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Melatonin decreases the amplitude of the b-wave of the human electroretinogram

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Abstract. In a double-blind placebo crossover study of 13 healthy volunteers, the pineal hormone melatonin (10 mg) was given at 4 pm, and the electroretinogram measured under conditions of dark and light adaptation. A significant diminution of b-wave amplitude was found under both photopic ($\Delta = 5.4$ μV, $p < 0.05$) and scotopic conditions ($\Delta = 7.4$ μV, $p < 0.01$). These data indicate that melatonin may transduce the dark signal at the level of the retina as well as the pineal. Acute administration of melatonin decreases sensitivity to light.
Key words. Melatonin; human electroretinogram, b-wave.

Introduction.
The pineal hormone melatonin is considered to be an "internal zeitgeber" for the circadian system, transducing the signal of darkness[1]. When given in the late subjective day, melatonin can phase-advance circadian rhythms in certain mammalian species[1]. Melatonin has important functions in the retina: it is involved in retinomotor movements[2], the retinal pigment epithelium cell membrane potential[3], and the regulation of photoreceptor disk-shedding[4]. Additionally, melatonin may also be a key component of the retinal biological clock (see ref. 5).
There is increasing interest in the potential use of melatonin (or its agonists or antagonists) in humans for the treatment of circadian rhythm-related sleep disturbances[6]. We therefore considered it important to elucidate the functional neurophysiological effects of melatonin on the human retina.

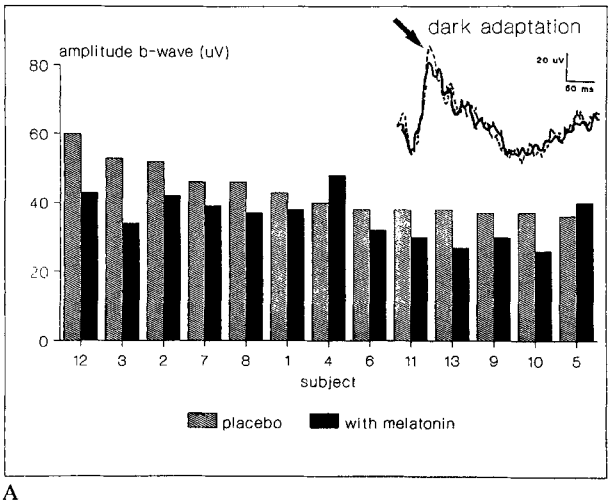
Materials and methods
Healthy subjects signed declarations of informed consent after the nature of the study had been explained. Approval for the oral administration of melatonin had been given previously by the University of Basel Medical School Ethics Committee[7]. The double-blind placebo crossover design used identical melatonin (10 mg) and mannite placebo pills, given to 8 women and 5 men (age range 22–48 years) in random order with an average of 3 days between the experiments. The study was carried out between the end of October and the end of December. The time of oral melatonin administration, 4 p.m., was chosen in view of the narrow temporal band of sensitivity to melatonin in the late afternoon[1]. The first electroretinogram (ERG) was measured after 20 min dark adaptation, at 5 p.m. and the second ERG after a further 20 min light adaptation. Maximum plasma levels occur between 60 and 150 min after oral administration of the formulation of melatonin we used[8].

The ERG was measured with an active electrode on the lower eyelid and a reference electrode at the outer eye[9], with impedances held at <5 kOhm. This technique permits measurement of amplitudes of maximally 100 μV. Stimulation was by means of a red LED-flash of 220 cd/m^2 intensity and 3 ms duration at 1.1 Hz. A Medelec MS 6 averaged every 64 responses with a duration of 512 ms in the frequency range of 0.8–160 Hz. This non-invasive experimental method of ERG registration with low flash intensity under scotopic conditions was chosen in order to reduce the negative effects of classic opthalmological ERG techniques on recruitment and cooperation of the volunteers.

Results and discussion
Analyses were made of the latency and amplitude of the a- and b-wave of the ERG. Melatonin had no effect on the latency of the a- or b-wave (table). There was a significant reduction of the amplitude of the b-wave without any modification of a-wave amplitude (table). This b-wave amplitude reduction occurred in 12 out of

Latency and amplitude of the a- and b-wave of the ERG [mean ± sd]

	Placebo	Melatonin	Wilcoxon
Latency [ms]			
a-wave			
photopic	23.2 ± 2.3	25.0 ± 2.5	ns
scotopic	23.6 ± 2.0	25.8 ± 3.0	ns
b-wave			
photopic	49.5 ± 2.4	49.2 ± 2.9	ns
scotopic	53.8 ± 2.3	54.4 ± 4.2	ns
Amplitude [μV]			
a-wave			
photopic	23.4 ± 2.3	24.8 ± 2.4	ns
scotopic	23.4 ± 2.1	25.4 ± 3.2	ns
b-wave			
photopic	39.9 ± 13.7	34.5 ± 10.4	$p < 0.05$
scotopic	43.4 ± 7.6	35.9 ± 6.7	$p < 0.01$



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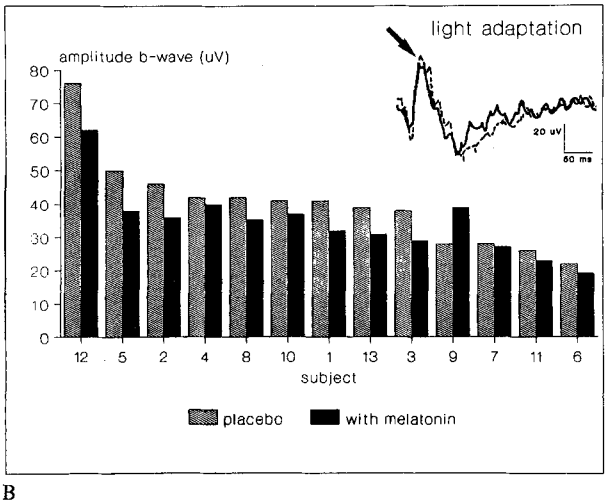

B

Individual ERG b-wave amplitude data from subjects taking placebo or melatonin. Figure A under scotopic conditions, Figure B under photopic conditions. An example of an individual trace of the ERG is shown in the inset (arrow indicates the b-wave), for subject # 2 under scotopic and photopic conditions.

13 subjects under light adaptation, and in 11 out of 13 subjects under dark adaptation (figure). This modified ERG technique does not permit differentiation between rod and cone activity (see similarity of signals under both conditions in figure and table).

This is the first demonstration of a direct effect of melatonin on the human ERG. Acute administration of melatonin in the late afternoon reduced sensitivity to light. In the cat retina a depression of b-wave amplitude has also been reported after melatonin administration[10]. An indirect ERG effect of plasma melatonin elevation – using psoralen administration – was found to be an augmentation of b-wave amplitude[11]. However, psoralen was given in the morning, and it has other photosensitizing effects that may explain this opposite result. Modification of b-wave amplitude has been regularly observed in conjunction with functional changes in the dopaminergic system, not only in animal experiments but also in humans. A diminution of b-wave amplitude is found in Parkinson patients[12], after treatment with dopamine antagonists (e.g. perphenazine)[13]; an augmentation occurs with dopamine agonists (e.g. 1-DOPA, bromocryptine)[13].

Retinal dopamine and melatonin have circadian rhythms opposite in phase. Dopamine turnover is stimulated in a dose-dependent manner by light, and inhibited by darkness or melatonin (see ref. 5). The human ERG has a circadian rhythm in b-wave amplitude, with an increase at the beginning of the subjective day[14,15]. This is the time when, in animal studies, dopamine synthesis and disk-shedding have been found to peak, and when endogenous retinal melatonin is declining[5]. Our finding that exogeneous melatonin can decrease b-wave amplitude in the late afternoon documents an important interaction of melatonin with mechanisms (presumably dopaminergic) generating ERG b-wave amplitude. Thus the postulated role for pineal melatonin as an internal representation of the dark phase[1] may be equally applicable to retinal melatonin[5]. Since melatonin was given at the circadian phase where it induces a phase-advance in some species[1], it remains to be elucidated whether this effect is mediated by a phase-advance of the circadian rhythm of light sensitivity in the retina.

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