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INHIBITION OF β-ADRENERGIC STIMULATION OF LYMPHOCYTE ADENYLATE CYCLASE BY PHORBOL MYRISTATE ACETATE IS MEDIATED BY ACTIVATED MACROPHAGES

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SUMMARY: Phorbol myristate acetate, a tumor promoter and lymphocyte mitogen, inhibits isoproterenol-stimulated adenylate cyclase in human peripheral blood mononuclear cells. Catalase and superoxide dismutase and depletion of macrophages from the cell preparations reversed this inhibitory effect. Phorbol myristate acetate did not inhibit isoproterenol-stimulated adenylate cyclase in purified T-cells or in membrane preparation from turkey erythrocytes. Thus, inhibition of adenylate cyclase activity by phorbol myristate acetate in human peripheral blood mononuclear cells is an indirect effect resulting from production of oxy radicals by activated macrophages.

A variety of cell membrane functions are altered following interaction with phorbol esters. These agents are potent tumor promoters (1) and induce mitogenesis in human lymphocytes (2,3). Recent reports have indicated that phorbol myristate acetate (PMA)[1] induces a loss of epidermal responsiveness to catechol amines in vivo via uncoupling of epidermal β-adrenergic receptors from adenylate cyclase (4,5). These observations prompted us to investigate the in vitro effect of PMA on isoproterenol-stimulated adenylate cyclase in human peripheral blood mononuclear cells (PBM). We found that PMA inhibited isoproterenol-stimulated adenylate cyclase in these cell preparations, but that the inhibition was not a result of the direct interaction of PMA with the responding lymphocytes. Oxygen-derived radicals produced by PMA-activated macrophages accounted for the inhibitory effect. In support of this conclusion is the finding that PMA did not inhibit isoproterenol-stimulated adenylate

[1]Abbreviations used: PMA, phorbol-12-myristate-13-acetate; PBM, peripheral blood mononuclear cells; SOD, superoxide-dismutase.

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cyclase in purified T-cells or in membrane preparations from turkey erythrocytes.

MATERIALS AND METHODS

PBM were isolated from heparinized blood of normal human subjects by Ficoll/Hypaque gradient density centrifugation, as previously described (6) and T-cells were isolated by a sheep erythrocyte rosetting method (7). Cells (10^7) were incubated in phosphate buffered saline containing Ca^{++} (1.0 mM), Mg^{++} (0.5 mM), glucose (1 mg/ml) and methylisobutylxanthine (20 µg/ml) with no further additions (basal), L-isoproterenol (10^{-6}M), or L-isoproterenol (10^{-6}M) with either PMA (100 ng/ml) or hemin (20 µM). Following 5 min incubation at 37°C, reactions were terminated by placing the tubes in boiling water for 3 min. Samples were then centrifuged (2000xg, 10 min) and 50 µl of supernate were used for determination of cAMP utilizing a commercially available radioimmunoassay kit (Amerscham).

Turkey erythrocyte ghosts were prepared and cAMP assayed as described by Steer and Levitzki (8). Preparations were incubated at 37°C for 20 min followed by heating at 100°C. The reaction was linear during 30 min of incubation. The complete incubation mixture contained, in a volume of 150 µl, Tris (40 mM), theophylline (0.4 mg/ml), bovine serum albumin (1.0 mg/ml), $MgCl_2$ (6.0 mM), creatinine phosphokinase (0.5 mg/ml), creatine phosphate (20 mM), ATP (2.3 mM), and 33 µg/ml of turkey erythrocyte membrane protein.

RESULTS

PMA inhibited isoproterenol-stimulated increases of adenylate cyclase in human PBM (Figure 1A), similar to its reported *in vivo* effect on mouse epidermis (4,5). We recently found that hemin shares some biologic properties with PMA. It is mitogenic for human lymphocytes (9), it activates oxidative metabolism in macrophages and induces luminol-dependent chemiluminescence in PBM (unpublished observations). We therefore determined whether hemin would mimic PMA in its inhibitory effect on isoproterenol stimulation of adenylate cyclase. The data depicted in Figure 1B indicate that this is indeed the case. Neither PMA nor hemin alone had a significant effect on basal levels of cellular cAMP.

Since PBM are a heterogenous population of cells, we determined the effect of PMA on isoproterenol-stimulated adenylate cyclase in purified T-cells and in the non-T-cell population. T-cells were purified by a sheep erythrocyte rosetting technique (7), and the remaining non-T-cells consisted of B-cells, macrophages and null cells. Isoproterenol stimulated an increase in adenylate cyclase in both sub-populations (Table 1A). However, while PMA markedly inhibited isoproterenol-stimulated adenylate cyclase in the non-T-cell

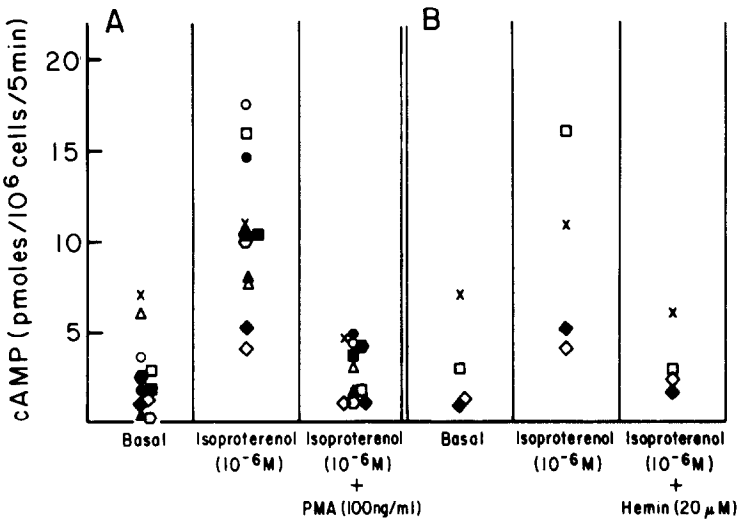

Figure 1. Effect of PMA and hemin on isoproterenol-stimulated increases in peripheral blood mononuclear cell adenylate cyclase. Each symbol represents the mean of duplicate results of separate experiments.

population, it resulted in little or no inhibition in the purified T-cells. In experiments 2 and 3 (Table 1A) PMA potentiated the effect of isoproterenol on T-cells and in experiment 1 (Table 1A) it resulted in some inhibition that was, however, less than that observed in the non-T-cell population.

The cell population in which PMA inhibited isoproterenol-stimulation of adenylate cyclase contained large numbers of macrophages. Since PMA (10) and hemin activate oxidative metabolism in macrophages and result in the generation of oxygen-derived radicals, we determined the effect of catalase and superoxide dismutase (SOD) on inhibition of isoproterenol stimulation of adenylate cyclase. Catalase decreased the inhibitory effect of PMA and eliminated that of hemin (Table 1B). SOD also decreased the inhibitory effect of these compounds, and the combination of SOD and catalase was most effective (Table 1B).

We next determined the effect of PMA on isoproterenol stimulation of adenylate cyclase in turkey erythrocyte membranes, a more well defined experimental system (8). Similar to our finding with the purified T-cell preparations, PMA had little inhibitory effect on the isoproterenol stimulated increase in adenylate cyclase (Table 2).

DISCUSSION

This study indicates that oxygen-derived radicals produced by PMA-activated macrophages are potent inhibitors of isoproterenol stimulation of

Table 1

A. Effect of PMA on isoproterenol-stimulated adenylate cyclase in sub-populations of human peripheral blood mononuclear cells.

	cAMP (pmoles/10^6cells/5 min) % of Control					
	Exp. 1		Exp. 2		Exp. 3	
Additions	Non-T Cells	T Cells	Non-T Cells	T Cells	Non-T Cells	T Cells
Isoproterenol (10^{-6}M)	548	567	615	571	536	202
Isoproterenol (10^{-6}M) +PMA (100 ng/ml)	110	268	111	1289	76	465

B. Effect of catalase and superoxide dismutase on isoproterenol-stimulated adenylate cyclase activity in PMA treated unfractionated human peripheral blood mononuclear cells.

	cAMP (pmoles/10^6cells/5 min) % of Control					
	Exp. 1			Exp. 2		
	Inhibitor			Inhibitor		
Additions	None	PMA (100 ng/ml)	Hemin (20/μM)	None	PMA (100 ng/ml	Hemin (20/μM)
Isoproterenol (10^{-6}M)	364	71	164	156	115	115
Isoproterenol + Catalase (1000 u/ml)	327	143	443	214	190	221
Isoproterenol + Superoxide dismutase (100 μg/ml)	-	-	-	184	222	162
Isoproterenol + Catalase +SOD	-	-	-	183	250	267

PBM and T-cells were isolated as described in the Methods. Results are expressed as % of control; that is as % of the cAMP value in cells containing no isoproterenol.

adenylate cyclase. The major inhibitory effect of PMA in our experiments appears to be related to its property of activating macrophages. Apparently PMA itself had little direct effect on the receptor mediated stimulation of adenylate cyclase. Previous studies have shown that treatment of spleen extracts with the oxidant dehydroascorbic acid also inhibits adenylate cyclase activity (11). It is of interest to note that guanylate cyclase and adenylate cyclase appear to be modified in opposite directions by oxidizing agents. The physiologic significance of our findings are unknown. However, since cAMP has a regulatory role in proliferation of lymphocytes and other cell types

Table 2
Effect of PMA on isoproterenol stimulation of adenylate cyclase in turkey erythrocyte membranes

	cAMP (pMoles/assay)			
	Exp. 1 Isoproterenol		Exp. 2 Isoproterenol	
Additions	-	+	-	+
None	1.1	10.2	2.4	7.0
PMA (100 ng/ml)	1.4	9.6	1.6	5.2

(12), some of the biologic effects of PMA may be related to its ability to activate oxidative metabolism in macrophages and thus influence cAMP levels in adjacent cells. Moreover, macrophages activated by other agents may participate in the regulation of inflammatory and immunologic responses via this mechanisms. It is possible that the reported effect of PMA on inhibition of isoproterenol-stimulated adenylate cyclase in epidermal cells is also related to PMA-induced activation of macrophage-like cells in the epidermis.

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