ACTIVATION OF CELL MEMBRANE ENZYMES IN THE STIMULATION OF CELL DIVISION*

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Summary - The tumor promoter, phorbol myristate acetate, increases the specific activities of the cell membrane enzymes Na^+-K^+-ATPase and 5'-nucleotidase in microsomal preparations from stationary cultures of BALB/c-3T3 mouse embryo fibroblasts. Under these conditions of *in vitro* exposure, the microsomal enzymes NADH diaphorase and glucose-6-phosphatase were not affected.

The potent tumor promoter for mouse skin, phorbol myristate acetate (PMA), binds to a cell membrane fraction of mouse embryo fibroblasts in culture (1). Further, PMA induces cells of a stationary monolayer of these fibroblasts to go through a division cycle (2,3). Since this induction of cell division is preceded by a remarkable increase in the refractility of the cells (4), we have attempted to determine whether the tumor promoter induces any functional alterations in cell membrane enzymes, in addition to the observed structural change. This report describes the enhancement of cell membrane enzyme activity by PMA in an *in vitro* system.

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MATERIALS AND METHODS

Cells of the BALB/c-3T3 line were used in this study (5). They were obtained from Dr. George Todaro, National Institutes of Health, and were propagated according to techniques described earlier (1,4,6).

Cells from monolayer cultures were harvested, disrupted and fractionated by procedures reported previously (1,4). The microsomal pellet (100,000 g x 1 hr) was used for the enzymatic assays. The assays for Na^+-K^+-ATPase and NADH diaphorase were carried out according to the methods of Wallach and Kamat (7). 5'-Nucleotidase activity was measured using the system described by Widnell and Unkeless (8) and glucose-6-phosphatase activity according to de Duve et al. (9). Protein assays were performed using the Lowry method (10) with bovine serum albumin fraction V as a standard. PMA was prepared as a concentrated solution (0.8 X 10^{-2}M) in dimethylsulfoxide and diluted to the appropriate concentration in the specific buffer for each enzyme assay.

RESULTS AND DISCUSSION

Figure 1 shows that addition of PMA directly to the incubation mixture for the assay of ATPase or 5'-nucleotidase resulted in an increase in specific enzymatic activity. These microsomal preparations are known to contain cell plasma membrane components (1,7). The enhancement of ATPase activity was nearly fourfold, while the activity of 5'-nucleotidase was increased by about 70 percent. The optimum effect with both enzymes was observed at a PMA concentration of 3.2 X 10^{-6}M. Preincubation of the microsomal preparation with PMA for up to one hour before assay for enzymatic activity gave similar results to those obtained when PMA was added at the time of assay.

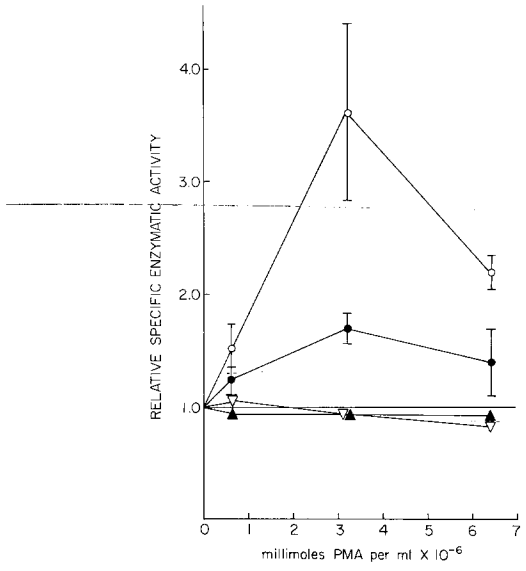

Figure 1 - Effects of PMA on cell membrane and microsomal enzymes. ATPase (0—0), control specific activity .073 µmoles phosphate released/mg protein/hour; 5'-nucleotidase (●—●), control specific activity .016 µmoles phosphate released/mg protein/min; NADH-diaphorase (▲—▲), control specific activity .057 µmoles NADH oxidized/mg protein/min; glucose-6-phosphatase (▽—▽), control specific activity .099 µmoles phosphate released/mg protein/min.

Over a wide dose range encompassing the effective concentrations of PMA for ATPase and 5'-nucleotidase activation, NADH diaphorase or glucose-6-phosphatase activities were unaffected (Figure 1). The absence of any increase of activity of the microsomal enzymes NADH diaphorase or glucose-6-phosphatase indicates that the observed increase in enzymatic activity was the result of a specific plasma membrane-PMA interaction.

The relationship between the activation of the membrane enzymes, Na^+-K^+-ATPase and 5'-nucleotidase, by the tumor promoter and the stimulation of cell division by PMA is not completely apparent from the available data. It is evident, however, that alterations of cell membrane interactions in several systems do have a marked affect on the controls that regulate cell division. Quastel and Kaplan (11), have observed that activation of lympho-

cytes with phytohemagglutinin (PHA) is dependent on an active transport of potassium ion mediated by a Na^+-K^+-ATPase. Further, they found that treatment of lymphocytes with the ATPase inhibitor, ouabain, prevented their transformation and cell division. Several reports have also implicated cyclic AMP as a mediator in PHA lymphocyte transformation suggesting the involvement of another cell membrane enzyme, adenyl cyclase, in this stimulation of cell division (12,13).

The role of the tumor promoter in the induction of division in stationary cell cultures appears to parallel the PHA induction of lymphocyte transformation and has several characteristics in common with it. These are 1) both cell populations are non-dividing, probably halted in the G_1 or G_o phase of the cell cycle (14,15); 2) the activating agents, serum or tumor promoters in monolayer cultures and plant lectins or antigens in lymphocytes, interact with the cell membrane (1,16,17); 3) enhancement of membrane enzyme activity is associated with induction of cell division (2,11).

Elucidation of the complete sequence of biochemical events linking the initial binding of an agent with a cell membrane to the nuclear events necessary for cell division will require considerable experimental effort. Yet, the first step in this sequence appears to involve enzyme activation induced by the binding of agentsto the cell membrane, possibly as a result of conformational changes, in appropriate proteins (17,18). Conformational changes associated with altered enzyme activity have been described for ATPase (19,20). Studies with appropriate enzyme inhibitors and additional cell membrane enzymes should aid in determining which of these enzymes may be important for the initiation of cell division.

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