In Vitro Modulation of Proliferation and Melanization of S91 Melanoma Cells by Prostaglandins

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ABSTRACT

The effects of prostaglandins (PGs) on the Cloudman S91 melanoma CCL 53.1 cell line indicate that melanogenesis and proliferation are regulated by separate mechanisms that are not necessarily cyclic AMP (cAMP) dependent. These cells responded to PGE1 and PGD2 in a dose-dependent manner, by an increase of tyrosinase activity and by inhibition of proliferation. PGA1 and PGD2 inhibited cellular proliferation and tyrosinase activity, while PGF2α had no effect after 24 h of treatment. PGE1, but not PGE2 or PGD2, increased cellular cAMP levels after 30 min of treatment. Treatment with 10 μg/ml PGE1 inhibited cellular proliferation after 4 h and enhanced tyrosinase activity after 12 h. Tyrosinase stimulation by PGE1 required de novo transcription and translation. Actinomycin D, cycloheximide, and the tyrosinase inhibitor phenylthiocarbamide blocked tyrosinase activation but did not alter the inhibitory effect of PGE1 on proliferation. Dibutyryl cAMP and 3-isobutyl-1-methylxanthine augmented tyrosinase activation by PGE1 without enhancing the inhibitory action of PGE1 on cell growth. Neither blockage nor enhancement of the PGE1 effect on tyrosinase altered the PGE1-induced retardation of proliferation. These results are in marked contrast to the traditional concept that elevation of cAMP levels in melanoma cells necessarily results in stimulation of melanogenesis and inhibition of proliferation. The data presented propose independent and possibly alternative pathways for the regulation of these two cellular events.

INTRODUCTION

The potential role of prostaglandins as effective chemotherapeutic agents against various tumor types has been well described (1-3). Particularly in the case of melanoma, prostaglandins have been shown to inhibit the growth of the murine S91 (4), B-16 (1, 2, 5), as well as of human melanomas (6-8).

Murine Cloudman S91 melanoma cells have been widely used to elucidate the regulatory mechanisms of melanogenesis and of pigment cell growth. These cells have been shown to respond to various melanogenic agents such as MSH,3 PGE1, dbcAMP, and the methylxanthenes by increased tyrosinase activity and melanin synthesis (9-11). It has been shown that both MSH and PGE1 increase cAMP levels in these cells (12). Cyclic AMP has been implicated as a regulator of melanogenesis and proliferation in pigment cells (9, 13). It has been proposed that stimulation of melanin synthesis results in the production of metabolic byproducts that are autotoxic (14).

Thus, an inverse relationship between melanogenesis and pigment cell proliferation has been suggested. However, α-MSH, the classic and best studied melanogenic agent, has been shown to elevate cAMP levels, increase melanin synthesis (9, 10) and to stimulate rather than inhibit melanoma cell proliferation (15).

We hereby report the effects of prostaglandins on both melanogenesis and proliferation of Cloudman S91 melanoma CCL 53.1 cell line. PGE1 and PGE2 stimulated tyrosinase activity and retarded cellular proliferation. PGA1 and PGD2 inhibited cellular proliferation as well as tyrosinase activity, while PGF2α was totally without effect after 24 h of treatment. PGE1 caused a marked increase of intracellular cAMP levels, stimulated tyrosinase activity, but inhibited proliferation. The increased tyrosinase activity in response to PGE1 could be either blocked or enhanced by various inhibitory or stimulatory agents without affecting the PGE1-induced reduction in proliferation. This indicates that the PGE1-induced tyrosinase stimulation and growth inhibition are not regulated by the same mechanism. Our results have important implications for cell types other than melanoma, and case doubt on the conception that a necessary relationship exists between cellular proliferation and phenotypic expression.

MATERIALS AND METHODS

Materials. Ham's F-10 medium was obtained from Flow Laboratories (McLean, VA). Horse serum, fetal bovine serum, and penicillin-streptomycin solution were purchased from GIBCO (Grand Island, NY). PGA1, PGD2, PGE1, PGE2, PGF2α, dbcAMP, IBMX, actinomycin D, cycloheximide, PTU, activated charcoal, and Dowex 50 W resin were all obtained from Sigma Chemical Company (St. Louis, MO). [3',5'-3H]tyrosine and the cAMP radioimmunoassay kit were purchased from New England Nuclear (Boston, MA).

Culture Conditions. The Cloudman S91 melanoma CCL 53.1 cell line was a generous gift from Dr. Mac. E. Hadley (Anatomy Department, University of Arizona). The doubling time of this cell line is 24 h. Cells grow logarithmically after 2 days in culture, and approach plateau phase of growth after 5 days in culture (16). These cells were maintained in monolayer culture in Ham's F-10 medium supplemented with 10% horse serum and 2% fetal bovine serum, both heat inactivated, and with 100 units/ml penicillin, 100 μg/ml streptomycin, at 37°C in a humidified incubator containing 5% CO2. Cells were subcultured weekly and were maintained in culture for only 10 passages to avoid phenotypic drift. Monolayer stock cultures were regenerated from cells that were cryopreserved in liquid nitrogen.

Experimental Procedures. Cells were harvested by replacing the culture medium with EDTA containing Tyrode solution. For most experiments, cells were seeded at a density of 0.2 × 10⁶ cells/25 cm² flask. Forty-eight h later, the medium in all flasks was replaced with medium containing 1 μCi/ml [3H]tyrosine (specific activity, 54.2 Ci/mmol) and the experimental groups received the appropriate treatment. All flasks were incubated for 24 h. Cells were then counted using a hemacytometer, and the medium was saved to be assayed for tyrosinase activity. All PGs were dissolved in ethanol, thus their effects on cell growth or tyrosinase are expressed as percentage of the ethanol-treated control.

For experiments involving different exposure periods to PGE1, all flasks received medium containing [3H]tyrosine, and the experimental groups were treated for 4, 6, 8, 12, 16, and 24 h with 10 μg/ml PGE1. At the end of each treatment time cells were counted and the medium was assayed to determine tyrosinase activity.

Tyrosinase Assay. A modified version of the Pomerantz charcoal absorption method (10, 17-20) was utilized to measure tyrosinase activity. The amount of H2O released into the medium when [3H]-
tyrosine was converted by tyrosinase to 3,4-dihydroxyphenylalanine
was measured. From each flask, duplicate 1-ml samples of "H-labeled
medium were placed into glass tubes. Each aliquot was treated with 1
ml of charcoal (10% v/v in 0.2 N citric acid), and the tubes were
centrifuged. One ml of the supernatant from each tube was passed
through a Dowex 50 W resin column, and the column was rinsed with
1 ml of 0.1 N citric acid. The eluents from each column were collected
into scintillation vials and were counted in a Packard Tricarb liquid
scintillation spectrometer. Tyrosinase activity was expressed as counts
per minute per 10^6 cells, and then as percentage of control.

Cyclic AMP Radioimmunoassay. Cyclic AMP levels were determined
by using a radioimmunoassay kit. Cells in logarithmic phase of growth
were harvested using a rubber policeman, and were counted, centri-
fuged, and resuspended in 1.5 ml of serum-free F-10 medium. Samples
(100 μl) of the cell suspension (1.5–2 x 10^7 cells) were aliquoted into
microcentrifuge tubes. The volume in each tube was adjusted to 250 μl
by adding the appropriate volume of medium. Duplicate samples were
treated for 30 min with the appropriate agent and were incubated at
37°C in a shaking water bath. At the end of the incubation period, 500
μl of cold (4°C) 0.05 M sodium acetate buffer containing theophylline
and sodium azide, pH 6.2, were added to each tube. The cells were then
sonicated for 30 s, maintained on ice, and centrifuged for 20 min in a
microcentrifuge at 4°C. Duplicate 100 μl samples of supernatant were
aliquoted into glass test tubes and each tube received 100 μl of 1^251-
labeled tracer that was initially mixed with normal rabbit serum, fol-
lowed by the addition of 100 μl of antiserum. The tubes were vortexed
and incubated at 4°C for 16–18 h. One ml of 0.05 M sodium acetate
buffer was added to each tube and the tubes were centrifuged for 20
min at 4°C. The supernatant was totally aspirated and the pellet in each
tube was counted in a y set counter. For each experiment, a standard
curve was prepared, and the cAMP values were first expressed as pmol
cAMP/10^6 cells, and then as percentage of control.

RESULTS

Effects of Prostaglandins on Proliferation, Tyrosinase Activity,
and Cyclic AMP Levels of S91 Melanoma Cells. Melanoma
cells incubated for 24 h with PGE1 and PGE2 at concentrations
and Cyclic AMP Levels of S91 Melanoma Cells. Melanoma
results of one experiment that was repeated at least twice with similar findings.

of 0.1, 1, and 10 μg/ml exhibited dose-dependent inhibition of
proliferation and stimulation of tyrosinase activity. PGE1, at 10
μg/ml caused a 50% inhibition of cellular proliferation and an
11-fold increase in tyrosinase activity compared to diluent-treated controls (Table 1, Fig. 1). By comparison, PGE2 at 10

<p>| Table 1 Effects of PGE1, PGE2, PGA1, PGD2, and PGE2 on the proliferation of S91 melanoma cells |
|---------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Cell no. (x10^6 ± SE)</th>
<th>% of ethanol control (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.713 ± 0.018a</td>
</tr>
<tr>
<td>Ethanol, 0.1%</td>
<td>0.751 ± 0.012</td>
</tr>
<tr>
<td>PGE1, 0.1 μg/ml</td>
<td>0.652 ± 0.007</td>
</tr>
<tr>
<td>PGE1, 1 μg/ml</td>
<td>0.605 ± 0.025</td>
</tr>
<tr>
<td>PGE1, 10 μg/ml</td>
<td>0.358 ± 0.020</td>
</tr>
<tr>
<td>PGE2, 0.1 μg/ml</td>
<td>0.740 ± 0.008</td>
</tr>
<tr>
<td>PGE2, 1 μg/ml</td>
<td>0.740 ± 0.026</td>
</tr>
<tr>
<td>PGE2, 10 μg/ml</td>
<td>0.617 ± 0.014</td>
</tr>
<tr>
<td>Control</td>
<td>0.873 ± 0.022</td>
</tr>
<tr>
<td>Ethanol, 0.1%</td>
<td>0.891 ± 0.034</td>
</tr>
<tr>
<td>PGA1, 0.1 μg/ml</td>
<td>0.985 ± 0.028</td>
</tr>
<tr>
<td>PGA1, 1 μg/ml</td>
<td>0.983 ± 0.035</td>
</tr>
<tr>
<td>PGA1, 10 μg/ml</td>
<td>0.300 ± 0.016</td>
</tr>
<tr>
<td>PGB1, 0.1 μg/ml</td>
<td>0.888 ± 0.032</td>
</tr>
<tr>
<td>PGB1, 1 μg/ml</td>
<td>0.915 ± 0.025</td>
</tr>
<tr>
<td>PGB1, 10 μg/ml</td>
<td>0.552 ± 0.019</td>
</tr>
<tr>
<td>Control</td>
<td>0.518 ± 0.020</td>
</tr>
<tr>
<td>Ethanol, 0.2%</td>
<td>0.583 ± 0.033</td>
</tr>
<tr>
<td>PGE2, 0.1 μg/ml</td>
<td>0.532 ± 0.029</td>
</tr>
<tr>
<td>PGE2, 10 μg/ml</td>
<td>0.580 ± 0.019</td>
</tr>
</tbody>
</table>

* Values, mean of 3–4 determinations ± SE.
† Values are not significantly different from their respective ethanol control at
P < 0.05 as determined by Student's t test.

The effects of PGE1 on cAMP levels in melanoma cells were
determined using a radioimmunoassay. PGE2, resulted in a dose-
dependent increase in cAMP levels after only 30 min of contact
with melanoma cells. At 10 μg/ml PGE1, a 9-fold increase in
cAMP level above basal level was observed (Table 2). Neither
PGE2 nor PGD2 stimulated cAMP level.

Response of Cells to Different Durations of Treatment with PGE1. When cells were treated for 4, 6, 8, 12, or 16 h with 10
μg/ml PGE1, retardation of cell growth was evident as rapidly as 4 h after treatment (Fig. 1). However, the PGE1-induced
increase in tyrosinase activity was first evident 12 h after
treatment. At this time period, treated cells were proliferating
at a slower rate but expressed greater tyrosinase activity than
untreated cells.

Effects of Actinomycin D, Cycloheximide, and PTU on PGE1 Action. In order to determine if the PGE1-induced increase in
zyminase activity required de novo transcription and translation,
some culture flasks were treated with 5 ng/ml actinomycin
D, an inhibitor of mRNA synthesis or with 25 ng/ml cyclo-
heximide, an inhibitor of protein synthesis, with or without 10 μg/
ml PGE1. In these experiments, PGE1 alone induced a 5-fold increase in tyrosinase activity (Table 3). Higher concentrations
of actinomycin D (0.05 μg/ml) and cycloheximide (0.25 μg/ml) have been reported to inhibit the MSH-induced increase in
zyminase activity in Cloudman melanoma cells (10, 19). These
concentrations, however, were cytotoxic to the cells utilized in
out studies. As our data indicate, actinomycin D at 5 ng/ml and
cycloheximide at 25 ng/ml inhibited basal tyrosinase level by
25 and 56%, respectively, but had no effect on cellular
proliferation. When added concomitantly with PGE1, actino-
mycin D or cycloheximide suppressed the PGE1-stimulated
zyminase level by 60 and 67%, respectively, but did not alter
the inhibitory effect of PGE1 on cell growth (Table 3).

The effect of PTU, a tyrosinase inhibitor (9, 20), on the
cellular response to PGE1 was investigated. PTU alone at 5 x
10^-4 M or at 5 x 10^-5 M decreased basal tyrosinase activity by
25 and 50%, respectively (Table 4). The proliferative rate of
cells treated with either concentration of PTU was not different
from that of untreated control cells. When PTU was added
simultaneously with 10 μg/ml PGE1, the magnitude of tyrosinase
stimulation by PGE1, was decreased 80% by 5 x 10^-4 M
PTU, and was totally abolished by 5 x 10^-5 M PTU (Table 4).
However, the inhibitory effect of PGE1 on cell growth was not
EFFECTS OF PROSTAGLANDINS ON S91 MELANOMA CELLS

![Graph showing effects of prostaglandins on tyrosinase activity](image)

**Fig. 1.** The effects of PGA₁, PGD₂, PGF₂α, PGE₁₀, and PGE₂ on tyrosinase activity of S91 CCL 53.1 melanoma cells. Cells were seeded at 0.2 × 10⁶ cells/flask and were treated 48 h later with 0.1, 1, and 10 µg/ml PG for 24 h. Tyrosinase activity was determined 24 h after the simultaneous addition of PGs and growth medium containing [³H]tyrosine. Values, mean of 8–12 determinations ± SE. Each PG was tested twice.

<table>
<thead>
<tr>
<th>Tyrosinase Activity (Percent of EtoH Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>PGA₁</td>
</tr>
<tr>
<td>PGD₂</td>
</tr>
<tr>
<td>PGF₂α</td>
</tr>
<tr>
<td>PGE₁₀</td>
</tr>
<tr>
<td>PGE₂</td>
</tr>
<tr>
<td>PGE₃</td>
</tr>
<tr>
<td>PG (µg/ml)</td>
</tr>
</tbody>
</table>

**Table 1.** Effects of PGE₁, PGE₂, and PGD₂ on cellular cAMP levels

Cyclic AMP levels were determined by a radioimmunoassay after 30 min of incubating the cells with PGE₁, PGE₂, or PGD₂. Values were first expressed as pmol cAMP/10⁶ cells, and then as percentage of control.

<table>
<thead>
<tr>
<th>PMOL/10⁶ Cells</th>
<th>% of Ethanol Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.7</td>
</tr>
<tr>
<td>Ethanol, 0.1%</td>
<td>16.1</td>
</tr>
<tr>
<td>PGE₁₀, 1 µg/ml</td>
<td>56.0</td>
</tr>
<tr>
<td>PGE₂, 1 µg/ml</td>
<td>319.0</td>
</tr>
<tr>
<td>PGE₂, 10 µg/ml</td>
<td>913.0</td>
</tr>
<tr>
<td>PGE₃, 0.1 µg/ml</td>
<td>13.7</td>
</tr>
<tr>
<td>PGE₃, 1 µg/ml</td>
<td>113.7</td>
</tr>
<tr>
<td>PGD₂, 0.1 µg/ml</td>
<td>14.7</td>
</tr>
<tr>
<td>PGD₂, 1 µg/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>PGD₂, 10 µg/ml</td>
<td>16.1</td>
</tr>
</tbody>
</table>

* Values, mean of six determinations. All SE were less than 10%.

**DISCUSSION**

The growing interest of cancer researchers in prostaglandins stems from the ability of these agents to inhibit tumor cell growth. In particular, these agents retard the growth of murine and human melanomas (1, 2, 4–8). Like α-MSH, prostaglandins have been reported to stimulate adenylate cyclase and to increase melanin synthesis in Cloudman melanoma cells (9, 12). However, prostaglandins, which are known to be formed in abundance in the epidermis, have not been systematically studied as potential regulatory agonists of epidermal melanocytes. We have undertaken this and other studies to identify agents other than MSH which might regulate melanocyte growth and function.

Using a moderately melanotic S91 melanoma cell line, CCL 53.1, we investigated the effects of PGE₁ on cellular proliferation and melanogenesis and compared them to the effects of other prostaglandins. Following 24 h of treatment with PGE₁ or PGE₂, the cells exhibited a dose-dependent stimulation of tyrosinase activity and decreased cellular proliferation (Table 1, Fig. 1). The magnitude of the PGE₁ effects was greater than that of PGE₂ (Table 1, Fig. 1). PGA₁ and PGD₂ inhibited tyrosinase activity and suppressed cell growth (Table 1, Fig. 1).

It has been shown that PGD₂ is degraded in serum-containing culture medium at 37°C and in human plasma to Δ⁵⁹-PGJ₂ (21, 22). This metabolite was found to be responsible for inhibiting the growth of PGD₂-treated murine leukemia cells in culture (21). This might also be the case of Cloudman melanoma cells, since retardation of cell growth was evident after a minimum of 12 h of contact with PGD₂ (data not shown), a lag period which is possibly required for degradation of PGD₂ to its active metabolite. This possibility is currently under investigation.
PGA-i seems to be a potent inhibitor of melanoma cell growth \textit{in vitro} and \textit{in vivo} (2, 4–8), but, in humans, the physiological relevance of this effect is yet to be determined. PGF$\textsubscript{2\alpha}$ had no effect on either tyrosinase activity or cell growth after 24 h of treatment (Table 3). Both agents, however, of protein synthesis, effectively blocked the PGE$\textsubscript{i}$-induced inhibition of mRNA synthesis, and cycloheximide, an inhibitor of protein synthesis, effectively blocked the PGE$\textsubscript{i}$-induced increase in tyrosinase activity (Table 3). Both agents, however, did not alter the inhibitory action of PGE$\textsubscript{i}$ on proliferation. A minimum of 12 h of contact with PGE$\textsubscript{i}$ was required to stimulate tyrosinase activity (Fig. 2). It has been shown that when Cloudman melanoma cells were exposed to α-MSH, an increase in the cAMP level was detectable within 20 min, and stimulation of tyrosinase activity was measurable after a minimum of 6–8 h of hormonal treatment (9, 10). In our case, PGE$\textsubscript{i}$, which as been assumed to mimic MSH action (9), induced an increase in cAMP levels within 30 min, but the cells required a minimum of 12 h of contact with PGE$\textsubscript{i}$, before any increase in tyrosinase activity could be measured (Fig. 2). Using a modification of the charcoal absorption method of Pomerantz, we have found that after this 12–h lag period, the degree of [$\text{H}$]tyrosine utilization and $^3$H$_2$O release, a reflection of tyrosinase activity, was much greater in PGE$\textsubscript{i}$-treated than in untreated cultures. The magnitude of tyrosinase stimulation increased with increasing the time of cellular exposure to PGE$\textsubscript{i}$ (Fig. 2). The effects of PGE$\textsubscript{i}$ were reversible since no residual effects were evident 48 h after removal of the prostaglandin from the culture medium (data not shown).

The stimulatory effect of PGE$\textsubscript{i}$ on tyrosinase activity, like that of α-MSH, requires induction of transcription and translation (9, 10). Unfortunately the specific proteins or mRNA induced by PG or MSH have defied isolation by all investigators. We are attempting in subsequent studies to identify the specific products of gene expression. Actinomycin D, a known inhibitor of mRNA synthesis, and cycloheximide, an inhibitor of protein synthesis, effectively blocked the PGE$\textsubscript{i}$-induced increase in tyrosinase activity (Table 3). Both agents, however, did not alter the inhibitory action of PGE$\textsubscript{i}$ on proliferation.

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<table>
<thead>
<tr>
<th>Table 3 Effects of actinomycin D and cycloheximide on PGE$_i$ action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell counts</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Cell no. (x10$^6$)</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Ethanol, 0.1%</td>
</tr>
<tr>
<td>PGE$_i$, 10 µg/ml</td>
</tr>
<tr>
<td>Actinomycin D, 5 ng/ml</td>
</tr>
<tr>
<td>PGE$_i$ + actinomycin D</td>
</tr>
<tr>
<td>Cycloheximide, 25 ng/ml</td>
</tr>
<tr>
<td>PGE$_i$ + cycloheximide</td>
</tr>
</tbody>
</table>

$^a$ Values, mean of three determinations ± SE.

$^b$ Values, mean of six determinations ± SE.

$^c$ Values are not significantly different from the ethanol control at $P < 0.05$ as determined by Student's $t$ test.

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The procedure for this experiment is the same as in Table 4. The data presented are representative of one experiment that was repeated twice with similar results.

Table 5 Effects of dbcAMP and IBMX on PGE$_1$ action

<table>
<thead>
<tr>
<th>Cell counts*</th>
<th>Tyrosinase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell no. (x10$^4$)</td>
<td>% of ethanol control</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Control</td>
<td>0.506 ± 0.038</td>
</tr>
<tr>
<td>Ethanol, 0.01%</td>
<td>0.500 ± 0.035</td>
</tr>
<tr>
<td>PGE$_1$, 1 mg/ml</td>
<td>0.304 ± 0.019</td>
</tr>
<tr>
<td>dbcAMP, 10$^{-4}$ M</td>
<td>0.516 ± 0.036</td>
</tr>
<tr>
<td>dbcAMP + PGE$_1$</td>
<td>0.296 ± 0.010</td>
</tr>
<tr>
<td>IBMX 2 x 10$^{-4}$ M</td>
<td>0.388 ± 0.018</td>
</tr>
<tr>
<td>PGE$_1$</td>
<td>0.228 ± 0.014</td>
</tr>
</tbody>
</table>

* Values, mean of six determinations ± SE.

To further demonstrate that tyrosinase activity and cellular proliferation are regulated independently, we studied the effect of PTU, an agent known to inhibit tyrosinase activity (9, 23). At 5 x 10$^{-5}$ M, PTU totally abolished the stimulatory effect of PGE$_1$, on tyrosinase activity, without affecting the PGE$_1$-mediated reduction in cell growth (Table 4). It has been suggested that stimulation of melanogenesis results in increased production of metabolites that are toxic to the growth of melanoma cells (14). However, this does not seem to be the case with PGE$_1$. Inhibition of cellular proliferation by PGE$_1$, treatment preceded tyrosinase stimulation (Fig. 2) and was not altered when tyrosinase activation was totally blocked by PTU (Table 4). Similar results were obtained by Lotan and Lotan (23) who investigated the effects of PTU on the action of retinoids on S91 melanoma cells. Retinoids increased melanin synthesis and inhibited cellular replication. PTU blocked the effect of retinoids on tyrosinase, but did not alter their effect on cell growth. These results further support the contention that phenotypic expression, i.e., melanin synthesis, and proliferation are independently regulated in Cloudman S91 melanoma cells.

The effects of the known melanogenetic stimuli, dbcAMP and IBMX, were also investigated. IBMX (2 x 10$^{-4}$ M) was more effective than dbcAMP (10$^{-4}$ M) in elevating tyrosinase activity and in enhancing the PGE$_1$, effect on this enzyme (Table 5). More importantly, the enhancing effect of dbcAMP and IBMX on tyrosinase was not correlated with an equally remarkable retardation of cell growth. The concomitant addition of IBMX and PGE$_1$, increased tyrosinase level 9-fold above that observed with PGE$_1$, treatment only. This effect was accompanied by only a 10% reduction of cellular proliferation below that seen with PGE$_1$, alone (Table 5). These results again strongly suggest that the modulation of growth and melanization in melanoma cells are not necessarily coupled events.

Cyclic AMP is known to be the second messenger for PGE$_1$, action (12). An increase in cAMP has been reported to be required for increased tyrosinase activity (9, 10). This cyclic nucleotide has also been suggested to play a role in regulating melanoma cell proliferation (2, 23). PGE$_1$, increased cAMP levels in a concentration-dependent manner (Table 2). However, PGE$_2$, which had similar effects as PGE$_1$, on melanoma cell proliferation and tyrosinase activity, had no effect on cAMP level. Also, PGD$_2$ inhibited both cellular growth and tyrosinase activity but did not stimulate cellular cAMP level (Table 2). It had been reported that other prostaglandins, such as PGF$_2$,$_{3a}$, increase cAMP levels, yet have no effect on melanoma cell growth (4). Also, it has been documented that α-MSH which elevates cAMP levels and increases tyrosinase activity (9, 10, 12) stimulates rather than inhibits the proliferative capacity of CCL 53.1 melanoma cells (15, 24). As for the role of cAMP in tyrosinase stimulation, factors such as retinoic acid and vitamin D, whose action does not necessarily involve activation of adenylate cyclase and increased cAMP levels, can stimulate tyrosinase activity (23, 25). These observations question the exact role of cAMP as a regulator of proliferation and melanin synthesis in murine melanoma cells and suggest separate mechanisms for the control of these two processes.

We are currently investigating whether or not an increase in the cAMP level is requisite for PGE$_1$, action on Cloudman S91 melanoma cells. Our preliminary data, using the adenylate cyclase inhibitor DDA (26), show that when the increase in cAMP level is blocked, the inhibitory effect of PGE$_1$, remains unaltered. Surprisingly, in the presence of DDA, the PGE$_1$,-induced tyrosinase stimulation is greatly enhanced. The effect of PGE$_1$, and DDA on the cAMP-dependent protein kinase is also being investigated.

ACKNOWLEDGMENTS

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REFERENCES


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