogen induces an increase in capillary permeability throughout the length of the uterus. We have shown that the rabbit blastocyst contains estrogen and suggested that this estrogen is synthesized by the blastocyst (*2*). Taken together, these findings led us to propose that the stimulus for the local increase in capillary permeability in the intact pregnant rat is estrogen secreted by the blastocyst, and that this stimulus is effective only in a uterus which has been properly primed with systemic progesterone and estrogen. The design of our experiments was based on this hypothesis.

The animals used were adult, virgin female rats of the Holtzman strain weighing 180 to 220 g. They were housed in temperature- and humidity-controlled quarters with lights on from 0600 to 2000 hours. To induce pregnancy, females