

III. Physiological Aspects

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Maternal Melatonin: A Source of Melatonin for the Immature Mammal

Steven M. Reppert

Children's Service, Massachusetts General Hospital, Boston, Mass., USA

Introduction

The concept that the melatonin generating system provides a model for study of the developing circadian time-keeping system in mammals is based on studies in the rat of the rhythm in pineal melatonin production. In this rodent, the rhythm in production, which is accurately reflected in the concentrations of the hormone in blood, cerebrospinal fluid and urine, appears to be generated by a biological clock located in the suprachiasmatic nuclei of the anterior hypothalamus. In contrast to other circadian rhythms, much is known about the other neural structures and the biochemical and molecular events involved in the generation and regulation of the rhythm. Important characteristics of the rhythm are that it appears to be entrained by only one environmental stimulus, the daily light-dark cycle, and that it is not affected by most forms of stress nor by end product feedback. The significance of the rhythm in melatonin production as a system for study of circadian function during development is highlighted by the fact that it is one of the first circadian rhythms manifested by the developing rat.

In the following sections, our current understanding of the events involved in the generation and regulation of the melatonin rhythm in the adult rat will be briefly reviewed and various aspects of the development of the melatonin generating system will be discussed. Next, I will describe the results of studies directed at maternal transfer of melatonin to the immature mammal and comment on the potential importance of maternal melatonin for the developing animal.

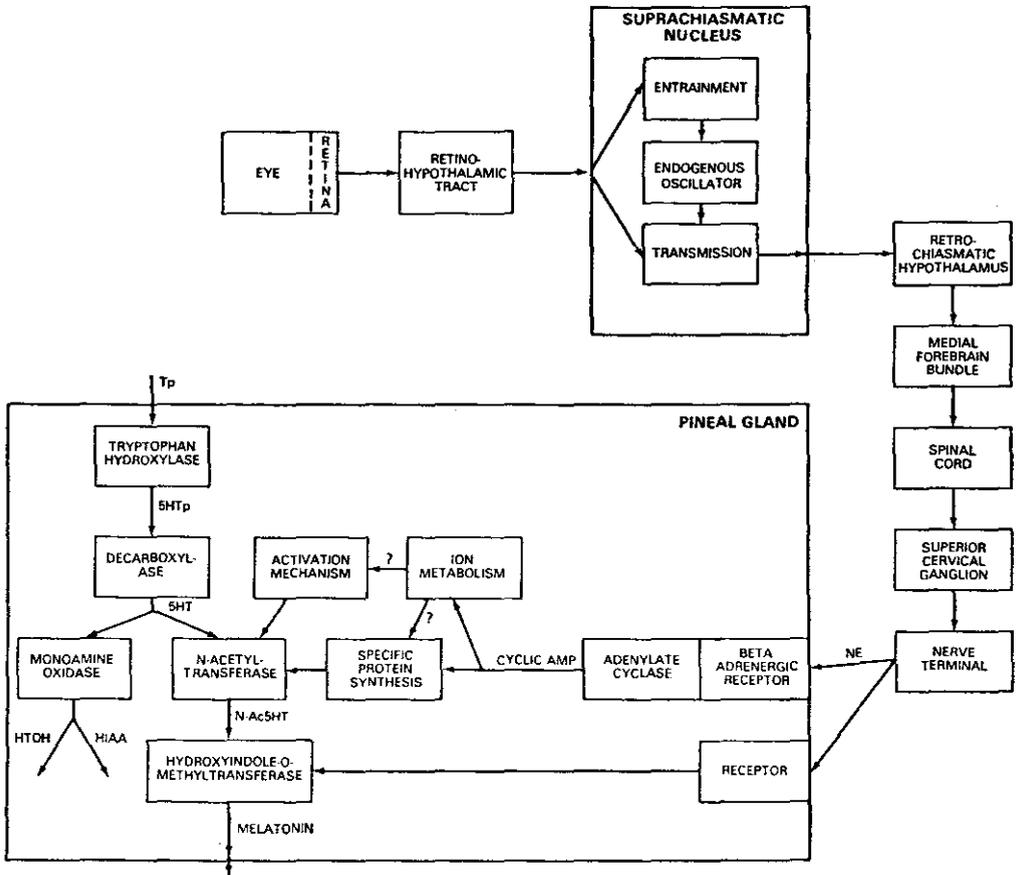


Fig. 1. Schematic representation of the melatonin rhythm generating system. NE = Norepinephrine; Cyclic AMP = adenosine-3',5'-cyclic monophosphate; Tp = tryptophan; 5HTp = 5-hydroxytryptophan; 5-HT = 5-hydroxytryptamine (serotonin); HIAA = 5-hydroxyindoleacetic acid; HTOH = 5-hydroxytryptophol; N-Ac5HT = N-acetyl-serotonin; ? = unproven hypotheses [from ref. 14].

Regulation of Melatonin Production in the Adult Rat

For more extensive reviews on the regulation of melatonin production in the adult rat (fig. 1), see Klein [12] and Reppert and Klein [23]. As already mentioned, neural signals that originate in the suprachiasmatic nuclei drive the daily oscillations in pineal melatonin production. A neural circuit that

passes through central and peripheral neural structures transmits daily oscillatory neural signals from the suprachiasmatic nuclei to the superior cervical ganglion which, in turn, sends projections into the pineal gland. Neural signals from the suprachiasmatic nuclei that result in an increase in melatonin production cause a release of norepinephrine from postganglionic sympathetic nerve terminals within the gland. Norepinephrine initiates a complex series of biochemical and molecular events that leads to a large increase in the activity of pineal *N*-acetyltransferase; this enzyme controls the major alternations in rat pineal melatonin production. The increase in *N*-acetyltransferase causes a decrease in serotonin levels in the pineal gland and a corresponding increase in *N*-acetylserotonin and melatonin.

The endogenous oscillations generated in the suprachiasmatic nuclei are precisely entrained to the 24-hour period by the daily light-dark cycle; lighting information passes from the eye to the suprachiasmatic nuclei by way of the retinohypothalamic projections. As a result, the daily rhythm in melatonin production is entrained to the 24-hour period with increased production occurring at night.

Developmental Aspects of the Rhythm in Melatonin Production

Expression of the rhythm in pineal melatonin production by the immature mammal is dependent on the functional maturation of each of the components necessary to generate the rhythm (table I). The last element to develop in the rat is the ability to convert pineal *N*-acetylserotonin to melatonin by hydroxyindole-O-methyltransferase; the enzyme is not detectable until the second week of life [1, 13, 28]. This situation is in marked contrast to that found in the sheep [9] and monkey [21] where detectable levels of the enzyme are easily measured late in fetal life. It is important to point out, however, that whether or not the latter two mammals can synthesize melatonin on a daily rhythmic basis at this early stage of development remains to be elucidated.

Development of the Pineal N-Acetyltransferase Rhythm

Before the development of hydroxyindole-O-methyltransferase, a rhythm in pineal *N*-acetyltransferase activity is detectable [6]. The enzyme rhythm is first seen at about the fourth day of postnatal life, which is at the time postganglionic sympathetic nerves begin to penetrate the

Table I. Development aspects of melatonin in the rat

Developmental stage	Pineal innervation	NAT ¹ activity	NAT rhythm	HIOMT ² activity	Potential source of melatonin	Mechanism of delivery
Prenatal (-1 day)		+			maternal	placental transport
Neonatal						
0-1 week	+	++	+		maternal	suckling secretion,
1-2 weeks	++	++++	++	+	endogenous, maternal	suckling secretion,
2-3 weeks	+++	++++	++++	++++	endogenous, maternal	suckling secretion,
Adult	++++	++++	++++	++++	endogenous	secretion

¹ *N*-acetyltransferase.

² Hydroxyindole-O-methyltransferase.

gland [8, 16]. Also, the entire neural circuit necessary to generate and regulate the rhythm, including the retinohypothalamic projections [26] and the suprachiasmatic nuclei [20], appear to be functional. Thus, the rhythm at this early age seems to reflect circadian hypothalamic output. In addition, the rhythm in pineal *N*-acetyltransferase activity is clearly evident well before the time that most behavioral rhythms and other hormone rhythms are expressed in the neonatal rat [2].

Development of the Rhythm in Pineal Melatonin Content

As predicted from the pineal enzyme studies cited above, a clear day-night variation in pineal melatonin content is first detectable at 8-10 days of age in the rat; a similar pattern of development occurs in the Syrian and Siberian hamsters [27].

Maternal Transfer of Melatonin

The inability of the fetal and neonatal rat to synthesize melatonin does not necessarily mean that the developing rat lacks melatonin. Indeed, evidence is available which indicates that the mother is an important source of melatonin for the developing fetus and neonate.

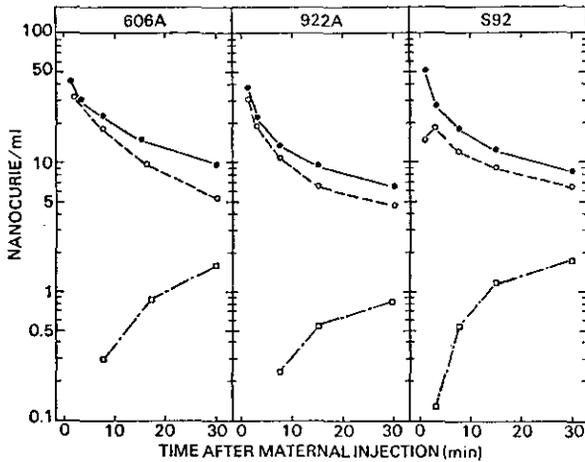


Fig. 2. Maternal plasma (●), fetal plasma (○), and amniotic fluid (□) $[^3\text{H}]$ -melatonin concentrations after maternal $[^3\text{H}]$ -melatonin injection [from ref. 21].

Placental Transfer of Melatonin

In three animal species studied, the rat [11], sheep [10] and rhesus monkey [21], placental transfer of melatonin has been demonstrated. Studies in the monkey have shown that placental transfer is very rapid and could result in transfer of a maternally generated daily melatonin rhythm. First, it was shown that a small amount of $[^3\text{H}]$ -melatonin injected i.v. into pregnant animals during the latter stages of gestation promptly appears in the fetal circulation and that the rates of disappearance of radiolabelled melatonin in the maternal and fetal circulation are parallel (fig. 2). In another experiment, a diurnal change in maternal melatonin, experimentally simulated on a reduced time scale, results in a rapid reflection of the rhythm in the fetal circulation (fig. 3). Rapid placental transfer of melatonin is quite predictable in view of the lipophilic, nonionized properties of this small molecule (mol. wt. 232; fig. 4).

Another important finding of the placental transfer study was that maternally derived $[^3\text{H}]$ -melatonin is recovered from fetal cerebrospinal fluid (CSF) [21]. This indicates that a maternally generated melatonin rhythm would be expressed in fetal CSF as well as in the circulation since it has been shown that in the adult mammal the rhythm in circulating levels is precisely reflected in CSF [24, 25]. Both routes may deliver the hormone

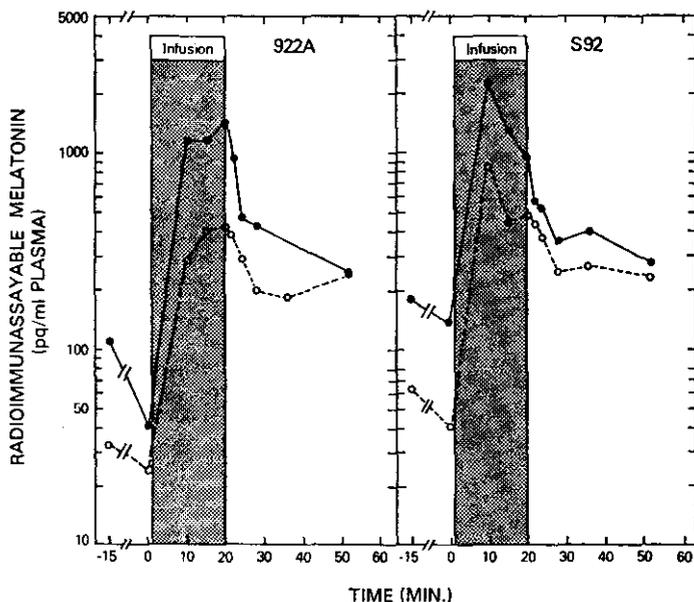


Fig. 3. Melatonin in maternal (●) and fetal (○) plasma before, during, and after an infusion of authentic melatonin to the mother [from ref. 21].

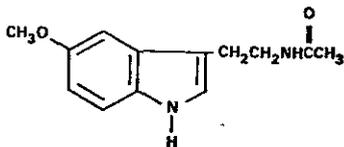


Fig. 4. Chemical structure of melatonin (N-acetyl-5-methoxytryptamine).

on a daily basis to fetal brain structures where melatonin could potentially exert its effects.

Milk Transfer of Melatonin

Not only is the mother a source of melatonin for the fetus, but she is also a source of melatonin for the neonate. Radiolabelled melatonin injected into the circulation of lactating rats is recovered from maternal breast tissue (fig. 5) and from the stomachs of the pups [22]. Also, when

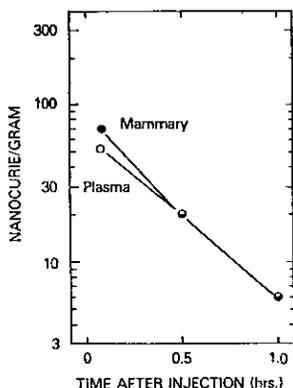


Fig. 5. [^3H]-melatonin in lactating mammary tissue and plasma following an i.v. [^3H] melatonin injection [from ref. 22].

the radiolabelled compound is infused into the neonatal stomach, melatonin is recovered from all tissues examined including brain. Although precise amounts of maternal melatonin transferred by milk have not been established, it is conceivable that through lactation a daily rhythmic source of melatonin could be provided to the neonatal animal.

Melatonin in the Immature Mammal

Based on the foregoing discussion, endogenous synthesis in some mammals and maternal transfer in all mammals are sources of the hormone for the immature animal (table I). In rodents such as the rat, it appears that the mother is the sole source of melatonin for the fetus and during the postnatal period until about day 10 of life. Also, the maternal hormone would augment endogenous levels from day 10 up to the time of weaning. On the other hand, higher mammals like the sheep and monkey have the enzymatic capability to synthesize melatonin during late fetal life and throughout the neonatal period. Maternal melatonin would supplement endogenous levels during those times and provide the sole source of melatonin during early fetal development.

As of this writing, the endogenous concentrations of melatonin in the circulation of the fetal and neonatal rat have not been reported. Circulating melatonin concentrations have been detected in the fetal sheep, rhesus monkey, baboon, and at the time of birth in the human neonate (table II)[21].

Table II. Melatonin in the fetus

Animal species	Gestation age % term	Plasma melatonin pg/ml	Pineal enzyme activity	
			NAT ¹	HIOMT ²
Rat	80	?	+	-
Sheep ³	90	10-30	+	-
Primate				
Rhesus	99	45	+	+
Baboon	80	40	?	?
Human	99 ⁴	18	?	?

¹ *N*-acetyltransferase.

² Hydroxyindole-O-methyltransferase.

³ Extracted from ref. 10.

⁴ Cord blood sample at the time of birth.

Potential Importance of Maternal Melatonin for the Immature Mammal

It is quite conceivable that maternal melatonin influences various aspects of development in the immature mammal. Evidence is available to suggest at least two potential physiological effects.

Melatonin as a Maternal to Fetal Zeitgeber

Prior to the time that circadian rhythms can be measured in the developing animal, it appears that the endogenous oscillator located in the suprachiasmatic nuclei is functional. This was first suggested by Moore [20] who hypothesized that detection of circadian rhythms is limited by maturation of the other neural structures necessary for the overt expression of the rhythms. Recently, Fuchs and Moore [7] have shown that the suprachiasmatic nuclei of the neonatal rat are capable of manifesting oscillatory metabolic activity on postnatal day 1. Furthermore, morphological studies in the rat indicate that most of the suprachiasmatic neurons have formed by the 18th day of embryonic life [15]. Thus, the question arises as to whether the suprachiasmatic nuclei are functional during fetal life.

Experimental evidence from an intriguing series of studies by Deguchi [3-5; this volume] suggests that indeed the fetal suprachiasmatic nuclei are functional. In addition, he found by monitoring the pineal *N*-acetyltrans-

ferase rhythm in the developing rat that the phase of the endogenous oscillations generated by the developing biological clock can be coordinated by the mother during both fetal and neonatal life. This set of observations, along with the evidence for transfer of a maternally generated melatonin rhythm suggests that maternal melatonin may be a chemical signal that acts to coordinate the phase of the developing biological clock. It is important to note, however, that there may be a number of small circulating maternal hormones, in addition to melatonin, that could act in concert to coordinate developing circadian rhythmicity.

Influence of Maternal Melatonin on the Developing Reproductive System

Martin et al. [17; this volume] and *Martin and Klein* [18] have shown in vitro that physiological concentrations of melatonin can inhibit, in a dose-related and specific manner, luteinizing hormone-releasing hormone stimulated luteinizing hormone release from the neonatal rat pituitary gland. This effect of melatonin has recently been confirmed in vivo [19]. An interesting aspect of these studies is that the inhibitory effect of melatonin on pituitary function is age-related and not evident in the adult rat. These observations, coupled with the evidence cited for milk transfer of melatonin, suggest that maternal melatonin may exert an antigonadal effect on the neonatal pituitary gland.

Closing Comments

The evidence cited in this review shows that the mother is a source of melatonin for the developing mammal. Although the precise functions of maternal melatonin have not been firmly established, further investigations of the proposed aforementioned functions of the hormone on development are clearly indicated.

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