

# CIRCADIAN RHYTHM IN PINEAL MELATONIN CONTENTS IN PLATEAU PIKA (*OCHOTONA CURZONIAE*) \*

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## Abstract

Pineal melatonin contents exhibited a circadian rhythm in plateau pikas (*Ochotona curzoniae*) maintained in natural light conditions ( $p < 0.001$ , the mean nighttime value vs. the mean daytime value). The results suggest that pika's pineal gland is sensitive to the photoperiods.

**Key words** Plateau pika (*Ochotona curzoniae*); Pineal gland; Melatonin; Circadian rhythm

There is a circadian rhythm in the production of the pineal hormone melatonin in all mammalian species (Reiter, 1990). Production is stimulated by darkness and inhibited by light. This provides a signal reflecting the cyclic changes of environmental lighting. In seasonally breeding mammals that use changes in the photoperiod to time their reproductive cycles, temporal signals to the reproductive system are controlled by the daily rhythm in melatonin production (Tamarkin et al, 1985).

Being a small endemic mammal in Qinghai — Xizang Plateau, plateau pika (*Ochotona curzoniae*) shows typical characteristics of seasonal reproduction (Wang et al, 1991). The present studies were performed to determine daily pattern of pineal melatonin content in plateau pikas maintained in natural light conditions and provided information about the role of pineal melatonin in regulating the seasonal reproduction.

## MATERIALS AND METHODS

**Animals:** Two groups of plateau pika were caught in the field of Guinan county, 3200m in altitude, Qinghai province respectively in January and October, 1991. They were sent to Northwest Plateau Institute of Biology, Academia Sinica (in Xining city) and housed in ironwire cages individually in a room with natural light through the windows. Food (carrot and cabbage) were supplied *ad libitum*.

**Collection of Pineal Glands:** In the first experiment, eighteen adult pikas (weigh-

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ing 122–164 g ) were chosen and sampled at 02:00, 09:00, 12:00, 18:00, 22:00 and 24:00 h through a 24-hour period on the 1st day of February. While in the second experiment, sixty adult animals ( weighing 102–153 g ) were chosen and sampled at 03:00, 06:00, 09:00, 12:00, 15:00, 18:00, 21:00 and 24:00 h through a 24-hour period on the 23th day of October. Three days after arrived in the institute, animals were sacrificed by decapitation at the time indicated above. If that occurred at the dark period, pikas were exposed to dim red light ( 10–20 lux ) for 1 minute prior to decapitation. Pineal glands were rapidly removed, immediately frozen in liquid nitrogen and stored at  $-20\text{ }^{\circ}\text{C}$  for the later analyses.

**Measurement of Melatonin:** Each pineal gland was homogenized by sonication ( Sonics and Materials INC. ) for 5 sec in 0.11 ml chilled perchloric acid ( 0.1 M, containing 0.1 % ascorbic acid ). Pineal melatonin was measured by high-performance liquid chromatography ( Varian 5000 ) with fluorimetric detection ( Shimadzu RF-530 ) described by Wakabayashi and Shimada ( 1986 ). The recovery rate of the pineal melatonin was  $96.2 \pm 3.2\%$ .

## RESULTS

### 1. Pineal Melatonin in February

In the first experiment, pineal melatonin exhibited a marked circadian rhythm in pikas maintained in the natural light conditions in February ( Fig. 1 ). Daytime levels

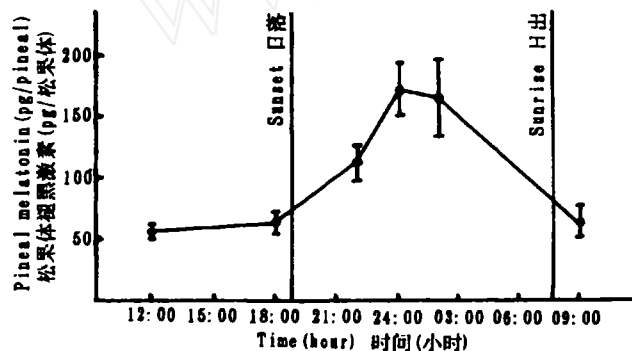


Fig. 1 Pineal melatonin contents throughout a 24-hour cycle in plateau pika exposed to natural light condition in February. Data are presented as the mean  $\pm$  SEM from two to six determinations.

图1 2月份自然光照条件下高原鼠兔松果腺中褪黑激素含量的昼夜变化。数值表示2-6个样品的平均值。

of pineal melatonin with 56–64 pg/gland rose to contents of 113–170 pg/gland during the dark phase (  $p < 0.001$  vs. the mean daytime value ). The evening increasing of the pineal melatonin content began at about 2–3 hrs after the sunset (  $p < 0.01$ , the mean value at 22.00 h vs. the mean daytime value ). Then the pineal melatonin contents increased more remarkably to the nocturnal peak with a content of  $169.7 \pm 25.2$  pg/gland (

mean  $\pm$  SEM,  $n=6$ ) at 24.00 h, 5 hrs after the sunset. The morning decline mainly occurred between 02.00 and 09.00 h. And then, the pineal melatonin content dropped to the lowest value  $55.5 \pm 1.0$  pg/gland (mean  $\pm$  SEM,  $n=2$ ) at 12:00 h ( $p < 0.05$  vs. other daytime values).

## 2. Pineal Melatonin in October

In the second experiment, pineal melatonin contents also exhibited a marked circadian rhythm in plateau pikas caught in October (Fig. 2). Daytime levels of pineal melatonin with 77–119 pg/gland rose to contents of 139–505 pg/gland during the dark phase ( $p < 0.001$  vs. the mean daytime value). The evening increasing of the pineal melatonin contents began at about 2–3 hrs after the sunset ( $p < 0.01$ , the mean value at 21.00 h vs. the mean daytime value). The increasing speed of pineal melatonin content from 18.00 to 21.00 h was 15 pg/hr. Then the pineal melatonin content increased more remarkably to 422 pg/gland at 24.00 h with a speed of 94 pg/hr. After that, it reached to the nocturnal peak with  $505.2 \pm 31.9$  pg/gland (mean  $\pm$  SEM,  $n=8$ ) at 03.00 h, 8.5 hrs after sunset. It was about five times of the mean daytime value. Following the peak, the pineal melatonin content declined significantly from 03.00 to 06.00 h ( $P < 0.001$ ). After falling to 119 pg/gland at 09.00 h, the melatonin content dropped to the lowest value:  $77.1 \pm 10.9$  pg/gland (mean  $\pm$  SEM,  $n=7$ ).

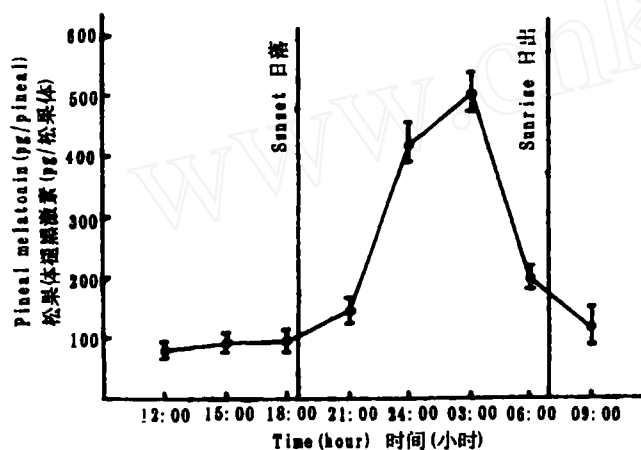


Fig. 2 Pineal melatonin contents throughout a 24-hour cycle in plateau pika exposed to natural light condition in October. Data are presented as the mean  $\pm$  SEM from seven to eight determinations.

图2 10月份自然光照条件下高原鼠兔松果腺中褪黑激素含量的昼夜变化。数值表示7-8个样品的平均值。

## DISCUSSION

The results of this study clearly indicate that a diurnal melatonin rhythm is present in pineal gland of the adult plateau pika. In addition, the daily patterns of pineal melatonin contents in plateau pikas maintained in natural light conditions showed some similarities in both February and October. They both exhibited pronounced circadian rhythms with the similar time of the lowest value (at 12.00 h) and the nocturnal peaks (24.00

or 03.00 h ). And also, both of the beginning time of the evening increasing in pineal melatonin contents were at 2—3 hrs after the sunset.

Some differences between the melatonin rhythms appearing in the two experiments were detected. (1) The mean pineal melatonin content was higher during October than that during February, and this difference was statistically significant ( Table 1 ). (2) The nocturnal peak of pineal melatonin appeared earlier in February ( at 24.00 h ) than that in October ( at 03.00 h ).

Table 1 Comparisons of pineal melatonin contents ( Mean+SEM ) in plateau pika maintained in natural light condition in between February and October

表1 2月份和10月份自然光照条件高原鼠兔松果腺褪黑激素含量(均值±标准误)的比较

Groups 组别	Melatonin content (pg/gland) 褪黑激素含量(微微克/松果腺)		Significant level 差异显著性水平
	In February 2月	In October 10月	
Light phase 白天	60.7±4.0 ( n=6 )	5.8±6.8 ( n=28 )	p < 0.05
Dark phase 夜间	154.6±15.9 ( n=12 )	316.1±30.1 ( n=32 )	p < 0.01
All day 全天	123.3±15.0 ( n=18 )	213.3±21.6 ( n=60 )	p < 0.05

The numbers of samples are in parentheses 括号中的数值为样品数

In general, the mammalian pineal gland generates a daily melatonin signal that can be altered by light in its phase and amplitude. Variations in the duration of elevated melatonin accurately reflect changes in the duration of darkness over most of the changes of natural day lengths ( Tamarkin et al. , 1985 ). The researches on the pineal melatonin in plateau pika showed that the nocturnal peak appeared earlier in February ( at 24.00 h ) than that in October ( at 03.00 h ), but the sunset time in February ( at 18.50 h ) is later than that in October ( at 18.30 h ). So that was not due to the different time in the sunset. When the peak melatonin contents at 24.00 h were compared to that at 02.00 h ( in February ) or 03.00 h ( in October ) individually, they did not showed statistically significant differences ( p > 0.05 ), especially that in February. These results suggested that every one of the three sampling time ( 24.00, 02.00 or 03.00 h ) may occur the nocturnal peak of the pineal melatonin in plateau pika.

The results of higher level melatonin secreted in October probably suggested that there was a stronger inhibiting effect on the neuroendocrine—gonadal axis from the pineal gland in plateau pika during the month than that in February. This is concordance with the reproductive characteristics of the animals in the wild. But the patterns of melatonin production may be similar in short— and long day breeders ( Tamarkin et al. , 1985 ). So it is necessary to test the photo sensitives of the neuroendocrine—gonadal axis in the animals. Plateau pikas live in the alpine meadow with 3 200— 5 200 m in altitude. They display annual cycle of fertility and infertility. The seasonal breeding cycle ensure that offspring, with a short period of gestation; twenty—two days ( Wang et al. , 1991 ), are produced at the time from April to July , that is optimal for survival. All these ecological characteristics mentioned above suggest that plateau pikas are similar to the long—day breeders. While " long day breeders " and " short—day breeders "

are classified depending on whether their gonads are active or inactive as day length increase. The specific strategies are also determined by the gestation period of each species, other aspects of their physiology, and the environment (Tamarkin et al., 1985). So further experiments should be performed to test the reproductive photo-responsivities in plateau pikas.

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## 中文摘要

### 高原鼠兔松果腺褪黑激素含量昼夜节律的研究

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自然光照条件下, 高原鼠兔 (*Ochotona curzoniae*) 松果腺褪黑激素 (Melatonin, MLT) 含量呈现明显的昼夜节律 ( $P < 0.001$ , 夜间组含量均值与白天组含量均值差异显著性比较)。在2月份的实验中, 对18只鼠兔(体重122-164克)松果腺的采样时间分别为02.00, 09.00, 12.00, 18.00, 22.00和24.00时。白天 MLT 含量波动为56-64微微克/松果腺, 夜间波动为113-170微微克/松果腺。夜间 MLT 含量高峰值出现在24.00时。在10月份的实验中, 对60只鼠兔(体重102-153克)松果腺的采样时间分别为03.00, 06.00, 09.00, 12.00, 15.00, 18.00, 21.00和24.00时。白天 MLT 含量波动为77-119微微克/松果腺, 夜间波动为139-505微微克/松果腺。夜间 MLT 含量高峰值出现在03.00时。将2月份和10月份高原鼠兔松果腺 MLT 含量进行差异显著性比较, 10月组显著高于2月组 ( $P < 0.05$ )。结果表明, 该动物的松果腺本身对光周期具有敏感性, 它能够感知环境光周期的变化, 成功地完成神经内分泌的转换。

**关键词** 高原鼠兔; 松果腺; 褪黑激素; 昼夜节律