Effect of Triamcinolone Acetonide on Tyrosinase Activity in a Human Melanoma Cell Line

Dennis M. DiSorbo,1 Nancy A. Harris, and Larry Nathanson

Oncology Research Laboratory [D. M. D., N. A. H., L. N.], Nassau Hospital, Mineola, New York 11501, and State University of New York [D. M. D., L. N.], School of Medicine, Stony Brook, New York 11790

ABSTRACT

The synthetic glucocorticoid, triamcinolone acetonide, was found to increase melanogenesis in the human melanoma cell line NEL. Treatment of NEL cells for 24 hr with triamcinolone acetonide (1 × 10^{-7} M) increased the activity of the enzyme tyrosinase by 43% and the incorporation of the melanin precursor, L-3,4-dihydroxyphenylalanine, by 23%. Additional studies revealed no change in cyclic AMP levels over an 18-hr test period. A 2-hr preincubation of NEL cells with actinomycin D (10 µg/ml) prevented the increase in tyrosinase activity by triamcinolone acetonide. When triamcinolone acetonide was added to a synchronized population of NEL cells, an increase in tyrosinase activity was observed at 16 hr, coinciding with the late S phase of the cell cycle. These results suggest that glucocorticoids are involved in the regulation of melanogenesis in NEL cells by increasing the activity of the rate-controlling enzyme tyrosinase.

INTRODUCTION

We have recently shown that a human melanoma cell line contains a significant amount of glucocorticoid receptors (7). In this study, we showed that treatment of these cells with the synthetic glucocorticoid triamcinolone acetonide produced a 30% reduction in cell growth. The exact mechanism by which triamcinolone acetonide produced the inhibition in cell proliferation was not ascertained, but experiments did reveal that incubation of these cells with triamcinolone acetonide resulted in a stimulation of DNA synthesis after 4 hr, followed by an inhibition of DNA synthesis after 18 hr of treatment. These results suggest that triamcinolone acetonide could be inducing the synthesis of proteins which are then involved in the growth regulation of melanoma cells.

In addition to the above study and other published reports (3, 6, 10) on the growth regulation of melanoma cells by glucocorticoids, several investigators have shown that the growth of melanoma cells can be regulated by MSH,2 cyclic AMP, and phosphodiesterase inhibitors. Kreider et al. (12) showed that treatment of B16 melanoma cells with cyclic AMP retarded cell replication and enhanced melanogenesis, while Wick (20) demonstrated growth inhibition of S-91 Cloudman melanoma cells by cyclic AMP phosphodiesterase inhibitors. Kreider et al. (12) showed that treatment of B16 melanoma cells with cyclic AMP retarded cell replication and enhanced melanogenesis, while Wick (20) demonstrated growth inhibition of S-91 Cloudman melanoma cells following treatment with MSH and theophylline. Additional studies by Kornor and Pawelek (11) and Pawelek (16) showed that increased melanogenesis is related to the activation of tyrosinase by a cyclic AMP-dependent protein kinase. Thus, there appears to be a close relationship between melanization and cell growth.

As stated above, melanogenesis is controlled by the enzyme tyrosinase. Since an increase in melanogenesis is associated with an inhibition in cell replication, agents which stimulate the activity of tyrosinase should also inhibit cell growth. Thus, the growth inhibition of human melanoma cells by glucocorticoid treatment could be related to the activation of tyrosinase and ultimately to an increase in melanogenesis.

The present study was undertaken to ascertain if glucocorticoid treatment of human melanoma cells (a) affected the enzymatic activity of tyrosinase and (b) altered the incorporation of L-DOPA into melanin. The results presented in this paper clearly show that glucocorticoids stimulate both the activity of tyrosinase and the incorporation of L-DOPA into melanoma cells.

MATERIALS AND METHODS

Materials. L-[3-{14C}]DOPA (10.9 mCi/mmol) and L-[3,5-3H]tyrosine (45 Ci/mmol) were purchased from Amersham/Searle Corp., Arlington Heights, IL. The cyclic AMP 125I radioimmunoassay kit was purchased from New England Nuclear, Boston, MA. Triamcinolone acetonide, L-tyrosine, L-DOPA, and actinomycin D were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were of reagent grade.

Cell Culture. Human melanotic melanoma cells, designated NEL, were maintained as described previously (7).

L-[3-{14C}]DOPA Incorporation. NEL cells were plated in T-25 sq cm flasks (0.5 × 10^6 cells/flask) and allowed to stabilize for 24 hr before experimentation. At the start of the experiment, medium was removed from all flasks and replaced with serum-free medium. Triamcinolone acetonide (1 × 10^{-7} M) was added to one-half of the flasks, and vehicle (ethanol 0.1%) was added to the remaining flasks. The cells were incubated for an additional 24 hr, after which the medium was removed, replaced with serum-free medium containing L-[3-14C]DOPA (2 µCi/ flask), and incubated for an additional 90 min. Medium was then removed, and the cells were washed twice in PBS (Grand Island Biological Co., Grand Island, NY). Cells were trypsinized off of the substratum, placed into conical tubes containing medium plus 10% serum, and centrifuged. The supernatant fraction was removed, 1.0 ml of cold 10% TCA was added to the cell pellet, and the tubes were kept on ice for 15 min and then centrifuged. The supernatant fraction was removed, and the pellet was washed once in 10% TCA and then centrifuged. The cell pellet was dissolved in 1 N NaOH. Two hundred-µl aliquots were taken from each test tube and placed into scintillation vials containing an equal volume of 1 N HCI. Scintillation fluid (10 ml of Scinti-Vers; Fisher Scientific Company, Pittsburgh, PA) was added to each vial and counted in a refrigerated SL4000 liquid scintillation counter.

Cyclic AMP Assay. Cyclic AMP was measured exactly as described in the New England Nuclear radioimmunoassay kit.

Tyrosinase Assay. Tyrosinase activity was measured as described by Pomerantz (17) and as modified by Montefiori and Kline (15). Essentially, NEL cells were sonicated in 80 mM sodium phosphate buffer, pH 6.8. Two hundred µl of the broken cell suspension were added to a test tube containing 0.12 ml of sodium phosphate buffer, 0.04 ml of L-DOPA (1 × 10^{-4} M), 0.04 ml of L-tyrosine (10 × 10^{-4} M), and 2 µCi of L-[3,5-3H]tyrosine. To correct for nonspecific counts, buffer was substituted for...
Table 1. The effect of triamcinolone acetonide on cyclic AMP levels was studied in NEL cells. Cells were incubated with (•) 1 x 10^-7 M triamcinolone acetonide for various times and then assayed for cyclic AMP. The response of NEL cells to 1 x 10^-7 M MSH (□) is also shown. The results are the average of 3 well treatments. Error bars represent ± S.E. for triplicate samples.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation time (hr)</th>
<th>L-[3,4-C]DOPA incorporation (dpm/mg of protein)</th>
<th>% treated control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>24</td>
<td>26,844 ± 341</td>
<td>400</td>
</tr>
<tr>
<td>Triamcinolone acetonide (1 x 10^-7 M)</td>
<td>24</td>
<td>35,018 ± 655</td>
<td>123</td>
</tr>
</tbody>
</table>

* Mean ± S.E. for triplicate samples.

† Difference was statistically significant (p < 0.001) from controls.

RESULTS

L-DOPA Incorporation. It has been shown that L-DOPA is selectively incorporated into pigmented cells (21). Thus, our initial experiment was to determine the effect of glucocorticoids on the incorporation of L-[3,4-C]DOPA into NEL cells. The results of this study are shown in Table 1. When NEL cells were incubated with vehicle or triamcinolone acetonide (1 x 10^-7 M) for 24 hr and then pulsed with L-[3,4-C]DOPA, a 23% increase in L-[3,4-C]DOPA incorporation was seen in the triamcinolone acetonide-treated group compared to the control flasks.

Cyclic AMP Levels. There have been reports that glucocorticoids increase cyclic AMP levels by a reduction in 3',5'-AMP phosphodiesterase activity (14, 18). Since cyclic AMP has been shown to control melanogenesis, the increase in L-[3,4-C]DOPA incorporation by triamcinolone acetonide could be due to an increase in cyclic AMP levels. To test this possibility, NEL cells were incubated in the presence and absence of steroid and assayed for cyclic AMP activity. As can be seen in Chart 1, there was no difference in the levels of cyclic AMP between control cultures and glucocorticoid-treated cultures. Included in Chart 1 are the levels of cyclic AMP after NEL cells were exposed to MSH for 10 and 30 min.

Tyrosinase Activity. Since tyrosinase is the key enzyme controlling melanogenesis, we measured the activity of the enzyme after incubating NEL cells with and without triamcinolone acetonide. The results of the study are shown in Chart 2. Incubation of NEL cells with steroid had no effect on tyrosinase activity after 2 or 6 hr of treatment. However, after 12 hr of exposure to triamcinolone acetonide, tyrosinase activity was increased by 24% over that of controls. Maximum stimulation of tyrosinase activity was seen at the 24-hr time point.

Actinomycin D Inhibition of Tyrosinase Induction. The results shown in Chart 2 indicate that tyrosinase activity was not altered by steroid treatment until cells had been exposed to the steroid for at least 12 hr. These data suggest that triamcinolone acetonide may be inducing the synthesis of new enzyme rather than activating existing enzyme. To test this possibility, we
Clustering NEL cells. Results are the average of 

various times as described in "Materials and Methods." Results are the average of synchronized NEL cells. Mitotic cells were incubated with (O) or without (•) 1 x 10^{-7} M triamcinolone acetonide and pulsed with [3H]thymidine (5 μCi/flask) at followed by a 24-hr incubation with triamcinolone acetonide. The 

exposed to actinomycin D, triamcinolone acetonide did not stim 

ulate an increase in tyrosinase activity. However, if the cells were first 

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acetonide suppressed [3H]thymidine incorporation as early as 8 

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cells up in GL Therefore, we investigated the effects of triamci 

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varies with the cell cycle, it is possible that glucocorticoid treat 

have been shown to block cells in the d phase of the cell cycle 

pretreated NEL cells for 2 hr with actinomycin D (10 μg/ml), 

followed by a 24-hr incubation with triamcinolone acetonide. The 

results are shown in Table 2. When NEL cells were exposed to 

triamcinolone acetonide for 24 hr, there was a 50% increase in 

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exposed to actinomycin D, triamcinolone acetonide did not stim 

ulate an increase in tyrosinase activity.

Tyrosinase Activity in Synchronized Cells. Glucocorticoids 

have been shown to block cells in the G_s phase of the cell cycle 

(4, 8). Since we have not ascertained how tyrosinase activity 

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throughout the cycle. With respect to the cell cycle, triamcinolone acetonide suppressed [3H]thymidine incorporation as early as 8 hr after plating of mitotic cells (Chart 3). Although this suppress 

ion was observed throughout the cycle, triamcinolone aceton-

id-treated cells apparently entered S phase at the same time as cells cultured in the absence of steroid.

We next studied the effect of triamcinolone acetonide on tyrosinase activity in synchronized NEL cells. As shown in Table 3, tyrosinase activity was not affected by steroid treatment at the 4-hr time point. After 12 hr of incubation, an 18% decrease in activity was observed in the treated group. However, after the cells had been exposed to triamcinolone acetonide for 16 hr, a 59% increase in enzymatic activity was seen.

**DISCUSSION**

In a previous report, we showed that the human melanoma cell line NEL contained a specific glucocorticoid binding protein (7). Additional experimentation on this cell line indicated that the synthetic glucocorticoid triamcinolone acetonide produced a 30% reduction in cell proliferation over a 72-hr incubation period. These data, in conjunction with other reports on the presence of glucocorticoid receptors in melanoma tissue (3, 5, 6, 9, 10), suggest that glucocorticoids are involved in the regulation of melanoma cell growth.

Since there have been many reports suggesting that melanogenesis proceeds at the expense of cell proliferation, our initial studies were directed toward determining the effect of glucocorticoid treatment on the activity of the enzyme which controls melanogenesis, namely, tyrosinase. The data presented in this report clearly show that glucocorticoids indeed have an effect on the enzyme tyrosinase and on melanogenesis.

The data shown in Table 1 demonstrate that treatment of NEL cells with triamcinolone acetonide increased the incorporation of L-DOPA into these cells. Furthermore, this increase in L-DOPA incorporation is accompanied by a stimulation of tyrosinase activity (Chart 2), which can occur either through an increase in the activity of existing enzyme or by an increase in the synthesis of new enzyme. Other studies have shown that glucocorticoids stimulate tyrosinase activity in the Harding-Passey melanoma and the Cloudman S-91 melanoma. Abramowitz and Chavin (1) have shown that, in the Harding-Passey melanoma, corticoster 

tone treatment results in an elevation of cyclic AMP levels, with maximum stimulation at 15 min. These authors also indicated that corticosterone stimulated tyrosinase activity at 5 min and 4 hr. With respect to the Cloudman S-91 melanoma, corticosterone depressed cyclic AMP levels over 4 hr but stimulated tyrosinase activity during this time period (2). Both reports conclude that the early stimulation of tyrosinase activity (15 min) reflects a
direct action of cyclic AMP on existing enzyme, while the increased activity observed at 4 hr is probably due to the synthesis of new enzyme.

When we pursued this question in NEL cells, we found that, although triamcinolone acetonide treatment resulted in an increase in tyrosinase activity by 12 hr of incubation, no change in the level of cyclic AMP was observed throughout an 18-hr incubation period. This suggests that the triamcinolone acetonide-induced stimulation of tyrosinase activity is not due to a change in the activity of existing enzyme and, therefore, synthesis of new enzyme may be occurring at an increased rate. This premise is also supported by 2 additional pieces of evidence. First, preincubation of NEL cells with actinomycin D blocks the increase in tyrosinase activity by triamcinolone acetonide, suggesting that RNA synthesis is required for the glucocorticoid effect to be seen (Table 2). In addition, we show that the increase in tyrosinase activity is not due to an enrichment of the enzyme in a specific phase of the cell cycle. The data depicted in Chart 3 and Table 3 clearly show that triamcinolone acetonide increases the activity of tyrosinase in the late S phase of the cell cycle. Thus, the triamcinolone acetonide-induced increase in tyrosinase activity in NEL cells is probably due to the synthesis of new enzyme. However, we cannot totally rule out the possibility that glucocorticoids promote the synthesis of proteins which are somehow involved in the activation of tyrosinase.

We conclude from the present study that glucocorticoids influence melanogenesis in a human melanoma cell line by increasing the activity of tyrosinase. The regulation of melanogenesis may be one mechanism by which glucocorticoids influence the rate of cell proliferation.

REFERENCES

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