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DEXAMETHASONE INHIBITION AND PHORBOL MYRISTATE ACETATE STIMULATION OF PLASMINOGEN ACTIVATOR IN HUMAN EMBRYONIC LUNG CELLS[1].

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SUMMARY

Treatment of human embryonic lung cells with dexamethasone resulted in a decrease in plasminogen activator activity measured in the fibrinolytic assay. The decrease in activity could at least partially be explained by the presence of an inhibitory substance(s) based on the following observations of lysates of dexamethasone-treated vs. control cells: a) an increase in specific activity following subcellular fractionation; b) an increase in fibrinolytic activity following separation by gel electrophoresis; c) an increase in fibrinolytic activity following mild acid-treatment; and d) a decrease in urokinase-directed fibrinolytic activity in mixing experiments. Phorbol myristate acetate increased plasminogen activator activity without affecting the level of inhibitory substance.

INTRODUCTION

Plasminogen activator (PA) is a serine protease which is increased dramatically in several cell types upon malignant transformation. This enzyme catalyzes the conversion of the serum zymogen, plasminogen, to the active protease, plasmin. Thus, controlled release of PA is thought to be an important factor in

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Abbreviations used: Plasminogen activator, PA; phorbol myristate acetate, PMA; human embryonic lung cells, HEL; urokinase, UK; sodium dodecyl sulfate-polyacrylamide gel electrophoresis, $NaDodSO_4$-PAGE.

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regulation of localized fibrinolytic events. The increased production in malignant cells may be related to the invasive and metastatic properties of these cells. For these reasons it has become important to understand the factors controlling the expression of this enzymatic activity.

This report describes the modulation of PA activity in human embryonic lung (HEL) cells and contrasts the regulatory features in these cells with those of 3T3 cells, which we have studied in detail (8,13,14). For this purpose we have studied induction of PA by the potent tumor promoter, phorbol myristate acetate (PMA) (which has been shown to induce PA in several cell types in culture (1-6)), and the decrease in PA by dexamethasone as characterized by Rifkin (7).

MATERIALS AND METHODS

Cell culture. HEL cells were used between passages 13 and 18. The cells were plated at 1×10^6 cells/60 mm dish in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and antibiotics.

Preparation of samples. The cells were allowed to attach for 24 hours. On the following day, fresh medium and the dexamethasone or PMA were added. At the appropriate times, cell lysates were collected (after rinsing 2 times) in 0.5 ml 0.1 M Tris (pH 8.1) containing 0.1% Triton X-100. The samples were centrifuged for 5 minutes at 800 x g and the supernatants were collected and frozen for later assays of PA and protein. For subcellular fractionation, homogenates were prepared and nuclei were removed by centrifugation as described (8). The postnuclear supernatants were then centrifuged at 100,000 x g for 60 minutes. Lysates were acidified to pH 2.7 with glycine as described by Loskutoff and Edgington. They were then incubated at 37° for 60 minutes.

PA assays. PA was assayed by measuring plasminogen-dependent fibrinolytic activity as described (8). Each sample was assayed at least in triplicate and the values obtained represent the mean with an error of no more than 15%.

Urokinase (UK) inhibition assays. The amount of inhibition of UK-directed fibrinolytic activity was calculated from $\frac{\%\ \text{hydrolysis (UK + lysate)}}{\%\ \text{hydrolysis (UK alone)}}$ during a 1 hour incubation at 37°. The amount of cell protein in each sample was 2.5 µg and under the conditions used contributed <10% hydrolysis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis ($NaDodSO_4$-PAGE). Cell lysates were prepared for electrophoresis as described (13).

Miscellaneous. Dexamethasone and PMA were purchased from Sigma. Protein was estimated using the method of Lowry (10). UK was obtained from Abbott.

RESULTS

Dexamethasone-treatment results in an inhibitor of the fibrinolytic assay. Previous studies by Rifkin (7) have documented the dexamethasone-induced

decrease in fibrinolytic activity in HEL cells. We obtained a similar dose-dependent decrease in PA activity as described in that report. Several laboratories have reported decreases in PA activity which have been associated with the presence of inhibitory substance(s)*. The presence of inhibitory substances has been postulated on the basis of several lines of evidence: a) an increase in fibrinolytic activity following subcellular fractionation which was attributed to removal of a soluble inhibitor (9); b) an increase in fibrinolytic activity following NaDodSO$_4$-PAGE which was attributed to separation of inhibitory substances from the PA (11); c) an increase in fibrinolytic activity following mild acid-treatment which was attributed to the destruction of an acid-labile inhibitor (9); d) a decrease in UK-directed fibrinolytic activity in mixing experiments with cell extracts which was attributed to the presence of inhibitors in the extracts (9,12). Each of these methods was employed as a means of testing whether the dexamethasone-related decrease in PA in HEL cells could be explained by the induction of an inhibitor.

Subcellular fractionation of control and dexamethasone-treated cells is shown in Table 1. In contrast to the small increase in specific activity in the pellet from control cultures, there was a 2.7-fold increase in specific activity in dexamethasone-treated cultures. This large increase in specific activity with centrifugation has also been reported for endothelial cells and was attributed to the removal of a soluble inhibitor (9). The recovery of PA from NaDodSO$_4$-PAGE in control and dexamethasone-treated cells is shown in Table 2. In each sample only one PA species (~60K Daltons) was observed. The large increase in recovered vs. applied activity in dexamethasone-treated vs. control samples is consistent with the separation of an inhibitory substance from PA during electrophoresis. Similar results have been reported for conditioned medium from transformed WI-38 cells in which no activity was observed before electrophoresis and substantial activity was observed after electrophoresis

* Because the fibrinolytic assay is a two-stage assay depending on the proteolytic activities of both PA (or UK) and plasmin, in this report we will refer to inhibitory substances as potential inhibitors of either activity.

TABLE 1: Subcellular distribution of PA

Sample	PA		
	% hydrolysis/μg[1]		
	Control	Dexamethasone-treated	PMA-treated
Post-nuclear supernatant	29.3 (30)	5.4 (178)	73.3 (30)
100,000 x g pellet	33.9 (0)	14.4 (18)	76.0 (0)
100,000 x g supernatant	26.2 (0)	1.9 (0)	27.0 (0)

Post-nuclear supernatants were prepared by centrifuging out nuclei from cells broken by Dounce homogenization. The postnuclear supernatant was then centrifuged at 100,000 x g for 60 minutes to collect a 100,000 x g pellet and supernatant fraction.

[1] Numbers in parentheses refer to the % increase in activity following acid treatment.

(11). These results were attributed to the presence of a substance which masked the fibrinolytic activity and was separated out during electrophoresis (11). The effect of mild acid-treatment of lysates on PA activity is shown in Table 1. The increase in fibrinolytic activity following acid-treatment of control and dexamethasone-treated samples was 30% vs. 178%, respectively, thus indicating an

TABLE 2: Recovery of PA from polyacrylamide gels

Sample	PA		% Recovered
	Total % hydrolysis		
	Total applied	Total recovered	
Control	1486	276	19
10^{-9} M dexamethasone-treated	995	375	38
10^{-8} M dexamethasone-treated	285	219	77
PMA-treated	6725	1413	21

Lysates from cell cultures were collected and prepared for electrophoresis. The fibrinolytic activities of the total lysate and the 60K Dalton species eluted from the gels were measured.

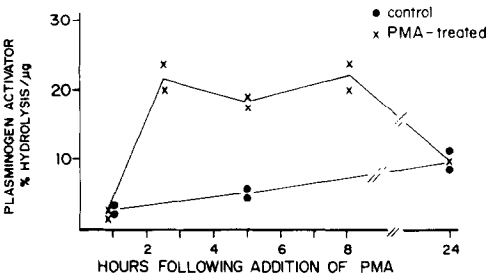

Figure 1. The effect of PMA on intracellular PA levels. Cells were treated with PMA (100 ng/ml) as described in Methods. Cell lysates were collected at several times following addition of PMA for measurements of fibrinolytic activity.

increase in the level of an acid-labile inhibitor in dexamethasone-treated samples. Endothelial cells have also been reported to contain an acid-labile inhibitor of fibrinolysis (9). Finally, the presence of an inhibitor was demonstrated through mixing experiments with cell lysates and UK. Using the same amount of cell protein in each assay, lysates from dexamethasone-treated cells resulted in 78% inhibition of UK. Lysates from control cells resulted in only 27% inhibition. Similar results have been reported for dexamethasone-treated vs. control cultures of hepatoma tissue culture cells (12).

PMA stimulates fibrinolytic activity. When HEL cells were exposed to PMA, there was an increase in PA within 2 hours (Fig. 1). Because this increase could be explained by increased PA, decreased inhibitor, or both, a comparison between control and PMA-treated cell samples was made with respect to subcellular fractionation, recovery from gels, and acid-treatment. The increase in specific activity of the 100,000 x g pellet vs. the post-nuclear supernatant was not substantial in either control or PMA-treated samples (Table 1). The recovery from $NaDodSO_4$-PAGE was the same for control and PMA-treated samples (Table 2). The amount of acid-stimulation in cell lysates of control and PMA-treated samples was 38 $\pm$ 7% and 54 $\pm$ 7%, respectively (mean $\pm$ SE, n = 8). These results indicate no significant change in the level of inhibitor accompanying PMA-treatment.

CONCLUSIONS

Treatment of HEL cells with dexamethasone leads to increased levels of an inhibitory substance which could be demonstrated by four independent assay procedures. The possibility remains open that dexamethasone-treatment also caused a decrease in enzyme levels because removal of inhibitory activity by centrifugation or acid-treatment did not restore activities of dexamethasone-treated samples to control levels.

These studies were initially undertaken to compare the regulatory features of PA in HEL cells to 3T3 cells. In contrast to HEL cells, we have found no evidence for the presence of an inhibitor in lysates of 3T3 cells either by increased specific activity with centrifugation or acid-treatment. The PA activity in 3T3 cells is associated with two molecular weight species, 75K and 49K Daltons. The ratio of 75K:49K activity is large in quiescent cells and small in mitogen-stimulated and growing cells (13,14). In contrast, HEL cells have one molecular weight species which does not change upon inhibition or stimulation. The regulation of PA in HEL and 3T3 cells, therefore, appears to occur through distinct mechanisms.

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