Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part A 142 (2005) 297 – 307
CBP
www.elsevier.com/locate/cbpa

Nocturnal patterns and up-regulated excretion of the melatonin metabolite 6-sulfatoxymelatonin in the diurnal rodent *Psammomys obesus* post-weaning under a short photoperiod☆

Alina Neuman [a,c], Yoav Gothilf [b], Abraham Haim [c], Gad Ben-Aharon [a], Nava Zisapel [a,*]

[a] *Department of Neurobiochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel*
[b] *Department of Zoology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel*
[c] *Department of Biology, University of Haifa at Oranim, Haifa, Israel*

Received 28 April 2005; received in revised form 18 July 2005; accepted 19 July 2005
Available online 19 September 2005

Abstract

The ontogeny of daily rhythms in body temperature (T_b) oxygen intake (VO_2) and urinary excretion of the major melatonin metabolite, 6-sulfatoxymelatonin (6SMT) was studied in the day-active rodent, *Psammomys obesus*. Generally, T_b and VO_2 were high during the light phase in this diurnal species. However, after weaning, and only under the short photoperiod, *P. obesus* individuals display elevated T_b and VO_2 levels during the dark phase, as in nocturnally active species. In parallel, 6SMT and nocturnal activity of pineal arylalkylamine *N*-acetyltransferase (AANAT) were greatly enhanced. The cDNA encoding *P. obesus* pineal AANAT was cloned and found to share 90.2% homology with rat and 83.8% with human AANAT, and based on homology modeling, to structurally resemble the ovine enzyme. A robust diurnal rhythm in *P. obesus* pineal AANAT-mRNA was found, with maximal levels at night. AANAT-mRNA levels were not enhanced in the post-weaning phase, suggesting post-transcriptional up-regulation of pineal AANAT activity. The photoperiod-dependent post-weaning change into nocturnal behavior and up-regulation melatonin production (as evidenced from the increase in both 6SMT and AANAT activity) represent a hitherto unobserved pattern of transition of a diurnal mammal into independent life. Possibly, this pattern may be physiologically important to facilitate T_b maintenance in the cold nights of winter in the desert.

Keywords: Circadian; Temperature; Rhythm; Pineal; Arylalkylamine *N*-acetyltransferase; Diurnal; Melatonin; Weaning

1. Introduction

All living organisms exhibit profound changes in physiological and behavioral variables during the 24-h day/night cycle. The production of the pineal hormone melatonin at night provides daily (clock) and seasonal (calendar) signals to the organism (Reiter, 1993). Because of its role in regulation of core body temperature (T_b) and activity–rest cycles in mammals (Arendt, 1995; Cagnacci et al., 1997; Saarela and Reiter, 1994), melatonin production may be physiologically important in young age, before T_b homeostatic regulation is fully established.

Melatonin is synthesized by the sequential action of two enzymes, arylalkylamine *N*-acetyltransferase (AANAT) and hydroxyindole-*O*-methyl-transferase (HIOMT) (Axelrod and Weissbach, 1961). Large changes in melatonin production are typically associated with similar changes in the activity of AANAT, which controls the rate at which serotonin is converted to *N*-acetyl-serotonin (see Ganguly et al., 2002 for review). The SCN-regulated increase in release of noradrenalin, from the sympathetic fibers in the pineal at night, causes an increase in cAMP and subsequently a robust increase in enzymatic activity of AANAT and melatonin production (Coon et al., 1995). AANAT activity declines rapidly upon exposure to light in the

☆ The nucleotide sequence reported in this paper have been submitted to GenBank with accession number DQ018371.

* Corresponding author. Tel.: +972 3 6409611; fax: +972 3 6407643.
E-mail address: navazis@post.tau.ac.il (N. Zisapel).

1095-6433/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2005.07.005

middle of the night (Klein and Weller, 1972). Consequently, the level of circulating melatonin is high at night and low during the day and serves as a diurnal and seasonal time cue in the organism (Reiter, 1993). Because of its rapid metabolism ($T_{1/2}$ 17–40 min) melatonin levels in the circulation decline rapidly and, consequently, the excretion of the major melatonin metabolite 6-sulfatoxymelatonin (6SMT) in urine closely reflects the timing and amount of melatonin production (Arendt, 1995).

The ontogeny of the melatonin rhythm has been studied in Syrian and Siberian hamsters, rats and humans (Ellison et al., 1972; Kennaway et al., 1992; Pfeffer and Stehle, 1998; Sivan et al., 2001; Tamarkin et al., 1980; Waldhauser et al., 1998). In hamsters and rats the melatonin rhythm was first detected during the second week of life (Tamarkin et al., 1980; Rollag and Stetson, 1981; Sato et al., 1989). Nocturnal melatonin synthesis peaks by the time of weaning (postnatal day 21) and is not significantly altered afterwards (Tang and Pang, 1988; Tamarkin et al., 1980). In humans, the melatonin rhythm is first detected at 6–8 weeks postnatal age (Kennaway et al., 1992). The nocturnal production then increases to level off at ca. 1 year of age when the pineal reaches its full capacity (Waldhauser et al., 1998).

We have recently reported on annual rhythms in the amount of 6SMT excreted in urine in infants (Sivan et al., 2001). Short photoperiod born infants excreted at the age of 8 weeks, on average, only one third of 6SMT compared with long photoperiod born infants. As 6SMT rhythm closely reflects melatonin production in the pineal gland (Arendt, 1995), these changes were thus interpreted in terms of seasonal changes in melatonin production in infants. Because melatonin lowers T_b in diurnally active and elevates T_b in nocturnal animals (Cagnacci et al., 1992; Stock and Rothwell, 1986; Zisapel et al., 1998), seasonal variations in melatonin production would presumably impinge on the ability of a diurnal mammal to maintain T_b homeostasis during the first stages of independent life under the cold nights in winter.

The fat sand rat, *Psammomys obesus*, is a diurnal desert-dwelling rodent (Harrison, 1991). Here we describe the daily rhythms in T_b, oxygen intake, urinary 6SMT excretion, pineal AANAT activity and mRNA in post-weaning and adult *P. obesus*, under short and long photoperiods.

We show that under short photoperiod, the 6SMT excretion in *P. obesus* is temporarily enhanced post-weaning and the animals display nocturnal activity patterns during this period. We hypothesize that these patterns have physiological implications with respect to body temperature homeostasis in the young.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC). Fat sand rats (*P. obesus* Crezschmar, 1828) from our breeding colony (University of Haifa-Oranim) were used. The animals were grown under short photoperiod (8 h light/16 h darkness cycles; lights on at 06:00) or long photoperiod (16 h light/8 h darkness cycles; lights on at 02:00) at a constant ambient temperature of 30±1 °C. Cool-white fluorescent illumination was used during the light hours while red dim light was kept on constantly. Each individual was kept in a separate cage bedded with sawdust and supplied with pellets (Coplek low calorie *Psammomys* pellets) and fresh *Atriplex halimus* leaves as a water source. Unless otherwise stated pups were separated from their mothers on day 21 after birth. Assessments were performed after weaning (age 35–42 days; body mass 50–80 g) and in the adult (age>60 days; body mass>110 g). In some cases assessments were performed before weaning (age 21–28 days; body mass 40–50 g). In these specific studies, the pups were separated from the mothers on day 20 after birth and measurements were conducted on day 21 after birth.

2.2. Oxygen consumption (VO_2) daily rhythms

Measurements were carried out inside metabolic chamber (2000 mL), using an open flow system (Depocas and Hart, 1957; Hill, 1972), with a flow of dried air (Silica gel, TamRod) at a rate of 1200 mL/min. The metabolic chamber was submerged into a regulated water bath (Neslab) with a connected Exocal system for cooling the water. O_2 concentration of the dried ex-current air was determined by using an Applied Electrochemistry A3 oxygen analyzer monitored on a Tek-Dyn-712 recorder and results were further processed by computer. Tested individuals were offered bedding, special low calorie rat pellets ad libitum and carrots as a source of moisture. Individuals were kept for 72 h of which the first 24 h were for habituation and 48 h for VO_2 recording as described earlier (Haim and Zisapel, 1995).

2.3. Body temperature (T_b) daily rhythms

Body (rectal) temperatures were measured using a copper-constantan thermocouple, and monitored on a TH-65 Wescor thermometer. T_b was recorded over 30 h at 6-h intervals. The thermocouple was inserted to a depth of 3 cm for no more than 30 s, while the tested individual was kept in a cotton bag. We favored this method over implanting transmitters as it has no long-term effect on the animal's physiology (Adams et al., 2001).

2.4. 6-Sulfatoxymelatonin excretion rhythms

Measurement of 6-sulfatoxymelatonin (6SMT, the major melatonin metabolite which accounts for 85–90% of the circulating melatonin) in the urine is a reliable and convenient method for assessing melatonin rhythms and is extremely useful in small animals that cannot be bled frequently (Haim and Zisapel, 1997). To assess urinary