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Research Article

Response of the Pineal Gland in Rats Exposed to Three Different Light Spectra of Short Periods

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Abstract: The pineal gland is a part of the photo-endocrine system. Photon energy is important for the function of this system, and affects the level of nocturnal melatonin. In mammals light-induced suppression of melatonin production is dependent on the intensity and wavelength of the light used. We studied the chronic effects of light wavelengths on the pineal gland in rats after exposure to a short photoperiod using 3 different light spectra with the same irradiance. Male Sprague-Dawley rats were used. The animals were divided into 5 groups. They were exposed 8/16 L:D periods in rooms under automatically regulated light and climate. Lee filters were used for the light spectra application. From animals at the age of 6 months blood samples were taken over a week at the third hour of the dark period under dim red light. The pineal glands were processed for electron microscopy. Melatonin levels in the blood were analyzed by ELISA. Melatonin levels were the higher in control group and group 5 (blue light spectra) compared to the others. We demonstrated that blue light spectra have a greater effect on melatonin production in rats.

Key Words: Light spectra, pineal gland, melatonin, short photoperiod

Kısa Fotoperiyodda Üç Farklı Işık Dalga Boyuna Maruz Bırakılmış Sıçanlarda Epifiz Yanıtı

Özet: Epifiz, foto-endokrin sistemin bir parçasıdır. Foton enerjisi bu sistemin fonksiyonu üzerinde çok önemlidir ve günlük melatonin üretimini etkiler. Memelilerde melatonin üretimi ışığın şiddeti ve dalga boyu etkisindedir. Yapılan bu çalışmada erkek Sprague-Dawley türü sıçanlarda kısa fotoperiotta (kış dönemi) ve sabit ışık şiddetinde üç farklı dalga boyunun, uzun süre kullanımda epifiz üzerindeki etkisi araştırılmıştır. Hayvanlar beş gruba ayrılarak 8 saat aydınlık ve 16 saat karanlık olmak üzere ışığı ve ısısı otomatik olarak ayarlanan odalarda büyütülmüşlerdir. Dalga boyları için Lee filtreler kullanılmıştır. Altı aylık yaşa ulaşan hayvanlardan karanlık periyodun 3. saatinde 3 lükslük kırmızı ışık altında bir hafta süre ile kan örnekleri alınmıştır. Kandaki melatonin miktarları ELISA metoduyla ölçülmüştür. Epifizler elektron mikroskobik gözlemler için hazırlanmıştır. Sonuçlarımızda kandaki melatonin değerleri, mavi dalga boyu uygulanan grupta ve kontrol grubunda diğer gruplara oranla yüksek bulunmuştur. Bu nedenle sıçanlarda melatonin üretimi üzerine mavi dalga boyu etkisinin diğer dalga boylarına göre daha fazla olduğunu düşünmekteyiz.

Anahtar Sözcükler: Işık spektrumu, epifiz, melatonin, kısa fotoperiyod

Introduction

The pineal gland is a circumventricular organ situated outside the blood-brain barrier. It is innervated by the fibers of the superior cervical ganglion (1). The gland synthesized melatonin hormone has very different effects on the organism (2). The pineal gland is a part of the photo-endocrine system. Photon energy is important for the function of this system, and affects the level of nocturnal melatonin (3,4). The most important effect of melatonin synthesized by the pineal gland is to have a diurnal rhythm influenced by light and dark periods (5,6). The blood concentration of melatonin increases in the dark period of the circadian rhythm and is inhibited by the light (7,8). Dark and light environments are

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differentiated through the eyes. Color sensation is determined by the wavelength and frequency of visible light, which is defined as an interval, not as color. All the wavelengths defining the color spectrum are present in daylight and their preference for the animal experiments have been determined for albino animal species with photopic (black/white) and scotopic (colored vision) electro-retinograms (9). The investigated animals perceive the light with their eyes and it reaches the hypothalamic suprachiasmatic nucleus via the retino-hypothalamic nerves. The signals are then conducted through the paraventricular nucleus, anterior median cerebral cluster, and reticular formation and medulla spinalis to the superior cervical ganglion. Impulses reaching the ganglion increase or decrease in response to the activity of the pineal gland. The synthesis of serotonin or specific methyl indoles begins or stops via the above mechanism (10,11).

All animal species living in the regions from the equator to the poles have fertile phases where light is the critical environmental factor regulating the seasonal model of coupling. The differences are regulated according to the seasons of summer and winter, and defined long and short photoperiods of days are initiated by setting up constant light arrangements. The synthesis and secretion of melatonin increase in winter months, i.e. in the short photoperiod, and decrease in summer months, i.e. in the long photoperiod (3).

The effect of photoperiod on the metabolism of melatonin and effects of light intensity and wavelengths on photo-periodical changes have been investigated for many species (12-14). The results determining the pineal gland activity are influenced by the light intensity and wavelengths in different ways with respect to the species but at the same level (11,15,16). Melatonin levels in various body fluids have been measured with different techniques (17). In recent years the morphology of the mammalian pineal gland has been extensively studied (1,2). Some investigators demonstrated that pineolocytes showed ultrastructural features, indicating an increased metabolic and synthetic activity of these cells in some species (18-21). The aim of this study was to investigate the long-time effect of light wavelengths on melatonin production and pineolocytes ultrastructure using 3 different light spectra (l max = 670 nm red, l max = 570 nm yellow, l max = 450 nm blue bandwidths).

Materials and Methods

Three generation male Sprague-Dawley rats weighing about 230 ± 250g were housed in clear polycarbonate cages in a windowless room. The cages we screened by metal lids, which caused no selective interference with the light transmission from overhead luminaries. The rats were kept in an air-conditioned room. Room temperature (22 ± 2 ºC) and humidity were automatically regulated. Food and water were supplied ad libitum (12,22). The animals were treated in accordance with the Guidelines for Animal Care and Use of the National Institutes of Health.

The animals was divided into 5 groups. The beginning of the gestation period was marked as day 0. The first born male rats were used in the experiment.

All new-born rats were isolated from their parents at the age of 1 month and subjected to the above protocol of illumination until the age of 6 months.

Group 1 (n = 10): Control (8 h of light: 16 of darkness)

Group 2 (n = 10): Dark (continuous dark period)

Group 3 (n = 10): Red light (Lee filter no: 182)

Group 4 (n = 10): Yellow light (Lee filter no: 101)

Group 5 (n = 10): Blue light (Lee filter no: 183)

Experimental design

The rats were exposed to fluorescent light (36 W) sources of 2500 lux during the experiment. Intensity was measured with a luxmeter (Foot Candle/ Extech instruments) inside the cages. Each cage was well ventilated with fans and coated with special filters. The distribution of the spectral power of the light sources was determined with a Lee filter (Figure 1). Ten animals were housed within each cage and adapted to an 8:16 h light:dark cycle (lights on from 08:00 to 16:00 daily) (15).

Determination of melatonin

When the animals had reached the age of 6 months, blood samples were taken over a week at the third hour of the dark period. At the end of each week, the animals were anaesthetized and intracardiac blood samples and tissues were taken under dim light during the dark period. Plasma samples were stored frozen until the time of assays (12). Melatonin levels were analyzed with ELISA (IBL-Hamburg, Germany) (17).

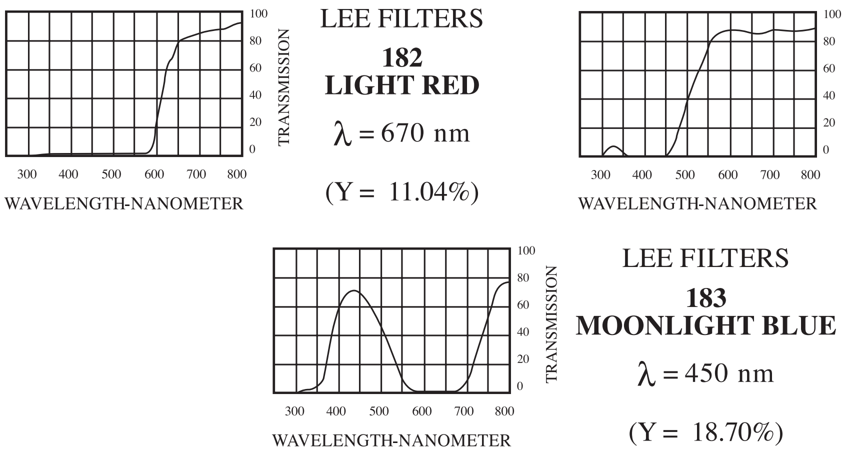

Figure 1. Lee filters used in the experiments.

The pineal glands were processed for electron microscopy (23). The examination was performed in a JEOL JEM 1220 electron microscope.

Statistical analysis

Melatonin levels of animals were compared by one-way ANOVA.

Results

Biochemical Results

We found some differences between the groups. The melatonin blood level was higher in the control group and group 5 than in the others (Table).

There were significant differences between the groups ($F_{4; 43}$ = 7.98 P < 0.01). There were differences between group 1 and groups 2, 3 and 4 and between group 2 and group 5 with respect to melatonin level measurements (P < 0.01), groups of 3, 4 and 5 showed no differences. (P > 0.05).

Histology

We observed a homogeneous population of pinealocytes in the pineal gland of this species. The Golgi apparatus, rough endoplasmic reticulum, mitochondria, lysosomes, clear vesicles, vacuoles containing flocculent material, microtubules and lipid droplets were consistent with the components of the pinealocytes cytoplasm: infrequently observed organelles including "synaptic ribbons". Pinealocytes contain a large number of mitochondria of generally moderate electron density in groups.

Figure 2 shows lipid droplets and mitochondria in the pinealocyte cytoplasm in group 3 (under growing red light spectra). Figure 3 shows in group 4 (under growing yellow light spectra) 2 pineolocyte irregularly shaped nuclei with prominent nucleoluses and small, peripheral clumps of chromatine. Figure 4 shows in group 5 (under growing blue light spectra) numerous mitochondria in pinealocyte cytoplasm.

Table. Melatonin values (μg/ml) in the blood.

Groups	Number of Rats	Mean and Standard Error
1	7	48.00 ± 6.29*
2	12	22.92 ± 3.04
3	11	27.91 ± 2.97
4	8	21.88 ± 2.42
5	10	39.30 ± 4.17*
Total	48	30.95 ± 2.10

Significance level * = P < 0.01

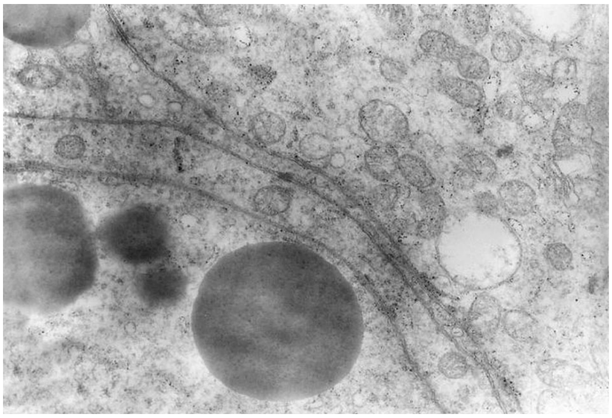

Figure 2. In group 3 (under growing red light spectra) lipid droplets and mitochondria (→) in pinealocyte cytoplasm. X 12.000

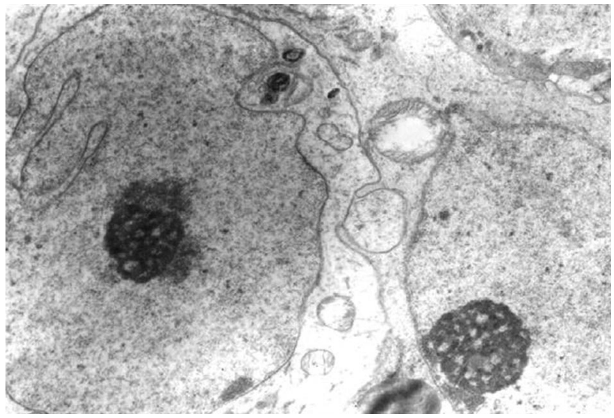

Figure 3. In group 4 (under growing yellow light spectra) two pinealocytes irregularly shaped nuclei have prominent nucleoluses and small, peripheral clumps of chromatine. X 5.000

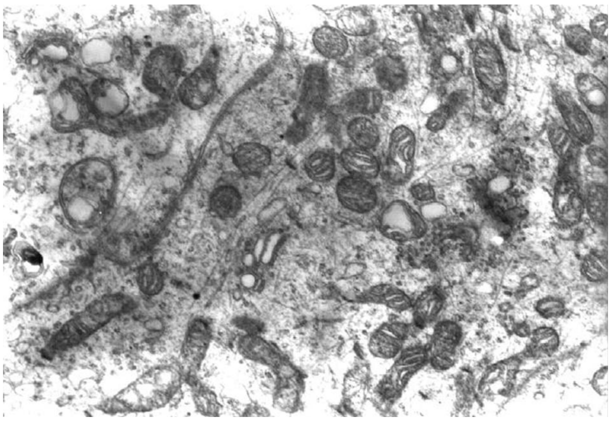

Figure 4. In group 5 (under growing blue light spectra) numerous mitochondria in pinealocytes cytoplasm. X 5.000

Discussion

In most mammalian species, the synthesis and release of melatonin increase at night and decrease with light. Light is the most important environmental factor regulating the melatonin generating system in the gland (3). In earlier studies light of wavelength has been shortly used at wavelengths 510-550 nm, 558-636 nm, 653-668 nm during the period of peak pineal melatonin production, (15) and at 435 nm, 530 nm, 590 nm, 660 nm for 96 hours of exposure to continuous light (22) at different times of light or dark period. The same methods have also been used for studying the effects of light stimuli (7,23,24,). It is reported that the intensity and wavelengths of light have the same effects the activity of the pineal gland (15,25). Furthermore, it is described that the wavelength of light has various effects that vary with the application of wavelengths and circadian phase (11,16,26). In generally, short and long day photoperiods have been used in experimental studies (3,11). It has been demonstrated that a long day photoperiod leads to dynamic procedures while a short day has contrasting effects. Therefore, in this study the method and duration of wavelengths were different.

In our study, in groups a homogeneous population of pinealocytes was present in the pineal gland of this species. The Golgi apparatus, rough endoplasmic reticulum, numerous mitochondria, lysosomes, clear vesicles, vacuoles containing flocculent material, microtubules and lipid droplets were consistent with the components of the pinealocytes cytoplasm: infrequently observed organelles including “synaptic ribbons”. These data correlated with some studies (27). The mammalian pinealocytes contain a large number of mitochondria, the matrix of which is generally of moderate electron density. Some changes in the mitochondria have been described in mammalian pinealocytes after various experimental procedures. According to some authors mitochondria are somehow involved in serotonin synthesis (3). In our study pinealocytes contain a large number of mitochondria which generally of moderate electron density.

Some studies reported that both serum and pineal melatonin concentrations were significantly higher at night than during the daytime and melatonin level was at its maximum in the third hour of the dark period in circulation. Melatonin has annual, seasonal and monthly rhythms as well as a daily rhythm (3). For this reason in this study all blood samples were taken at the same hour and at the third hour of the dark period.

We found some differences between the groups in melatonin levels. The melatonin blood level was higher in the control group and group 5 than in the others. Groups 3, 4 and 5 showed no differences ($P > 0.05$). Recently, several studies reported that novel blue sensitive pigment is not only present but also fully functional in controlling the circadian biological clock in chickens (28) and humans (29). Some studies show that a single photo pigment may be primarily responsible for melatonin secretion, and its peak absorbance appears at 446-477nm or at 459 nm (30). Our data are in good agreement with previous investigations and demonstrated that the response of the pineal glands of the rats to various light spectra was proved quantitatively to be very sensitive to blue light wavelengths of $\lambda_{max} = 450$ nm.

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