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Actionspectra for Circadian Melatonin Rhythms in the Avian Pineal In Vitro

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Abstruct : The avian pineal as well as the retina has been known to contain several types of photoreceptors with different visual pigments such as rhodopsin, iodopsin and the pineal specific opsin, pinopsin. These organs are also known to have circadian clock to regulate melatonin production. Exposure of animals to light causes a decline of the melatonin level and the phase shifts of melatonin rhythms in the pineal and retina. Therefore, the circadian clock system of these organs seem to consist of three elements, i.e., light input, oscillator and melatonin output systems. In birds, it was suggested that rhodopsin might be involved in the entrainment of pineal melatonin rhythms from the action spectrum experiment for controlling NAT activity rhythms. However, there are much more pinopsin-immunoreactive (Pino-IR) cells than rhodopsin (Rho-IR) and iodopsin (Iodo-IR) cells in the avian pineal. We found that Pino-IR cells appeared earlier embryonic stages than Rho-IR and Iodo-IR cells. So, we tried to identify the visual pigments involved in the circadian melatonin rhythms in the pineal and retina. Organ cultured pineals were exposed to monochromatic light to find out which opsin participates in regulation of melatonin rhythms. The action spectra showed a peak at 475nm, suggesting that pinopsin is the major photopigment to regulate melatonin production in birds.

Key words: pineal, avian, embryo, melatonin, organ culture, action spectra.

INTRODUCTION

The avian pineal and retina have been well known to function as the oscillators of circadian locomotor rhythms and to secrete melatonin in a circadian manner which is entrained by light-dark (LD) cycles [1, 2]. These organs consist of three elements, i.e., light input, oscillator and melatonin output systems.

Deguchi [3, 4] reported that rhodopsin might be involved in the entrainment of pineal melatonin rhythms from the action spectrum experiment for N-acetyltransferase (NAT) activity rhythms. However, Okano et al. [5] suggested that pinopsin which was cloned in the chicken pineal might be the photoreceptor pigment for the light-input system because of its abundance. Therefore, we tried to identify the photoreceptor pigments involved in the circadian melatonin rhythms in the pineal and retina. Our previous study in Japanese quail embryos revealed that immunoreactive cells to rhodopsin and iodopsin appeared at embryonic day (E) 13-15 in the retina and at E 14 in the pineal, whereas pinopsin appeared at E 8-9 in the pineal [6]. It was also shown that the action spectra for melatonin inhibition in the pineal and retina of quail embryos indicated a peak at 475nm.

In the present study, we investigated the melatonin rhythms in Japanese quail and chick pineals in vitro. We, then, tried to irradiate the pineals in vitro by monochromatic light in searching the photopigments responsible for inhibition of melatonin production.

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MATERIALS AND METHODS

a: Culture

The chicken eggs were set in an incubator with temperature at 38 ± 0.5°C and humidity at about 50% under light-dark (LD) cycle (LD=12:12). Light (fluorescent lamp) intensity was 450-1300 lux. On embryonic day 19 (E19), 70 chicken embryos were sacrificed by decapitation in the day time, the pineal organs were removed and washed with Hank's salt solution. The pineals were cultured in membrane cups (40 μm pore) on the dishes (IWAKI 6 hole), using medium DMEM: Dulbecco's medium powder (Wakenkagaku), sodium hydrogen carbonate (1.4mg/ml), glucose (0.6%), kanamycine (60μg/ml), pyruvate (5mg/ml). Before culture, 10% fetal bovine serum_calf (Hyclone) was added. The organs were placed in an incubator at 37°C with 95% air/5% CO_2. The culture dishes were maintained in LD=12:12. One ml of the culture medium was taken out for later radioimmunoassay (RIA) and new medium was added every 4 hours.

b: Monochromatic light irradiation

Culture dishes were set in the Okazaki Large spectrograph room [7]. The wavelengths applied were 400, 450, 475, 500, 550nm. Intensity of light was adjusted to be 10.0 μmol/m^2 • s. The medium for 2 hours before irradiation and the medium during 2 hours of irradiation were taken out and stored at -80°C until RIA.

c:Melatonin radioimmunoassay (RIA)

^{3}H labeled melatonin and melatonin were purchased from Daiichi Kagaku and Sigma, respectively. Rabit anti-melatonin, anti-serum and normal rabit serum were supplied by Gunma Univ (ID: H-23, 971126). The upper and lower limit of the assay was approximately 2000 and 16pg/ml, respectively.

RESULTS

The action spectrum experiment using Okazaki large spectrograph revealed a peak at 475 nm for the melatonin rhythms in the chick pineal in vitro (Fig.1).

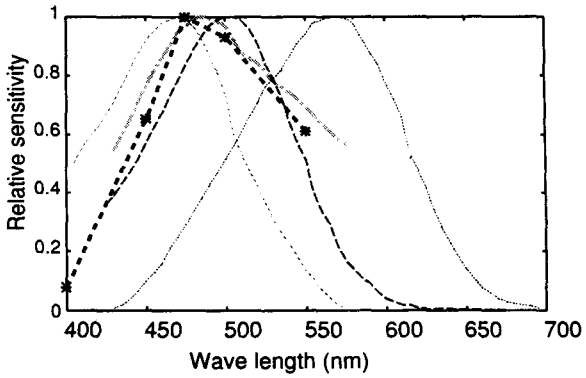

Fig. 1 The action spectrum for acute supression of melatonin synthesis in the pineal of chick embryos (✱---✱). Relative sensitivities of iodopsin (·····) [8] and rhodpsin(------) [9] and pinopsin (·····) [5] were added in the graph.

Pinopsin +Iodopsin+Rhodopsin (-·-·-)

The action spectrum obtained in vitro was similar to that obtained in vivo with a peak at 470 nm [6].

Using a fitting curve including pinopsin, pinopsin was suggested to participate in the major part of melatonin inhibition, and iodopsin and rhodopsin may act minor parts (Fig.1).

DISCUSSION

Two mechanisms have been reported in the melatonin rhythms in the pineal [10, 11]. One is the acute suppression of melatonin which is blocked by pertussis toxin and another is the phase-shifting effect on melatonin rhythms.

Pinopsin was suggested to be the photoreceptive pigment for the quail pineal and blue and green pigments for the retina [6]. Daily rhythmicity in chick pineal NAT activity was found at E18 in incubated embryos under LD=12:12 and LD=16:8 but not in constant darkness (DD) [12].

In the pineal of quail embryos, daily melatonin rhythms appeared at E9, while circadian rhythms in DD appeared at E15-16 just before hatching [13]. In the retina, the photosensitivity and circadian rhythms appeared a few days later than in the pineal. Yamao [6] tried to obtain actionspectrum for direct melatonin suppression in the pineal and retina of quail embryos, and found a peak at 470 nm in both organs. The results suggest that the photoreceptive pigment in the pineal

is pinopsin and that in the retina is blue and green pigment [6].

We confirmed the result in the pineal [6] by in vitro experiment, and estimated the photo pigments involved in the acute suppression of melatonin level by curve fitting analysis.

We suggest that the major photopigment is pinopsin and iodopsin (and rhodopsin) might also be involved in photoreception.

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