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Control of the Rat Pineal Gland by Light Spectra

(melatonin/hydroxyindole-*O*-methyl transferase)

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ABSTRACT Control of pineal hydroxyindole-*O*-methyl transferase (*S*-adenosylmethionine:*N*-acetylserotonin-*O*-methyl transferase; EC 2.1.1.4) by light spectra was determined by placing groups of rats previously housed in continuous darkness under one of seven light sources for 96 hr; rats were exposed to the same intensity of irradiation. Activity of the enzyme was lowest in rats maintained under green light (λ peak = 530 nm); blue and yellow light were somewhat less effective; red and ultraviolet light did not significantly lower the enzyme activity. The suppression of pineal hydroxyindole-*O*-methyl transferase by full-spectrum light sources could be correlated with the proportions of their spectral outputs in the blue–green–yellow range. These observations suggest that the retinal photopigment that mediates pineal responses to light in rats is rhodopsin or another compound with similar absorption properties.

The pineal gland is a neuroendocrine transducer; it converts neural signals that are generated in the retina by light, and transmitted to it by postganglionic sympathetic neurons, into a hormonal output, i.e., the secretion of melatonin (1). Both the morphology and the biochemical activity of rat pineal gland exhibit a marked dependence on the light to which the animal is exposed. For example, pineals of rats kept under continuous light weigh less than those of controls (2), display lower *in vitro* activities of two of the enzymes involved in melatonin biosynthesis: hydroxyindole-*O*-methyl transferase (HO-IndMeTrase; *S*-adenosylmethionine:*N*-acetylserotonin-*O*-methyl transferase; EC 2.1.1.4) (3–5) and serotonin-*N*-acetyltransferase (EC 2.3.1.5) (6), and contain less melatonin (7).

In all published studies on the photic control of the mammalian pineal organ, researchers have manipulated the time of exposure, but have not compared the effects of spectral variations or magnitude of irradiation. Inasmuch as the retinal photoreceptors that mediate vision display characteristic action spectra, it seemed likely that the distribution of spectral power of a light source would also influence the extent to which that source modified pineal functions. By using light sources that emit radiation in specific regions of the visible and near-ultraviolet spectra, we have attempted to define in rats the magnitude of inhibition of pineal HO-IndMeTrase by light spectra. Our studies suggest that the same or closely related photopigments mediate both the visual and the neuroendocrine effects of light in rats.

Abbreviations: HO-IndMeTrase; hydroxyindole-*O*-methyl transferase (*S*-adenosylmethionine:*N*-acetylserotonin-*O*-methyl transferase; EC 2.1.1.4.)

MATERIALS AND METHODS

Sprague–Dawley male rats, 2–25 months old, (230–250 g) were housed in polycarbonate cages covered with screened metal lids that caused no selective interference with the light transmission from overhead luminaires. Rats were kept in an air-conditioned room (20 ± 2°), and given food (Big Red Lab Chow, Mother Hubbard, Lowell, Mass.) and water ad libitum.

Seven experiments were performed, and seven different sets of bulbs were used (Table 1). The distribution of spectral power of the light sources was determined with a double quartz prism spectroradiometer (9).

Groups of rats were initially maintained in continuous darkness for 7 days, and then placed under one of the seven light sources for 96 hr. Rats were exposed to the same intensity irradiation in all seven experiments (65 μW/cm^2), as measured by an Eppley thermopile (Eppley Lab., Newport, R.I.) connected to a model 148 Keithley nanovoltmeter (Keithley Instruments, Inc., Cleveland, Ohio). Irradiation was measured with the thermopile inside the cages and with the wire top in place. In order to achieve equal magnitudes of irradiation, the distance between the cages and the luminaires was varied.

It had been demonstrated that after 96 hr of continuous exposure to cool-white light, the daily variations in the rat pineal HO-IndMeTrase disappear and enzyme activities are low (4, 5). Groups of seven rats were killed by cervical dislocation at the end of the dark period, and after exposure to each light for 96 hr. Pineal HO-IndMeTrase activity was assayed *in vitro* by measurement of the transfer of a [^{14}C]-methyl group from *S*-adenosylmethionine to *N*-acetylserotonin (10). The radioactive methylated product was identified as melatonin by thin-layer chromatography (11). Results were analyzed by analysis of variance (12).

RESULTS AND DISCUSSION

Decline in the activity of pineal HO-IndMeTrase of rats exposed to each light source is shown in Table 1. Green light was most effective in decreasing the activity of HO-IndMeTrase, while light that peaked in the blue or yellow band of the spectrum was 75 and 46% as effective, respectively. In contrast, neither red nor ultraviolet light significantly suppressed the melatonin-forming activity of the pineal organ. The inhibitory activity of cool-white light was greater than that of Vita-Lite, but less than that of green light (Table 1).

It is generally assumed that the sole photoreceptor in the rat retina is the rod and that the sole visual pigment is

TABLE 1. *Spectral dependency of pineal HO-IndMeTrase activity in rats*

Light source	λ peak (nm)	Half-peak bandwidth (nm)	HO-IndMeTrase activity (pmol of melatonin/hr per pineal)		% Inhibition	Significance* t-test
			0 hr	96 hr†		
I. Ultraviolet	360	34	373 ± 32‡	314 ± 14§ II[a], III[a], IV[a], VI[b] VII[a]	16	n.s.
II. Blue	435	54	319 ± 19	137 ± 12 I[a], III[b], IV[b], V[a], VI[a]	57	$P < 0.001$
III. Green	530	45	323 ± 16	76 ± 9 I[a], II[b], IV[a], V[a] VI[a], VII[a]	76	$P < 0.001$
IV. Yellow	590	80	302 ± 13	191 ± 21 I[a], II[b], III[a], V[a] VI[a]	37	$P < 0.001$
V. Red	660	19	325 ± 14	326 ± 16 II,[a] III[a], IV[a], VI[a], VII[a]	—	n.s.
VI. Vita-Lite	¶	¶	327 ± 18	256 ± 21 I[b], II[a], III[a], IV[a], V[a], VII[a]	22	$P < 0.02$
VII. Cool-white	‖	‖	360 ± 19	155 ± 10 I[a], III[a], V[a], VI[a]	57	$P < 0.001$

* Comparing 0 against 96 hr; n.s., not significant.
† Hours of exposure to continuous light.
‡ Mean ±SE, $n = 7$ in each group.
§ Analysis of variance: Roman numerals indicate groups whose means are significantly different and italics designate P value, e.g.; 314 (I) is different from 137 (II), 76 (III), 191 (IV), 256 (VI), and 155 (VII). [a] $P < 0.01$, [b] $P < 0.05$.
¶ Broad-spectrum light source approximating sea level solar radiation (8).
‖ Broad-spectrum light source with very little long-wave and no erythemal ultraviolet radiation (8).

rhodopsin (13). The absorption spectrum of rhodopsin presents a peak at about 505 nm (14–16), and is generally similar in shape to the sensitivity function for the photic inhibition of pineal HO-IndMeTrase observed here. This correlation is evident in Fig. 1, where the decrease in HO-IndMeTrase after exposure of rats to each light source is plotted against change in the absorption spectrum of rhodopsin (14). (The present data do not allow us to discriminate between rhodopsin and other hypothetical photopigments that might also absorb maximally in the green band of the spectrum.)

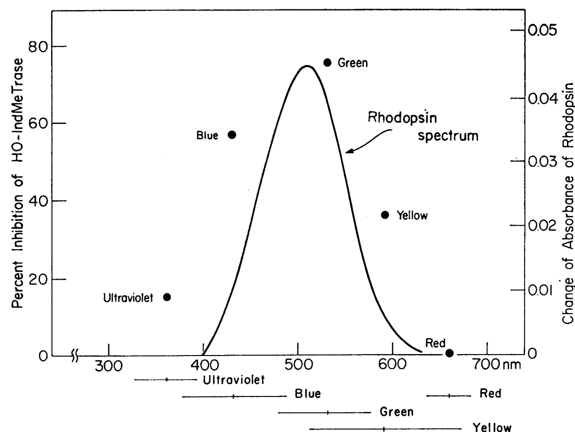

FIG. 1. Spectral dependency for photic suppression of pineal HO-IndMeTrase activity compared with absorption spectrum for rhodopsin (14). *Bars* express λ peak and half-peak bandwidth for each light source.

The significant differences between the effects of exposure to cool-white and Vita-Lite on rat HO-IndMeTrase could be explained in a similar manner, i.e., by consideration of the proportion of the total energy emitted in the spectral band corresponding to the absorption of rhodopsin (58 and 49%, respectively) (17). Rats born and reared under Vita-Lite bulbs were shown to develop larger gonads and smaller spleens than rats raised under cool-white light (8). These differences may also result from the different capacities of the visible radiation from the two light sources to interact with rhodopsin; however, an effect of the ultraviolet radiation emitted by Vita-Lite cannot be ruled out.

The present data, thus, suggest that the curve representing inhibition of rat pineal HO-IndMeTrase by light spectra is similar to that representing absorption spectrum for rhodopsin. Inasmuch as rhodopsin may be the sole visual pigment in the rat (13), our data support the hypothesis that the same photopigment mediates both the visual and the neuroendocrine responses to light.

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