Transient Changes in Phospholipid Methylation Induced by Dexamethasone in Lymphoid Cells

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ABSTRACT

We have investigated the effect of dexamethasone on phospholipid methylation by chronic lymphatic leukemia cells in culture. Methyl transfer from S-adenosyl[methyl-3H]methionine into lipid fraction showed a sharp rise within 2 to 3 hr of dexamethasone treatment. After 6 hr of dexamethasone treatment, however, methylation decreased below the control levels and remained lower thereafter. Analysis of the lipid components indicated that the formation of phosphatidylmonomethylethanolamine was not affected by dexamethasone. However, phosphatidylcholine synthesis by the transmethylation pathway showed an initial increase followed by a decrease. The results point to the possibility that this effect may have physiological significance in the lymphocytolytic effects of glucocorticoids.

INTRODUCTION

Glucocorticoids have a variety of effects on the metabolism of various cell types. One of the consequences of treatment of steroid-sensitive lymphoid cells, lymphomas, and leukemias with glucocorticoids is cell lysis. The biochemical mechanism underlying this effect is poorly understood. Evidence from our past studies suggests that perhaps the lymphocytolytic effect of glucocorticoids is related to their action on the cell surface, since in several cell lines glucocorticoids cause profound changes in membrane components (12-14, 17, 19). Furthermore, marked changes in the physical properties of plasma membrane including increased fluidity have recently been noticed in dexamethasone-treated cells (2, 14).

In several membrane systems, changes in phospholipid methylation have been implicated in the alteration of various membrane-related functions such as signal transmission (9), beta-adrenergic receptor function (8), viral transformation (16), and mediation of vasopressin and angiotensin action (1). Phospholipid methylation is also known to increase membrane fluidity in some conditions (5, 8). Since dexamethasone increases membrane fluidity and alters the cell surface components, the possibility that this steroid affects phospholipid methylation was investigated. The results presented suggest that dexamethasone elicits changes in phospholipid methylation in CLL cells.

MATERIALS AND METHODS

Materials. PE, PME, and PDE were obtained from Grand Island Biological Co. (Grand Island, N. Y.). Sigma Chemical Co. (St. Louis, Mo.) supplied PC, SAM, S-adenosyl homocysteine, and other chemicals. [methyl-3H]SAM (64 mc/mmol) and [14C]choline chloride (52 mc/mmol) were purchased from New England Nuclear (Boston, Mass.). Amersham Corp. supplied [8,10-3H]oleic acid which was used for the synthesis of 2-[3H]oleophosphatidylcholine according to the method of Suzuki et al. (21).

Cell Culture. Cultured CLL cells of B-cell origin, kindly provided by Dr. Tsuneo Suzuki of this Department, were used in this study. The cells were maintained in this laboratory in suspension culture in Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 15% horse serum. The cells were split one to three weekly.

Assay of Methylenetransferase Activity. Determination of phospholipid methylation in cell microsomal fractions was based on the method of Crews et al. (3) as modified by Sastry et al. (20). Unless otherwise stated, the assay system contained 200 ¡ÀM [methyl-3H]SAM (5 ¡ÀM), 10 nm MgCl2, 0.1 nm EDTA and various concentrations of protein in a total volume of 50 ¡Ìl of 50 ¡ÌM Tris-HCl buffer (pH 8.0). Incubations at 37° for various periods were stopped by the addition of 3 ml of chloroform:methanol:HCl (1:1:0.02, by volume), and the mixture was shaken for 10 min. The extract was washed twice with shaking (2 x 10 min) with 2 ml of 0.1 M KCI in 50% aqueous methanol. The upper aqueous phase was aspirated off, the chloroform phase was evaporated to dryness at 80°, and the dried material was dissolved in 1 ml of methanol and transferred to liquid scintillation counting vials for measurement of radioactivity.

In experiments where the incorporation of the 3H-methyl group into various lipid components was examined, 5 ¡Ìg each of carrier PME, PDE, and PC were added to the chloroform extract before evaporation. The lipid components were separated by thin-layer chromatography on Silica Gel G Uniplates (Analtech, Newark, N. J.). The chromatograms were developed in a solvent system consisting of propanol:propionic acid:chloroform:water (2:2:1:1, by volume). The spots were identified with I2 vapors and were scraped into scintillation vials for radioactive determination.

Assay of Phospholipase A2 Activity. Measurement of phospholipase A2 activity was carried out by the method described by Suzuki et al. (21) using 2-[3H]oleophosphatidylcholine as the substrate. Radioactive oleic acid formed during the incubation was extracted and separated from other lipids by thin-layer chromatography on Silica Gel G in a solvent system consisting of chloroform:methanol:acetic acid:water (100:60:16:1.5, by volume).

Dexamethasone Treatment and Processing of Cells. Cells were inoculated (1 million cells/ml) in blake bottles containing 100 ml Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 15% delipidized horse serum. An ethanolic solution of dexamethasone was added to one set of bottles at a final concentration of 1 ¡Ìg/ml. An equivalent volume of ethanol was added to each control.

In those experiments where the incorporation of [14C]choline into cellular lipids was studied, the cells were treated with 2.5 ¡Ìg each of [14C]choline (specific activity, 52 mc/mmol). The cells were harvested at regular intervals and placed in 5 ml of chloroform:methanol (2:1, v/v). The total cellular lipids were isolated by the method of Folch et al. (4).

For the measurement of methyltransferase activity, cells were harvested by sedimentation at regular intervals after dexamethasone treatment. The cells were homogenized in a buffer (pH 7.4) containing 0.32 M sucrose, 1 mM potassium phosphate, and 1 mM MgCl2. The homoge-
nates were subjected to differential centrifugation for the isolation of microsomes. The microsomes were stored at -20° until use.

RESULTS

Dexamethasone at 1 µM levels decreased the cell growth of the CLL cells used in this study 24 hr after its addition (data not given). We observed no morphological changes in these cells during the period of this experiment. In this work, attention has been focused on the dexamethasone-induced biochemical changes which precede any noticeable effect on the growth.

Chart 1 shows that dexamethasone had no effect on the incorporation of choline into total lipids. However, both control and dexamethasone-treated cells responded to the addition of delipidized serum by increasing lipid synthesis.

We then examined the incorporation of radioactivity from [methyl-3H]SAM into the total lipid fraction by microsomes isolated from cells treated with dexamethasone for various time intervals. As shown in Chart 2, the transfer of methyl group into the lipid fraction increased significantly in microsomes isolated from dexamethasone-treated cells. This effect was noticeable as early as 2 hr after the addition of dexamethasone and peaked at 2 to 3 hr. This sharp increase was followed by a drop in the methylation to a level below that seen in control cultures. After the initial changes, the methyl incorporation remained consistently lower than the controls. It was also noticed that the presence of 10 mM S-adenosyl homocysteine blocked the methyl transfer (data not given). The data presented in Chart 2 also indicate that the magnitudes of the initial increase and the subsequent decrease in transmethylation elicited by dexamethasone are similar.

On analysis of the lipid fraction, most of the radioactivity could be recovered in the PC (Chart 3). Data presented in this chart represent the kinetics of the methyl transfer reaction. In these experiments, microsomes isolated from cells grown in the presence or absence of dexamethasone for 6 hr were used as the enzyme source. At the end of incubation, the lipid fraction was separated by thin-layer chromatography, and the radioactivity associated with PC was determined. The methyl transfer reaction increased with the time of incubation (Chart 3A). At all time intervals, the dexamethasone-treated cells showed lower levels of enzyme activity. Chart 3B shows that the reaction followed linearity with the amount of protein up to 300 µg. A marked decrease in the enzyme activity was also evident in dexamethasone-treated cells. A concentration dependence on the substrate was also noticed in the methyl transfer reaction (Chart 3C). Under all conditions, dexamethasone treatment caused a markedly lower enzyme activity. It was consistently noticed that a small percentage of the radioactivity was associated with PME (data not shown).

Studies conducted in Axelrod’s laboratory (6) have shown that the enzyme that catalyzes the formation of PME can be assayed by switching the optimal conditions used above to measure the methylation. Accordingly, the methyltransferase assay was carried out to measure the methylation of PME. The result is presented in Chart 4. Under the conditions of the experiment, the main product formed was PME. No difference between the control and dexamethasone-treated microsomes was noticed in their ability to form PME.

Table 1 compares the methyltransferase activity in microsomes isolated from cells treated with dexamethasone for 2 and 6 hr. Under all conditions, approximately 90% of the label was recovered in PC. Dexamethasone treatment for 2 hr stimulated methyl incorporation into PC by about 40%. On the other hand, when the cells were harvested 6 hr after the dexamethasone treatment, the methyl incorporation into PC appeared to be lower than the controls. A similar but small change in PDE was also noticed (Table 1). A minor increase in incorporation of the label

![Chart 1](https://example.com/chart1.png)

**Chart 1.** Incorporation of choline into total lipids. Cultured CLL cells of B-cell origin were grown in the presence of 1 µM dexamethasone in Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 15% delipidized horse serum. The cell density was 1 million/ml medium. Each flask received [14C]choline chloride (0.5 nCi/ml; specific activity, 52 mCi/mmol) at the time of incubation. The cells were harvested and the lipids were extracted according to the method of Folch et al. (4). Points, average of triplicate determinations.

![Chart 2](https://example.com/chart2.png)

**Chart 2.** Time course of the effect of dexamethasone on phospholipid methylation. Cells were set up as described in the legend to Chart 1. The cells were harvested at indicated time intervals after the addition of dexamethasone. Microsomes isolated from these cells were used in the enzyme assay. The reaction consisted of 200 µg of microsomal protein, 10 mM MgCl2, 0.1 mM EDTA, and 200 µM [3H]SAM, all in a final volume of 50 µl of 50 mM Tris-HCl buffer (pH 8.0). Incubation at 37° for 30 min was stopped by the addition of chloroform:methanol:HCl (2:1:0.02, by volume). The lipid extract was washed twice with 0.1 M KCl in 50% aqueous methanol. The radioactivity was determined in the chloroform phase as described in “Materials and Methods.” Points, average of triplicate determinations.
Glucocorticoid Effects on Phospholipid Methylation

Table 1: Effect of dexamethasone on methyl transfer

<table>
<thead>
<tr>
<th>Duration of treatment (hr)</th>
<th>Control (2)</th>
<th>Dexamethasone (2)</th>
<th>p</th>
<th>Control (6)</th>
<th>Dexamethasone (6)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>PDE</td>
<td>PME</td>
<td>PC</td>
<td>PDE</td>
<td>PME</td>
</tr>
<tr>
<td></td>
<td>(cpm x 10^9)</td>
<td>mg protein</td>
<td></td>
<td>(cpm x 10^9)</td>
<td>mg protein</td>
<td></td>
</tr>
<tr>
<td>Control (2)</td>
<td>21.37 ± 1.15</td>
<td>1.78 ± 0.13</td>
<td>0.97 ± 0.05</td>
<td>29.56 ± 0.84</td>
<td>2.39 ± 0.29</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>27.64 ± 0.61</td>
<td>2.50 ± 0.20</td>
<td>1.17 ± 0.75</td>
</tr>
<tr>
<td>Control (6)</td>
<td>23.18 ± 1.80</td>
<td>1.75 ± 0.06</td>
<td>2.86 ± 0.10</td>
<td>23.18 ± 1.80</td>
<td>1.75 ± 0.06</td>
<td>2.86 ± 0.10</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Note: Mean ± S.D. of triplicate assays.

A similar inhibition of the activity of this enzyme in glucocorticoid-treated cells would then lead to error in the measurement of PC. We therefore measured the activity of phospholipase A2 in these cells at 2-hr intervals up to 24 hr of dexamethasone treatment. No change in the phospholipase A2 activity was noticed during this period as a result of the hormone treatment (data not given).

To determine if there was any relation between glucocorticoid activity and the effect on transmethylation, several steroids at 1 µM concentrations were added to the cultures. After 6 hr of the hormone treatment, dexamethasone, prednisolone, and cortisol inhibited the methyl transfer while progesterone and deoxycorticosterone were not effective. The inhibitory effect at a concentration of 1 µM paralleled glucocorticoid potency: dexamethasone (51%); prednisolone (46%); and cortisol (32%). (In order to establish the specificity of these steroids, more studies at the early time intervals are needed. However, preliminary studies indicate the possibility of a structure function relationship. It was also noticed that addition of dexamethasone directly to the enzyme assay did not have any effect on the methyltransferase activity.)

**DISCUSSION**

Hirata and Axelrod (6) have implicated phospholipid methylation in various cellular regulatory processes. Evidence has been provided by these workers which indicates that the stepwise methylation of PE to form PC is catalyzed by 2 enzymes having distinct kinetic properties (20). Our experiments also point to a possibility that CLL cells possess 2 distinct methyltransferases.

Although these results indicate that dexamethasone does not...
appear to influence the formation of PME, it does seem to cause a dramatic fluctuation in the conversion of PME to PC. The physiological significance of this transient change in transmethylation is not clear but could result in changes in intramembrane levels of PME and PDE. Since accumulation of PME within the lipid bilayer can increase membrane fluidity, various related functions could be affected by this process.

Although membrane fluidity has not been directly measured in these cells, our previous studies in other cell lines have indicated that dexamethasone increases membrane fluidity (2, 14). Endocytosis, a process that is intimately related to the membrane fluidity, is also affected in dexamethasone-treated cells (18).

An effect on phospholipid methylation by dexamethasone was noticed as early as 2 hr after treatment. Honma et al. (10) noticed a decrease in phospholipid methylation in dexamethasone-treated mouse myeloid leukemia cells during differentiation. Our results are in agreement with this work. Since Honma et al. (10) were interested in the long-term effect of dexamethasone, they might have overlooked potential initial fluctuations in the methyl transfer. Since choline incorporation into lipids remained unaltered in dexamethasone-treated cells (Chart 1), the result reported here is not a general effect on the overall synthesis of phospholipids.

Moreover, the effect by dexamethasone on phospholipid methylation could be noticed even when the cells were grown in the presence of serum. Thus, the possibility that the observed changes are due to an unstable status of lipid synthesis due to the addition of delipidized serum is ruled out.

The physiological significance of this effect of dexamethasone remains to be explored. It is too speculative at present to propose that alteration of phospholipid methylation is a mechanism of glucocorticoid-induced growth inhibition of lymphoid cells. However, this paper gives further evidence suggesting that the plasma membrane may have a crucial function in the biological effects of steroid hormones, and it is possible that regulation of phospholipid methylation by glucocorticoids is relevant to their lymphocytolytic effects.

Acknowledgments

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REFERENCES

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