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Indoleamines and the UV-light-sensitive photoperiodic responses of the melanocyte network: a biological calendar?

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Abstract. The pineal, serotoninergic and pigmented neurons are associated with light-dependent sleep/arousal, serving as a biological clock with a circadian rhythm. This rhythm is maintained by melatonin which serves to recognise the 'dark' phase. The neural network that responds to seasonal variations in day/night length has not been identified. The present study demonstrates that melanocytes in human skin respond to changes in the duration of UV exposure, and can serve as a biological calendar. These responses are mediated by two indoleamines, serotonin and melatonin. Higher melatonin levels correspond to long nights and short days (short UV pulse), while high serotonin levels in the presence of melatonin reflect short nights and long days (long UV exposure). This response recapitulates the sleep/arousal patterns in animals exposed to large variations in day/night cycle that cause changes in coat colour from pure white in winter to complete repigmentation in summer.
Key words. Serotonin; melatonin; melanocyte network; biological clock; UV light-sensor; dark sensitivity.

In the central nervous system, the pineal, serotoninergic and pigmented neurons are associated with the light-dependent sleep/arousal cycle involving the tryptamine derivatives melatonin and serotonin[1]. In certain animals, it has been observed that skin pigmentation is related to annual hibernation and arousal in areas where the day-night cycles have a large annual variation[2]. This study was performed to assess whether there is a similar relationship between melanocyte function and tryptamine, and its derivatives serotonin and melatonin, in humans.

Materials and methods

Whole skin samples from 54 vitiligo cases were cultured. The tissues were transported in MEM medium. Samples were cut into 3 pieces under sterile conditions. Each piece was 2 mm in width, including the marginal zone between the pigmented and vitiliginous zone. 29 biopsies were incubated in tryptamine, 11 biopsies in serotonin, and 14 biopsies in melatonin (Sigma). One piece from each sample was immediately immersed in cold buffered formol-glutaraldehyde as a control. Incubation was done in micropetridishes, each containing 2 ml MEM and tryptamine 1 mg/ml, serotonin 0.5 mg/ml and melatonin 0.25 mg/ml. All three agents were dissolved directly in MEM tissue culture medium. The second piece was incubated at 37 °C in the dark while the third was exposed to a pulse of 120 s of UV at 2 h and reincubated, to be harvested at 3 h. The UV tube was 15 W, emitting at 280–400 nm. Photometric readings showed 11.85 mA at the point of exposure, 30 cm from the tube.

All tissues were cut into serial frozen sections 5 u in thickness on a Lipshaw Cryostat at − 25 °C and stained with Hematoxylin + Eosin, catecholoxidase activity, and monoclonal antibodies against serotonin and melatonin (DAKOPATS). These antibodies are specific and do not show crossreactivity. The percentage of melanocytes that were positive was calculated, taking into account 5 marginal melanin units in each case.

For comparison, proliferative lesions with highly dendritic melanocytes and melanomas were also stained for the presence of melatonin and serotonin.

Immunohistochemical staining: ABC method. 1) Hydrate slides; 2) Block in 1.5 ml H_2O_2 + 50 ml methanol; 3) Wash with PBS buffer (3 washes) of 5 min each; 4) Apply 4–6 drops of normal swine serum (1:50 with PBS) and incubate for 1 h at room temperature; 5) Apply primary antibody (mouse) and incubate at 2–8 °C overnight; 6) Wash with PBS (3 washes); 7) Apply 4–6 drops of biotinylated swine anti-rabbit antibody (diluted appropriately). Incubate at room temperature for 1 h; 8) Wash with PBS (3 washes); 9) Apply 4–6 drops of avidin biotin complex (mixed and diluted appropriately at least 30 min before use) and incubate at room temperature for 1 h; 10) Wash with PBS (3 washes); 11) Apply substrate-chromogen solution and incubate till desired colour intensity has developed (DAB-2–5 min); 12) Rinse with distilled water; 13) Counterstain with haematoxylin; 14) Cover with coverslip.

Statistical analysis was done to show the significance of these data and are indicated in the graphs.

Results

Melanocyte morphology and melatonin-serotonin positivity

Serotonin. On incubation with serotonin, the melanocytes are positive for serotonin (fig. 1) in the dark with

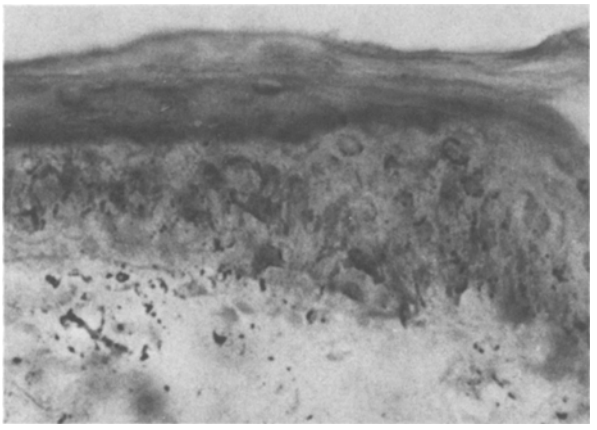

Figure 1. Marginal melanocyte demonstrating serotonin positivity on incubation with serotonin (mABSer × 400).

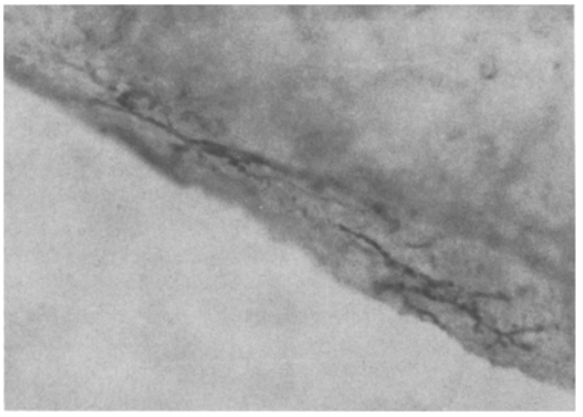

Figure 2. Marginal melanocyte showing melatonin positivity, on incubation with melatonin (mAb Melt × 400).

a prominent increase on UV exposure. Very low basal levels of melatonin are seen. The control serotonin is 8.3%, melatonin is 16.7%. On dark incubation the serotonin increases to 33.3% ($p < 0.01$) while melatonin levels do not change. On UV exposure the cells are 70% ($p < 0.001$) positive for serotonin and 25% ($p > 0.05$) for melatonin (fig. 3).

The dendricity is 45.5% in controls, following dark incubation as well as on UV exposure; there is no change even after UV exposure (fig. 3).

Melatonin. Melatonin binds strongly to the melanocytes both during the dark incubation and on UV exposure (fig. 2). The melatonin positivity which is 16.7% in controls rises to 66.7% ($p < 0.001$) on dark incubation, with a further rise to 75% ($p < 0.001$) on UV exposure. The serotonin positivity is 8.3% in control, 16.7% both in the dark and on UV exposure (fig. 4).

The dendricity is 26.7% in controls, 28.3% (no significant difference) in the dark, and falls to 15% ($p > 0.05$) on UV exposure (fig. 4).

Tryptamine. The melanocytes are positive for both serotonin and melatonin. Serotonin is particularly

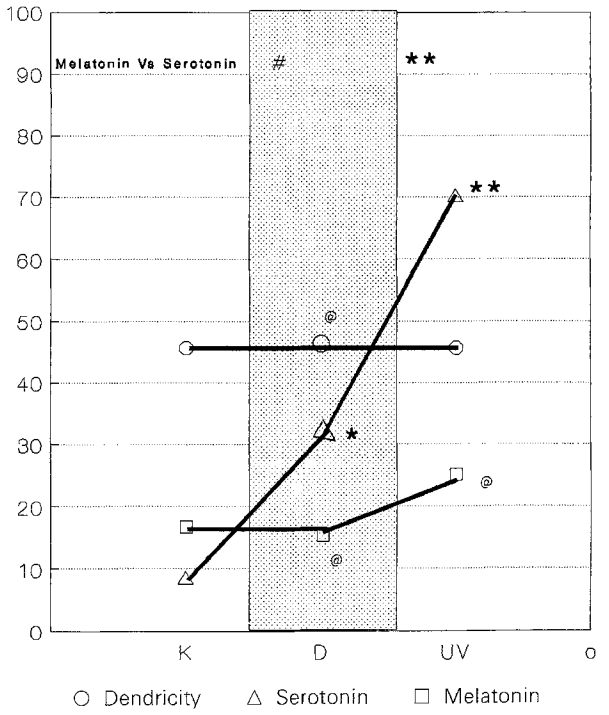

Figure 3. Incubation with serotonin. Graph showing serotonin and melatonin positivity and dendricity on incubation with serotonin. The serotonin positivity rises more on UV exposure. There is no change in melanocyte morphology.

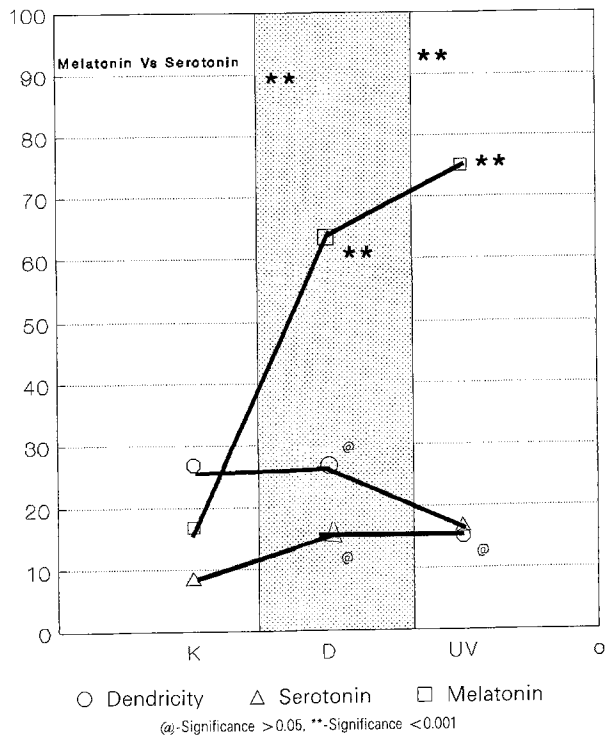

Figure 4. Incubation with melatonin. Melatonin positivity increases in dark and after UV when skin is incubated with melatonin. There is a fall in dendricity coinciding with melatonin binding on UV exposure.

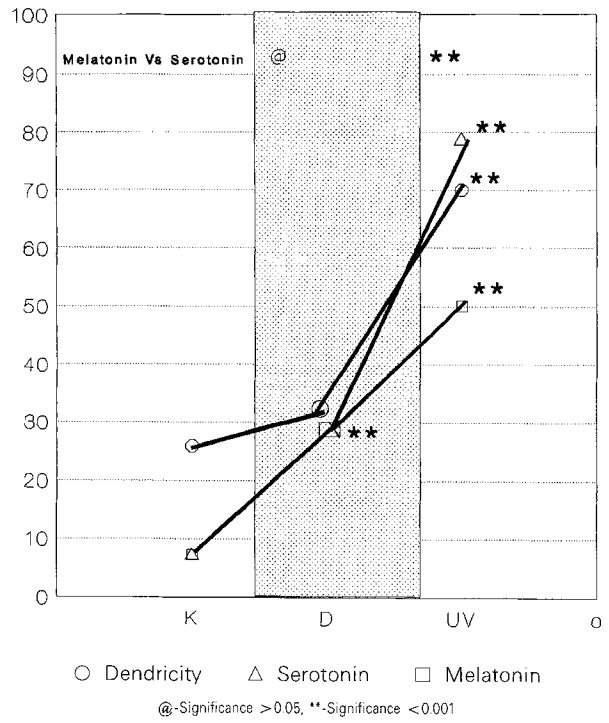

Figure 5. Incubation with tryptamine. Both melatonin and serotonin positivity increase on incubation with tryptamine, the serotonin levels rising prominently on UV exposure, as does dendricity.

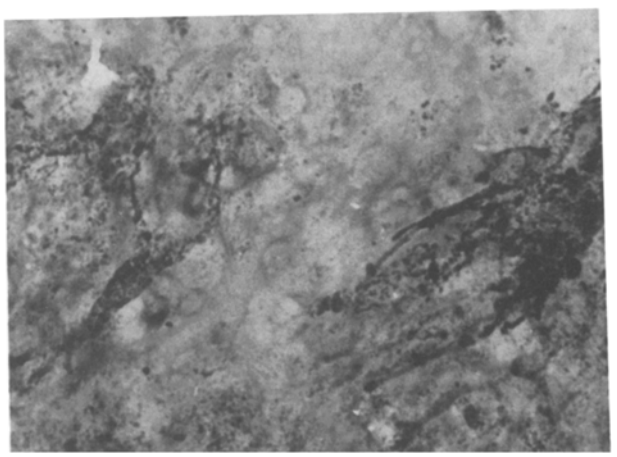

Figure 6. Serotonin positivity in the highly dendritic melanocytes in seborrheic keratosis (mAb serotonin × 400).

prominent after UV exposure. The signal is associated with both cytoplasm and membrane. The serotonin and melatonin positivity are 7.14% in controls and 28.6% ($p < 0.001$) in the dark. On UV exposure there is a marked increase in serotonin positivity to 78.6% ($p < 0.001$) and a mild increase of melatonin to 50% ($p < 0.001$) (fig. 5).

The melanocytes show 25.8% dendricity in controls, 32% ($p > 0.05$) on dark incubation while 70% ($p < 0.001$) are positive on UV exposure indicating a marked increase in dendricity (fig. 5).

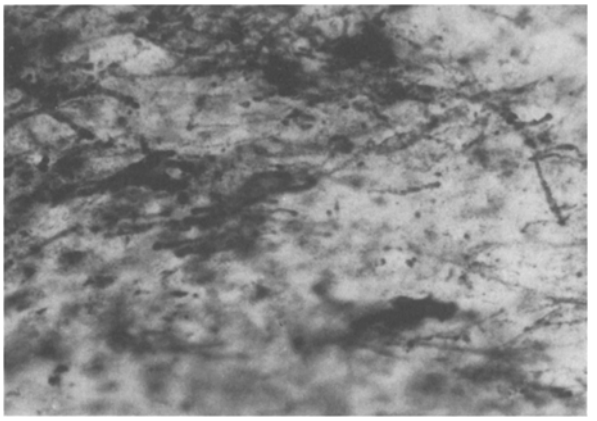

Figure 7. Melatonin positivity in the dendritic melanocytes in the next serial section in the same case as in figure 6 (mAb melatonin × 400).

Proliferative lesions. The highly dendritic melanocytes in a rapidly growing seborrheic keratosis, pigmented basal cell carcinoma and epidermis overlying melanomas, are positive for serotonin and melatonin (figs. 6, 7).

Discussion

Marginal zone melanocytes in vitiligo can be used as a human model for animals such as the polar fox[2] as there is active depigmentation and repigmentation in these cells in the same manner as seen in animals responding to variations in day/night cycles. It is observed that melatonin and serotonin are low in these melanocytes, which show between 7.14% and 16.7% positivity.

On incubation with serotonin or melatonin, the marginal melanocytes are positive, indicating the presence of receptors to the indoleamines. Serotonin binding does not change the morphology of melanocytes, dendricity being maintained at control levels, while melatonin lowers dendricity on UV exposure as in depigmenting vitiligo[3].

From present observations it appears that the melanocytes utilise tryptamine to produce melatonin and serotonin. Another explanation could be that tryptamine displaces serotonin from intracellular binding sites, thereby making it available for melatonin synthesis and detection. Both melatonin and serotonin increase from 7.14% in controls to 28.4% on dark incubation, while melatonin rises to 50% and serotonin to 78.6% on UV exposure ($p < 0.001$). This corresponds to chemical changes in the pineal gland in response to light entrainment[4]. As UV-sensitive dendricity is a G_2-phase function[3], the prominent dendricity (70%) indicates that serotonin, in the presence of melatonin, pushes the melanocytes into the S/G_2 phase on UV exposure. This phenomenon is well observed in the highly dendritic melanocytes of proliferation lesions

where they are positive for both serotonin and melatonin.

The two indoleamines drive the melanocyte cell cycle through aplasia in the dark with melatonin, and into the G_2-phase and proliferation with serotonin accumulation on UV exposures. Thus the melanocytes are photosensitive cells responding to the nuances of UV variations[6] as do retinal and pineal cells[7]. A similar cell-cycle effect and corresponding pigmentation is observed in planaria, where fission takes place at night[8]; melatonin inhibits both fission and pigment formation during the environmental photoperiod on UV exposure[9].

Lerner (1958) identified melatonin as a skin-lightening indoleamine[10], which acts on development, cell division, axoplasmic flow and secretion[11]. Melatonin binds to calmodulin and modulates the cytoskeletal proteins[12] controlling pigment aggregation and cell shape. Serotonin is known for its effects on regeneration and RNA production in planaria[13].

In this study it is observed that melanocytes act as a peripheral neural network sensing variations in UV light with the same chemical indicator of 'dark' melatonin[14]. The network may be an additional extrapineal source of melatonin as are the chromaffin cells of the gut[14]. This ability of melanocytes to process tryptamine and modulate the melatonin levels in response to UV exposure might explain the efficacy of whole body light therapy used for SAD and MDS[15].

The melanocyte network may function as an endogenous biological 'calendar' sensing seasonal variation in the night/day length. When melatonin binding exceeds that of serotonin, melanocyte dendricity decreases on exposure to a pulse of UV, corresponding to the long nights followed by the short days of winter, with associated depigmentation. When serotonin exceeds melatonin, prominent dendricity results on UV exposure with increased pigmentation, simulating the lengthening summer days with increasing serotonin and low melatonin. The human mechanism seen in this study is very much accentuated in animals exposed to large variations in the day/night cycles, such as the polar fox and weasel[2].

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