6-Sulphatoxymelatonin secretion in different locomotor activity types of the blind mole rat

**Spalax ehrenbergi**

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Abstract: 6-Sulphatoxymelatonin (aMT6S) excretion was examined in the urine of rhythmic and arrhythmic blind subterranean mole rats (Spalax ehrenbergi) to test the correlation between melatonin secretion (as represented by aMT6S) and variability in circadian locomotor activity. Activity pattern was tested in four males, first for a week under short photoperiod [light:dark (LD) 10:14], followed by 10 days in constant darkness (DD). After several months the experiment was repeated under long photoperiod (LD 14:10), followed by DD conditions. Under LD conditions all animals exhibited aMT6S excretion during the dark phase, with a decline just before the onset of light. No correlation was found between activity pattern and melatonin secretion. The animal with the highest melatonin secretion both under LD and DD had an arrhythmic locomotor pattern. The results suggest that in mole rats melatonin secretion and circadian locomotor activity are controlled by two different mechanisms. There were large differences in the aMT6S levels among individuals, suggesting the importance of duration of melatonin secretion over amplitude for gonadal development and thermoregulatory changes. During summer, i.e., before the breeding season, the animals keep a more stable aMT6S secretion than in winter, and the amplitude of secretion is higher under DD vs. LD conditions.

**Introduction**

In mammals melatonin is rhythmically synthesized in the pineal gland and is secreted during hours of darkness into the bloodstream. The hormone influences the circadian and seasonal timing of a variety of physiological and behavioral processes [Arendt, 1995]. Melatonin regulates the reproductive alteration in response to changes in day length in seasonally breeding vertebrates. The role of melatonin within mammalian circadian systems has long been postulated, largely through circumstantial associations [Rusak, 1982; Cassone, 1990].

Pinealectomy has little effect on the circadian rhythms of rats and hamsters [Cassone, 1990; Steinlechner, 1996]. On the other hand, injection of melatonin at the same time every day entrains the circadian locomotor patterns of free-running rats in constant darkness [Redman et al., 1983]. In humans, the administration of melatonin at specific times significantly increases fatigue and sleepiness [Dollins et al., 1994; Terlo et al., 1995]. A specific 2-[125I]iodomelatonin binding has been identified by in vitro autoradiography in the hypothalamic suprachiasmatic nucleus (SCN), the site of the circadian clock, of several mammals [Vanecek et al., 1987; Cassone, 1990; Reppert et al., 1994]. The circadian effects of melatonin appear to be mediated by melatonin receptors in the SCN [Reppert et al., 1994].

The effect of melatonin administration on circadian behavior, its effects on SCN function in vivo and in vitro, and the distribution of 2- [125I]iodomelatonin binding within the mammalian SCN, point to some role for the hormone in the circadian
domain [Cassone, 1990]. In the blind mole rat, Spalax ehrenbergi, we found a natural variability in the circadian locomotor activity (i.e., rhythmic and arrhythmic phenotypes among individuals [Ben-Shlomo et al., 1995]). Thus, mole rats can provide an opportunity to test the correlation between melatonin secretion and circadian activity without using any invasive procedure.

The mole rat, Spalax ehrenbergi, is a solitary subterranean mammal that spends its life in underground burrows [Nevo, 1991]. Mole rats are totally blind; structural and molecular investigations of the atrophied eye, however, suggest a functional role for the retina in light perception [Sanyal et al., 1990; de Jong et al., 1990; Cooper et al., 1993a,b]. The animals do perceive photoperiodic changes [Haim et al., 1983; Pevet et al., 1984; Rado et al., 1991]. Acclimation of cold sensitive individuals to a short photoperiod increases their thermoregulatory capacity in cold conditions [Haim et al., 1983], and the effects of melatonin administration
on body temperature of mole rats kept under a long photoperiod simulate the effects of a short photoperiod [Heth et al., 1986]. In Spalax, the severe regression of thalamic and tectal structures involved in the perception of form and motion is coupled to the hypertrophy of structures subserving photoperiodic functions [Cooper et al., 1993a,b].

Adult mole rats exhibit a multiphasic mode of activity [Nevo et al., 1982], both diurnally and nocturnally. Free-running locomotor activity experiments showed that more than 20% of the individuals were arrhythmic, and only 27% were totally rhythmic [Ben-Shlomo et al., 1995]. Mole rats have one breeding season per year, from December through March [Nevo, 1991]. In the present study we examined the secretion of 6-sulphatoxymelatonin, the chief metabolite of melatonin, in rhythmic and arrhythmic mole rats in long photoperiod (summer), short photoperiod (winter), and free-running light regimes.

Materials and methods
Activity pattern

The examination of circadian locomotor activity patterns was conducted on adult mole rats that had...
been captured in the field (Carmel Mountains, Dalia population, 2n=58 chromosomal species) during autumn, after at least 4 weeks of acclimatization to laboratory conditions. The animals were singly housed under a short (LD 10:14) photoperiod, at constant room temperature of 20°C, and were fed with carrots. The test apparatus simulated an underground runway of a solitary occupant in which the locomotor activity was continuously monitored using light beams [for details, see Ben-Shlomo et al., 1995]. In the present study the activity pattern was tested in four males, first for a week under short photoperiod (LD 10:14), followed by 10 days of free run in constant darkness [DD, (during December 1993-January 1994)]. After several months (in July-August 1994), during which the light regime in the animal facility followed natural conditions, the experiment was repeated under a long photoperiod (LD 14:10) and DD conditions.

Three animals were tested in both experiments. One animal died during the first experiment and was replaced in the long photoperiod experiment. Another animal died during the second experiment. For urine collection, the animals were placed in metabolic chambers supplied with food. Urine samples of each animal were collected every hour for an entire day under LD (following a minimum of a week of recording activity) and DD (following a minimum of 10 days of free run) conditions. The total urine collected from each animal was unusually high (41–118 ml/24 hr in the short photoperiod and 19–52 ml/24 hr in the long photoperiod). The samples were centrifuged and frozen (at -80°C) until the 6-sulphatoxymelatonin analysis.
Radioimmunassay (RIA), using standard tracer and antibody (Stockgrand, Ltd., Guildford, UK), following Aldous and Arendt [1988]. Fifty microliter mole rat urine was assayed in duplicates and calculated against the aMT6s standard curve that was measured in buffer and triplicates. A standard curve was performed with additional 50 µl daytime urine, and there was no change in binding behavior. When the urine samples showed too high amounts the determination was repeated with 5 µl urine. The amounts of aMT6s in urine were calculated to the whole hourly fraction volume. The intraassay coefficient of variation was 5.96%, calculated from ten samples of 10 pg. The interassay coefficient of variation was 8.52%, calculated from ten RIAs.

Discussion

While nocturnal and diurnal species have quite different phase relationships with the day/night cycle, the internal structure of the circadian timing system and the nocturnal production of melatonin within the pineal gland are similar [Reiter, 1991]. Other reported sites of melatonin synthesis are the retina, the Harderian gland, the gut, and blood platelets. These, in mammals, are not believed to make a major contribution to melatonin level in blood but may be of local importance [Arendt, 1995]. In mole rats that show a polyphasic activity pattern, both diurnally and nocturnally, melatonin production increases during the dark phase and decreases just before light onset. This supports the earlier conclusion that mole rats do perceive light [Haim et al., 1983; Sanyal et al., 1990; Cooper et al., 1993a,b]. In blind rats or blind humans the rhythm of melatonin free-runs with a period slightly greater than 24 hr [Reiter, 1991; and references therein]; under these conditions the peak pineal melatonin content coincides with high locomotor activity. In the naturally blind mole rats no such correlation has been found: in constant darkness, as well as in light/dark cycles, there was no correlation between the level of activity and the level of melatonin.

The influence of the pineal gland and melatonin secretion on mammalian locomotor activity is still under dispute. Although pinealectomy has little effect on the circadian rhythm of rodents, melatonin appears to act directly through the SCN receptors to entrain circadian rhythms [Cassone, 1990; McArthur et al., 1991; Reppert et al., 1994]. Melatonin can induce sleep in humans; however, this effect is likely distinct from the effect of melatonin on circadian rhythms and it may occur through thermoregulation [Dollins et al., 1994; Reppert and Weaver, 1995].

In the mole rat we found regular aMT6s secretion during the dark phase, regardless of the circadian type of the tested animals. Both rhythmic and arrhythmic animals showed a nocturnal increase in melatonin production. Hence, circadian locomotor activity seems to be controlled by a different mechanism than melatonin secretion. It is suggested that although the rhythmic source of melatonin may be essential for circadian organization in mammals, it is not sufficient for the generation of circadian locomotor activity in mole rats.

Reppert et al. [1994] detected melatonin receptor mRNA in the SCN of Siberian hamsters and rats. In both species it appeared that melatonin receptor mRNA was distributed throughout the SCN. Melatonin receptor gene expression in the SCN of two rodent species in which melatonin has clear circadian effects strengthens the notion that melatonin acts directly through the SCN receptors to modulate circadian rhythm [Reppert et al., 1994]. In our experiment two output parameters of the SCN were 247
measured: locomotor activity and pineal melatonin secretion. The fact that one of these outputs can be rhythmic while the other is arrhythmic suggests that the SCN itself remains intact, and that the two components are controlled independently. It would be of interest to compare the existence of melatonin receptors in the SCN of different locomotor activity types of mole rats.

One of the well-documented physiological roles of melatonin is its regulation of seasonal responses to changes in day length in seasonally breeding mammals [Hoffmann, 1985; Reiter, 1991; Steinlechner and Niklowitz, 1992]. The nocturnal secretion of melatonin, which provides a measure of night length, regulates seasonal changes in reproductive status. The photoperiodic responses are dramatic in some mammals and include changes in reproductive status, body weight, coat color, and behavior.

There are three alternative explanations to the seasonally appropriate reproductive, metabolic, and behavioral responses [Reiter, 1991; and references therein]. The most common explanation is the duration hypothesis, which claims that it is the duration of the elevated nocturnal melatonin that determines its ability to modify endocrine physiology. The second explanation is that of internal coincidence, claiming that there are two important rhythms for determining the response of a given system to melatonin: the melatonin cycle and the rhythm in sensitivity. In this model the duration of the period of elevated melatonin is important only to the extent that it increases the chance that the elevated melatonin will overlap with the time of sensitivity. The third explanation is the amplitude hypothesis, which claims that the action of melatonin depends on the magnitude of the nocturnal melatonin peak.
Bartness et al. [1993] used the time infusion paradigm to show that the duration of melatonin stimulation is critical for both inhibitory and stimulatory effects. The results of the present study support the conclusion of the importance of the duration of secretion: there is very high variability in amplitude between different mole rats. While one animal showed a considerable difference in amplitude between photoperiods (Fig. 1c), another animal exhibited similar amplitudes (Fig. 2). Moreover, the differences in amplitude between animals were higher than the differences between photoperiods in the same animal. On the other hand, the duration of secretion depended on the length of the dark phase in all animals.

Mole rats spend their life in underground borrows and rarely come to the surface [Nevo, 1991]. During the dry and hot summer, the tunnel system and nest are deepened, particularly in deep soils, and both digging and food hoarding activities are reduced, though they do not stop completely. It is not yet clear how frequently the animals sample the length of the day above ground and whether the photoperiod is sensed in their burrows; however, in the lab mole rats react primarily to changes in the photoperiod, not to temperature [Haim et al., 1983]. Transfer mole rats to a short photoperiod, without changing the ambient temperature, caused a significant increase in the resistance to cold. Yet a drop of 5°C in the ambient temperature, and the extension of the photoperiod, decrease significantly the resistance to cold. Thus, the change in day length may be the cue for seasonal changes. An approach to the surface to sample day length should be relatively rare. However, during the summer, the estimation of day length may be important to initiate the development of the gonads before the mating season.

The photoperiodic system in the mole rats, sustaining appropriate reproductive and thermoregulatory responses, has been selectively expanded [Cooper et al., 1993a,b]. The results of the present study showed that while during a short photoperiod (simulated winter), after 10 days of DD, there was no measurable melatonin secretion (Fig. 2 and in an additional animal; data not shown); in a long photoperiod (simulated summer) the secretion continued after 12 DD days. Moreover, the level of melatonin secretion during DD following a long photoperiod was higher in both animals (up to more than two-fold) than during the former LD light regime (Figs. 1c, 2). These results, although based on only four animals and are thus preliminary, suggest the existence of additional factors that affect the duration and level of melatonin secretion.

Melatonin secretion in the blind mole rat

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Literature cited

Ben-Shlomo et al.


