Stimulatory Effect of Prostaglandin E_2 on the Configuration of Normal Human Melanocytes In Vitro

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Normal human epidermal melanocytes became swollen and more dendritic when they were cultured for 6 days with prostaglandin (PG) E_2, but not with PGE_1, although the amount of immunoreactive tyrosinase in the melanocytes did not appear to be increased markedly after these treatments. From these data we suggest that PGE_2 may be one of the factors responsible for the induction of postinflammatory hyperpigmentation of the skin. *J Invest Dermatol 89:299–301, 1987*

Melanocytes have a highly specialized function: to make the melanin pigment in the skin. The regulation mechanism of melanin synthesis, including hyperpigmentation after skin inflammation, has not yet been clarified, however. Prostaglandin (PG) E_1 and PGE_2 were reported to stimulate in vitro the melanogenesis in human cultured hair bulbs [1] and in mouse retinal pigment cells [2]. Recently Nordlund and colleagues reported that PGE_2, but not PGE_1, stimulated the proliferation of the epidermal melanocytes in vivo [3]. Hence, PGE_1 and/or PGE_2 are suggested to be involved in the postinflammatory skin pigmentation, since prostaglandins are known to be important endogenous regulators of inflammatory diseases in the skin [4]. There is a lack of information about the direct effect of these PGEs on the human epidermal melanocytes, however. In this study, we have examined the direct effect of PGE_1 and PGE_2 on normal human melanocytes in vitro.

MATERIALS AND METHODS

Melanocyte Culture The isolation procedure of human melanocytes from the roof of suction blisters raised on the skin of a 21-year-old Oriental male volunteer is as described previously [5]. Melanocytes were cultured in Eagle's minimal essential medium (GIBCO Laboratories, Grand Island, New York) supplemented with 10% fetal bovine serum and 10 ng/ml phorbol-12-myristate-13-acetate (PMA) for the first 6 days and then the medium was changed to a PMA-free medium. Three days after culturing in the latter medium, 5.6 μM of PGE_1 or PGE_2 (Sigma Chemical Company, St. Louis, Missouri) was added to the culture medium, and the culture was continued for 6 days.

Immunofluorescence Staining Cultured melanocytes were stained immunohistologically according to the method described previously [6] using rat monoclonal antibody against tyrosinase [7] and fluorescein isothiocyanate (FITC)-conjugated second antibody directed to the monoclonal antibody (TAGO Inc., Burlingame; California). The intensity of fluorescence of FITC in each melanocyte was measured at 520 nm by Olympus multimicrospectrophotometer (model MMSP) [8].

Morphometry Stained melanocytes were photographed microscopically with Vanox-T (Olympus, Tokyo). From the enlarged photographs, the shape of all the melanocytes were transferred to a sheet of tracing paper, and the cell perimeter, area, and number of dendrites of each melanocyte were determined with an image scanner (NEC, PC-IN 502) connected to a microcomputer (NEC, PC-9801VM) and appropriate software.

RESULTS

After 6-day culturing in the presence or absence of PGE_1 or PGE_2, the melanocytes were immunohistochemically stained with the monoclonal antibody to tyrosinase. They showed no significant differences in fluorescence intensity irrespective of the culture conditions as shown in Table I. Prostaglandin E_2, however, changed the configuration of the melanocytes into a more swollen and dendritic one (Fig 1); there was an increase in the number of dendrites, which appeared more slender and extended than those of the controls. We also determined these morphologic changes quantitatively by 3 parameters obtained with the image scanner as shown in Table I. The melanocytes cultured with PGE_2 showed a significant increase in all the parameters: an increase of 60%, 15%, and 100% in their cell perimeter, area, and number of dendrites, respectively, as compared with those of the melanocytes cultured in the absence of PGEs. On the other hand, PGE_1 did not induce these changes in the melanocytes (Fig 1 and Table I).

DISCUSSION

Under an electron microscope, Sauk and associates [1] observed the stimulative action of PGE_1 and PGE_2 on maturation of melanosomes, orientation of microfilaments in dendritic processes, and melanosome complexing in melanocytes of the hair bulbs of white humans within 24 h in vitro. Garcia and coworkers [2] also

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Abbreviations:
FITC: fluorescein isothiocyanate
PG: prostaglandin
PMA: phorbol-12-myristate-13-acetate

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Table I. Relative Intensity of Fluorescein Isothiocyanate in Immunofluorescence Staining of Human Melanocytes Cultured With or Without PGE_1/PGE_2, and Perimeter, Area, and Number of Dendrites of These Melanocytes

Treatment	Number of Cells Examined	Relative Fluorescent Intensity at 520 nm		Perimeter (μm)		Area (μm^2)		Number of Dendrites	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
None	100	20.7	7.0	184.9	69.2	890.5	413.2	2.8	1.0
5.6 μM PGE_1	100	22.6	6.0	182.5	58.9	695.5	249.3	2.8	1.0
5.6 μM PGE_2	100	23.6[a]	6.5	300.0[b]	145.6	1021.6[a]	407.1	5.5[b]	2.7

Melanocytes were prepared as described in Methods and Materials. They were cultured for 6 days in MEM with 10% fetal bovine serum without PMA in the presence or absence of 5.6 μM PGE_1/PGE_2.

[a] PGE_2 vs none, $p < 0.05$ by Student's t test.

[b] PGE_2 vs none, $p < 0.001$ by Welch's t test.

MEM = minimal essential medium; PG = prostaglandin; PMA = phorbol-12-myristate-13-acetate.

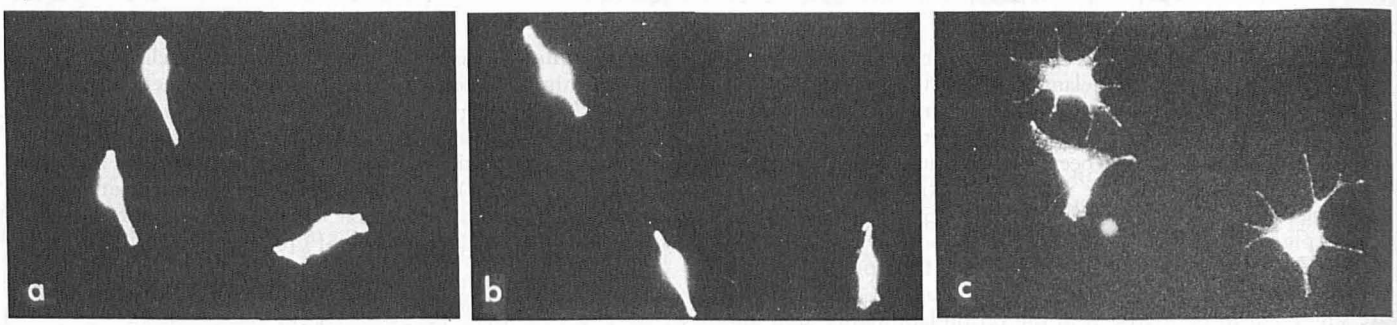

Figure 1. Human melanocytes cultured with or without PGE_1/PGE_2. The melanocytes cultured (*a*), without (*b*), with 5.6 μM PGE_1, or (*c*), with 5.6 μM PGE_2 as described in *Table I* were photographed microscopically. × 190.

found that the addition of either PGE_1 or PGE_2 to culture medium accelerated the increase in melanin content of cultured retinal pigment cells obtained from chicken embryo. Recently, Nordlund and colleagues [3] reported that PGE_2, but not PGE_1, stimulated the proliferation of melanocytes in the pinnal epidermis of a mouse in vivo. In this communication we have demonstrated for the first time the stimulatory effect of PGE_2, but not of PGE_1, on the cell configuration on isolated normal human epidermal melanocytes in vitro. The disagreement about the effect of PGE_1 among various reports may be due to marked differences in the studied cell source, experimental condition, and methodology.

The elongation and increase in the number of dendrites of melanocytes are observed in the human epidermis when hyperpigmentation occurs after sunlight exposure of the skin [9]. These morphologic changes are thought to be necessary for the transfer of the melanosomes and melanin granules to the surrounding keratinocytes [9]. In our experiment, PGE_2 increased not only the number of dendrites but also the size of the cells. Since it is difficult to precisely measure each length of all dendrites, in the present study we also measured the perimeter of the cell as a substitute for the sum of length of all dendrites in each melanocyte, and found that PGE_2 increased the perimeter of the cell to 160% of the control. We think that this increase in the perimeter of the melanocyte by PGE_2 reflects not only the elongation of each dendrite but also the swelling of the cell body as well as the increase in the number of dendrites. Although the effect of PGE_2 on the cell shape is similar to that of vitamin D_3, which we have preliminarily reported [8], in contrast to the latter, PGE_2 did not increase markedly the amount of immunoreactive tyrosinase. Thus, the mechanism of the PGE_2 action on the melanocytes is distinct from that of vitamin D_3, which is thought to play a major role in the skin pigmentation after UV irradiation.

Prostaglandin E_2, a constituent commonly found in the inflammatory exudate from the skin after injury due to UV radiation [10], burn [11], or allergic contact dermatitis [12], has been suggested to take part in the postinflammatory hyperpigmentation in the human skin. Our present study has provided, for the first time, direct evidence to support such a hypothesis.

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