THE UV RESPONSIVE MELANOCYTE SYSTEM: A PERIPHERAL NETWORK FOR PHOTOPERIODIC TIME MEASUREMENTS - A FUNCTION OF INDOLEAMINE EXPRESSION

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Melanocytes are photoresponsive cells as evidenced by tanning on exposure to sunlight This is expressed as dendricity on UV exposure (Iyengar 1992b) in invitro organ culture studies. The melanocytes show photoresponsiveness in the G_2-phase of the cell cycle(Iyengar 1992a). This response is dose related. Photoresponse in the neural systems is a function of the indoleamines and depends on the light sensitive enzymes NAT and HI0MT in the pineal (Sugden, 1989).

Earlier studies have indicated the involvement of indoleamines in the photoresponse of melanocytes and their role in modulating the cell-cycle (Iyengar 1994, Iyengar et al 1996). It was observed that on incubating whole skin organ cultures with serotonin, melatonin and tryptamine melanocytes are traversed into G_1 -phase by melatonin and into G_2-phase by serotonin in the presence of a low level of melatonin(Iyengar, 1994) on UV exposure. Since 80% of marginal melanocytes show photoresponse in the G_2-phase on incubating with adriamycin, the present work was undertaken to study the location of indoleamine expression during photoresponse.

MATERIAL AND METHODS:

Whole skin organ culture was done on biopsies from 14 cases of vitiligo. Biopsies, 1.5cm x0.5cm in size were taken from the marginal zone to include both pigmented and non-pigmented areas. The tissues were transported in sterile MEM medium. These were cut into 5 bits each, 2mm in width to include the marginal zone between the pigmented and vitiliginous zone under sterile conditions.

One bit from each biopsy is immediately fixed in cold buffered formol-glutaraldehyde to serve as control. The rest were immersed in 2000µl MEM containing 200µg of adriamycin as described earlier (Iyengar 1992) to synchronise melanocytes in the G_2-phase and incubated at 37°C in the dark. One bit each was exposed to a pulse of 30s, 60s, 90s and 120s of UV in the 14 biopsies at 2h of incubation. The tissues were reincubated in the dark for 3h. All organ cultures were harvested at 5h of incubation.. The UV tube is of 15w emitting 280-390 nm. Photometric reading shows 11.85 mamps at the point of exposure, 30cm from the tube.

All specimen are fixed in cold buffered formolglutaraldehyde overnight, to prevent diffusion of enzymes and neurotransmitters, while retaining their activity. Formolglutaraldehyde is prepared in phosphate buffer (pH 7.2), with 10% formalin (4% formoldehyde) and 1% glutaraldehyde. Serial,

5μm thick frozen sections are cut on a Lipshaw cryotome at -25° C. Serial sections are stained for HE, without substrate to assess pigment and with dopa as substrate for dopaoxidase (Pearse,1980).
Immunohistochemistry was done by the avidin biotin method (Iyengar, 1994) using monoclonal antibodies (mAb) against serotonin and melatonin (DAKO). The primary antibody was excluded to serve as a negative control, while mAb serotonin and melatonin served as positive controls for each other.

Immunohistochemical staining
ABC Method using Vectastain detection kit-PK6102 (Vector Laboratories, Burlingame) :

Hydrate slides : Block in 1.5ml H_2O_2 + 50 ml methanol; Wash with PBS buffer; Apply 4-6 drops of Normal Horse Serum (1:50 with PBS) and incubate for 1 hour at room temperature; Primary antibody (mouse, 1:50 dilution) applied and incubated at 28°C overnight; Wash with PBS; Apply 4-6 drops of biotinylated horse anti-mouse antibody; Incubate at room temperature for 1 hour; Wash with PBS; Apply 4-6 drops of Avidin Biotin complex(mixed and diluted appropriately at least 30 min before use); incubate at room temperature for 1 hour; Wash with PBS; Apply substrate chromogen solution and incubate till desired colour intensity has developed (DAB-2.5 mins); Rinse with distilled water and counter-stain with Haematoxylin; Mount in DPX.

The changes in 5 marginal zone melanocytes have been studied in the following, since this is the zone which shows active depigmentation and repigmentation.

Photoresponses:

Melanocyte morphology is assessed on dopa staining . The marginal melanocytes show prominent dendricity on UV exposure when arrested in G_2 phase (Iyengar, 1992a). The number of melanocytes (of 5 marginal melanocytes) showing dendricity at each exposure is depicted as percentage (total 70). 11 cases show photoresponse.

Indoleamine Positivity:

Melanocyte positivity for serotonin and melatonin was assessed in serial sections, in 5 marginal melanocytes for each exposure, in the 11 cases which showed photoresponse i.e. 55 for serotonin positivity and 55 for melatonin positivity. The total indoleamine positivity was calculated out of the total 110 melanocytes at each exposure by adding the serotonin (SER) and melatonin (MLT) positive melanocytes. The percentage expression of serotonin and melatonin at each exposure has been calculated to show the conversion of serotonin to melatonin or its inhibition in relation toUV exposure. This was calculated taking the total indoleamine positive cells as 100% in each group.

Statistical analysis for significance was done by the Student's t-test. The 3 non responsive cases are shown for comparison.

RESULTS:

Dendricity:

The dendricity is 45% in controls, 5% on 30s UV exposure, 30% after 60s UV,65% after 90s UV and 80% after 120sUV. Thus the melanocyte dendricity in G_2 phase correlates with increasing UV exposure (Table 1). There is a statistically significant increase in dendricity on 90s UV and 120s UV ($p < 0.001$) as compared to 30s UV.

Photoresponders (11 cases)

Serotonin positivity:(Fig. 1& 2)

.Eleven marginal melanocytes show serotonin positivity in controls. On UV exposure the serotonin positivity is seen in 9 melanocytes after 30s UV,37 after 60s UV, 50 after 90s UV and 33 after 120sUV. Thus serotonin positivity increases with increasing UV exposure the peak being at 90s UV exposure.[Table I]. The values at 60s, 90s and 120s are statistically significant ($p < 0.001$).

Melatonin positivity (Fig. 3)

. In control skin melatonin positivity is seen in 11 marginal melanocytes. The melatonin positivity is seen in 28 melanocytes after a 30s pulse of UV, 35 after 60s UV, then falls to 26 after 90s UV and 7 after 120s UV out of 55 marginal melanin units counted. Significant difference is seen on 30s UV and 60s UV ($p < 0.001$) and on 90s UV exposure ($p < 0.01$).

Total Indoleamine positivity:

The total indoleamine positive cells include both the serotonin and melatonin positive melanocytes at each exposure out of a total of 110 melanocytes.Thus there are 22 positive melanocytes in controls, 37 on exposure to 30s UV, 72 on 60s UV, 76 on 90s UV and 40 on exposure to 120s UV.[Table I]. Thus there is a significant increase in positivity beyond 60s UV exposure.

Serotonin :. Melatonin ratio: (Fig. 4)

The ratio of serotonin:melatonin positivity indicates the percentage conversion to melatonin positivity in relation to the UV exposure. The serotonin:melatonin ratio is 50:50 in controls; 24.3:75.7 on 30s UV($p < 0.001$); 51.4:48.6 on 60s UV; 65.8:34.2 on 90s UV($p < 0.01$)and 82.5:17.5 on 120s UV($p < 0.001$). The comparative graphs shows the inhibition of melatonin on increasing UV exposure.

Non responders (3 cases):

Indoleamine positivity:

In controls there are 2 positive, 1 for serotonin and 1 for melatonin; On 30s UV exposure 2 are positive, 2 for serotonin and 0 for melatonin; on 60s UV 3 are positive 1 for serotonin and 2 melatonin; on 90s UV, 1 melanocyte is positive for melatonin; and on 120s UV exposure 3 melanocytes are positive 2 for serotonin and 1 for melatonin.

DISCUSSION:

Melanocytes are photoresponsive cells as evidenced by tanning on sun exposure. This is also observed in animals which show coat color changes in response to large variations in day-night cycles.. The marginal melanocytes in vitiligo have been used as the model in this study, since these cells undergo active repigmentation and depigmentation. The marginal melanocytes show prominent dendricity during photoresponse in the G_2-phase as seen in earlier studies(Iyengar 1992). The present work was taken up to study whether indoleamines are involved in this response. In controls, the marginal melanocytes show very low positivity both for serotonin and melatonin, while on UV exposure in the G_2 phase, the melanocytes are positive for both serotonin and melatonin. The total indoleamine positivity rises to a peak level on 90s UV exposure of the marginal melanocytes.

On comparing serotonin and melatonin positivity it is observed that there is a gradual increase in serotonin positivity with a peak at 90s UV, while melatonin positivity declines after 60s UV. In the pineal gland, melatonin is produced from serotonin during the dark phase by the action of the enzymes NAT and HIOMT(Underwood, 1989 Gaudet et al 1993).These enzymes are inhibited, on exposure to light, with the accumulation of serotonin(Armstrong, 1989). Since the photoresponsive melanocytes are positive for both serotonin and melatonin, a similar photo inhibitor activity is possible within them, if indoleamines are being metabolised by them.

The percentage conversion of serotonin to melatonin, in relation to the dose of UV exposure would indicate if photoinhibition occurs in melanocytes. This effect is well brought out by assessing the ratio of serotonin vs melatonin expression in the total indoleamine positive cells in relation to UV exposure. From the present findings, a very short pulse of UV exposure(30s) stimulates indoleamine expression but does not inhibit the conversion of serotonin to melatonin as indicated by the large proportion of melatonin positive cells, (SER:MLT:: 24.3:75.7) indicating that the enzymes NAT and HIOMT remain active. The percentage serotonin expressed, increases proportionate to increasing UV while that of melatonin is inversely related to the UV exposure. Thus the conversion of serotonin to

melatonin is inhibited in the melanocytes on increasing UV exposure,(SER:MLT::82.5:17.5 on 120s UV) as observed in the pineal on light entrainment (Sugden, 1989).

Melatonin is known to inhibit melanin production (Slominski et al 1989, Slominski and Pruski, 1993a). This is well illustrated when the melatonin positivity is compared with pigment donation (Unpublished data). The pigmentation is inversely proportionate to melatonin positivity.. This is accompanied by poorly dendritic melanocytes.

As discussed by Herbert (1989) the neural net for the detection of small alterations in photoperiod has not yet been identified From the above findings it appears that the melanocytes form a peripheral photoresponsive neural network (Iyengar, 1992b; Iyengar, 1996b), photosensitivity being a function of indoleamine expression. On UV exposure the melanocytes express indoleamines and show dendricity indicating G_2 traverse,the varying serotonin:melatonin ratio reflecting sensitivity to the amount of UV exposure. The melanocyte network expresses POMC(Iyengar, 1995, 1996c, Slominski et al 1993b) and the hormone PRL during photoresponse to UV and is thus linked to the opioid system and is associated with regulating gonadotropins. This is accompanied by a dose related increase in pigment donation. These features are also reflected by the association of coat color changes and the estrus cycle(Niklowitz and Hoffmann, 1988; Altmeyer and Holzman 1985: Rust and Meyer, 1969) in animals in association with the seasonal photoperiod. Thus the melanocyte network in man responds to nuances of UV variation in the sunlight and thus to the annual seasonal circadian utilising the photosensitive indoleamine metabolism.. From the present and earlier studies (Iyengar, 1994; 1992b, 1995), the melanocyte network in the skin, including the hair follicles, can serve as the peripheral neural-net for photoperiodic time measurements - the biological calender.

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LEGENDS:

Table I: Table showing dendricity, serotonin and melatonin positivity with total indoleamine positivity in marginal melanocytes in response to different exposures of UV.

Figure 1: Section from marginal zone vitiligo, showing no positivity for indoleamines in controls. (mAb serotonin x 400)

Figure 2: Dendritic melanocytes showing serotonin positivity in 3 out of 5 marginal melanocytes in skin exposed to a 90s pulse of UV (mAb serotonin x 400) (Figure 2a: Camera Lucida diagram of the same field).

Figure 3: Poorly dendritic melanocytes showing melatonin positivity in 3 of 5 marginal cells on exposure to a UV pulse of 30s (mAb melatonin x 400) (Figure 3a: Camera Lucida diagram of the same field).

Figure 4: Comparison of serotonin and melatonin expression by marginal melanocytes in vitiligo during increasing and decreasing UV exposure in the G_2-phase.