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THE EFFECT OF MSH ON THYMIDINE INCORPORATION BY KERATINOCYTES IN THE EPIDERMAL MELANIN UNIT

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Epidermal melanocytes were observed in the black but not in the white skin of black-and-white spotted guinea pigs. In experiments designed to determine whether melanocyte-stimulating hormone (MSH) affects the incorporation of thymidine by keratinocyte nuclei of the epidermal melanin unit, the labeling index was the same in all skin before MSH administration. After MSH injections, the level of [^{3}H]thymidine incorporation in keratinocytes increased significantly in black skin but not in white. We suggest that through the mediation of melanocytes MSH indirectly affects keratinocytes in the epidermal melanin unit.

Keratinocytes, melanocytes, and melanosomes are closely related in vertebrates. An organizational theory emphasizes a structural and functional relation between keratinocytes and melanocytes (the epidermal melanin unit), which is expressed at a higher level than the individual cells [1,2]. According to this theory, keratinocytes are actively engaged in regulating melanin synthesis, and any factor capable of stimulating melanocytes must also affect keratinocytes. This hypothesis, that a modification of one component of the epidermal melanin unit affects the others, may be involved in certain inflammatory conditions [3] and in human tanning [2].

MSH affects the activity of mammalian melanocytes [4]. In the guinea pig, for example, it causes an increase in the length and complexity of the melanocyte dendrites as well as in the amount of melanin in the surrounding keratinocytes [5,6]. Similar processes lead to hyperpigmentation in human [7–9] and rabbit [10] skin. If melanocytes and keratinocytes are a functional unit, both should be affected by the systemic administration of MSH. The studies reported here were designed to determine whether MSH and increased melanin production affect thymidine incorporation by keratinocytes in a cell population of keratinocytes with and without melanocytes.

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Abbreviations:
dopa: L-3,4-dihydroxyphenylalanine
[^{3}H]TdR: tritiated thymidine
MSH: melanocyte-stimulating hormone
UV: ultraviolet

MATERIALS AND METHODS

Animals

Nine of 10 adult male black-and-white spotted guinea pigs received a daily subcutaneous injection of 40 μg of active racemized MSH (courtesy of Dr. A. B. Lerner) in 16% gelatin (vehicle, Calciton diluent B, lot #K614-029, Armour Pharmaceutical Co.) for 21 days; the 10th control animal received a daily subcutaneous injection of only the vehicle for 21 days. Full-thickness skin biopsies were removed from both the white and the black skin of the back before the first MSH or vehicle injection and after 7, 14, and 21 injections.

Autoradiography

Biopsies removed before the administration of MSH and after 7 and 21 injections were processed for autoradiography. The samples were incubated with 1 μCi/ml of [^{3}H]TdR (New England Nuclear, sp act 6.7 Ci/mM) in TC Hanks' solution (Difco Labs) for 2 hr at 37°C. To distinguish between melanocytes and keratinocytes, specimens were washed, fixed for 45 min in 10% buffered neutral formalin, and incubated in 0.1% dopa in phosphate buffer for 5 hr at 37°C. The samples were postfixed, dehydrated, cleared, embedded in paraffin, and serially sectioned. The slides were dipped in Kodak NTB-2 emulsion, exposed for 1 month at 4°C in light-proof boxes, and developed [11]. Autoradiographs were stained with Harris's hematoxylin and eosin.

To determine the labeling index, the number of [^{3}H]TdR labeled malpighian cell nuclei (more than 10 grains) was counted in 8 histologic sections; the percentages for each biopsy were based on about 5,000 malpighian cells. The data were subjected to one-way analysis of variance.

Electron Microscopy

Blocks of tissue were fixed in a 2.5% glutaraldehyde + 2% paraformaldehyde in phosphate buffer [12] overnight, washed in Milloning's phosphate buffer, and postfixed in

1% OsO_4 for 2 hr. After osmication, the tissues were dehydrated through graded ethanol to propylene oxide and embedded [13]. For light microscopy, 1-μ sections were stained with toluidine blue. Electron microscopic sections were stained with lead citrate and uranyl acetate and viewed with a Philips 200 electron microscope operating at 60 kv.

RESULTS

Autoradiography

Table I shows the epidermal labeling index of both the black and the white skin before MSH. Before treatment this index was not significantly different (Tab. II, $p = 0.51$). The mean number of labeled keratinocyte nuclei in black and white skin after MSH treatment is also shown (Tab. I). These labeled cells were predominantly in the basal layer. An analysis of variance established a statistically significant difference among treatment groups for the black skin ($p = 0.04$) but not for the white ($p = 0.54$). In addition, there was a significant difference between white and black skin after both 7 and 21 doses of MSH (Tab. II). The level of [^{3}H]TdR incorporation increased in black but not in white skin.

Electron Microscopy

Dendritic cells observed in the normal white skin included Langerhans and indeterminate cells; in addition the black skin contained melanocytes (Fig.). After the administration of MSH (7, 14, and 21 doses), the epidermis of the white skin still contained no melanocytes but indeterminate and Langerhans cells were observed. In the black skin, active melanocytes were seen.

DISCUSSION

MSH-induced hyperpigmentation has been well documented in amphibian and mammalian skin. How it is mediated by melanophores is fairly well understood, but many questions about melanocyte hyperpigmentation remain unanswered. According to Snell [6], MSH does not increase the number of melanocytes in guinea pigs but does affect their size, the number of dendrites, and the amount of melanin in the skin. The failure of MSH to form melanocytes or to increase mitosis in white skin suggests that it has an indirect effect on keratinocytes when epidermal melanin units are present. The increase in labeling index after MSH treatment in black but not white skin from the same animal indicates that mitosis of the keratinocytes is effected through the mediation of the melanocytes.

These results, however, suggest that keratinocytes do play an active role in pigmentation after MSH activation. Although MSH does not affect the ability of keratinocytes to phagocytize melanin-laden dendrites, it does indirectly stimulate mitosis and, therefore, provides more vehicles for removing melanosomes. Hadley and Quevedo [2] have suggested a mechanism of human tanning after UV irradiation, that is, the proliferation of keratinocytes provides more vehicles for melanin transport and results in hyperpigmentation. UV irradiation does not seem to stimulate melanogenesis in cultured melanoma cells, an indication that

TABLE I. *Mean labeling indices[a] in skin of guinea pigs before and after MSH administration*

Animal #	White skin[b]			Black skin[c]		
	Control	MSH		Control	MSH	
		7 doses (days)	21 doses (days)		7 doses (days)	21 doses (days)
1	0.786	0.902	0.830	0.694	1.155	1.359
2	1.584	0.750	0.511	1.361	1.468	1.226
3	0.628	0.681	0.189	0.703	1.235	0.488
4	0.190	1.423	0.302	0.342	1.280	1.581
5	0.304	0.591	0.244	0.446	1.358	1.794
6	0.078	0.658	1.025	0.354	0.894	0.607
7	0.332	0.579	0.443	0.620	1.173	0.869
8	0.961	0.699	0.850	0.488	0.894	0.821
9	0.319	0.299	0.566	1.460	1.036	0.858
Mean L.I.	0.575	0.731	0.551	0.718	1.166	1.067
ANOV[d]	$F = 0.64$			$F = 3.63$		
F test	$df_1 = 2$	$df_2 = 24$	$p(0.64) = 0.54$	$df_1 = 2$	$df_2 = 24$	$p(3.63) = 0.04$

[a] Labeling index (L.I.) = number of [^{3}H]TdR labeled malpighian cells/total number of malpighian cells

[b] Vehicle only (1 animal): 0 dose = 0.534, 7 doses = 0.507, 21 doses = 0.522

[c] Vehicle only (1 animal): 0 dose = 0.494, 7 doses = 0.617, 21 doses = 0.558

[d] One-way analysis of variance

TABLE II. *Comparison of black and white skin of guinea pigs after the same number of treatments*

	Control	MSH	
		7 doses	21 doses
ANOV F test[a]	F = 0.46 p (0.46) = 0.51	F = 12.86 p (12.86) = 0.001	F = 8.38 p (8.38) = 0.01

[a] Degrees for freedom are the same for all three tests, $df_1 = 1$, $df_2 = 16$.

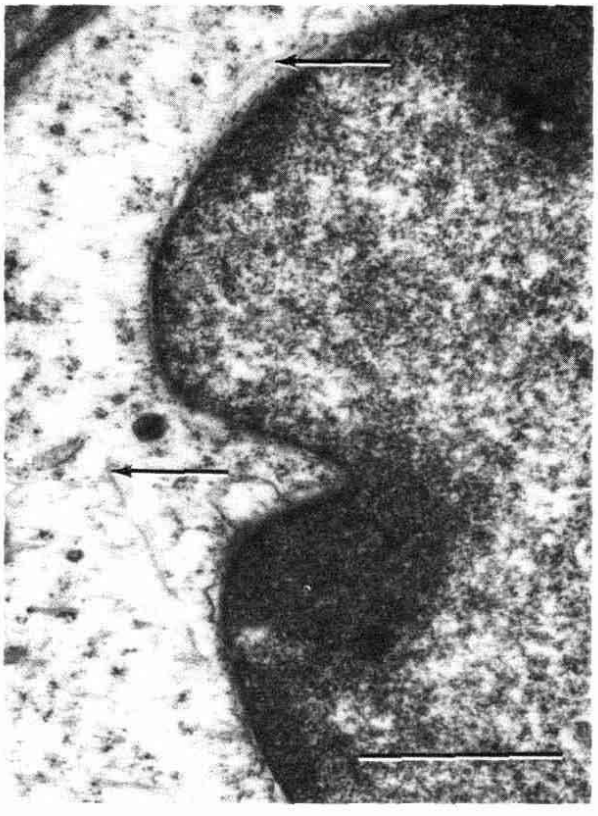

FIG. Indeterminate cell of black skin. These cells were always present in both black and white skin. In the latter they were not stimulated by MSH to become melanocytes (× 25,000). Scale bar 1,000 nm.

UV light probably has no melanogenic effect on cells grown in vitro [14,15]. On the basis of results from melanocyte-keratinocyte culture experiments, Prunieras [16] suggested that keratinocyte multiplication inductively affects melanocyte morphology as well as the ability of this cell to enter mitosis. Keratinocytes in culture actively participate in pigment transfer by melanocytes [17]. Dibutyryl adenosine 3',5'-cyclic monophosphate significantly increases the rate of pigment donation as well as the percent of melanocytes donating pigment in epidermal cell cultures [18]. MSH, however, appears to increase tyrosinase activity in melanoma cells in culture [19] and in tumor-bearing animals [20]. Thus, work done on melanocytes in vitro and the use of known melanogenic stimuli in vivo suggest that both melanocytes and keratinocytes are involved in hyperpigmentation.

In some systems, UV light may induce indeterminate cells to become melanocytes [21]. In the white skin of guinea pigs, MSH apparently does not stimulate the indeterminate cells, probably because the latter were predetermined to become Langerhans cells. Thus, cell interactions not only occur but are very important in the skin. Keratinocytes appear to be more involved in pigmentation than just acting as a passive storehouse for melanin; they play an important role in the epidermal melanin unit.

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