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Activation of protein kinase C inhibits prostaglandin- and potentiates adenosine receptor-stimulated accumulation of cyclic AMP in a human T-cell leukemia line

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Accumulation of cAMP in the human T-cell leukemia cell line Jurkat was stimulated by the adenosine analogue 5′-*N*-ethylcarboxamidoadenosine (NECA) and by prostaglandin E_2 (PGE_2). Addition of two phorbol esters, PDiBu and TPA, markedly enhanced the NECA-stimulated accumulation of cAMP whereas the PGE_2-stimulated cAMP accumulation was substantially reduced. The non-tumor-promoting phorbol ester, 4α-PDD, had no effect on either NECA- or PGE_2-stimulated cAMP accumulation. The ability of PDiBu to inhibit the effect of PGE_2 and to stimulate the effect of NECA remained in the presence a low concentration of forskolin (0.3 μM), which per se increased both NECA- and PGE_2-stimulated cAMP accumulation. Our results suggest that the effect of PK-C-activating drugs on receptor-mediated cAMP accumulation is entirely dependent on which receptor is being stimulated

Lymphocyte; Phorbol ester; Phosphorylation; Adenylate cyclase; Forskolin

1. INTRODUCTION

Activation of PK-C has been shown either to potentiate [1–4] or to inhibit receptor-stimulated accumulation of cAMP [5]. PK-C can be activated by stimulation with tumor-promoting phorbol esters, synthetic diacylglycerol derivatives and by receptor-mediated activation of phospholipase C leading to the formation of diacylglycerol from phosphatidylinositol [6,7]. It has been suggested that the explanation of the PK-C enhancement of cAMP accumulation is that the guanine nucleotide-binding protein (N_i or G_i), which mediates inhibition of adenylate cyclase, is inactivated by phosphorylation [4,8,9], but there are probably also other mechanisms [3,8–10]. For example, it was recently shown that purified PK-C can directly stimulate adenylate cyclase in membrane preparations from rat fat cells [15].

We recently showed that activation of PK-C in the human T-cell leukemia cell line Jurkat markedly potentiated the cAMP response induced by adenosine receptor stimulation [12]. By contrast, in the murine interleukin-2-dependent T-cell line CT6 both prostaglandin receptor-stimulated and basal levels of cAMP were reduced by IL-2 and phorbol ester, and by incubating membranes with semipurified PK-C [5]. By studying the effect of tumor-promoting phorbol esters on PGE_2 and adenosine receptor-mediated cAMP accumulation in a single line, Jurkat, we found that this dif-

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Abbreviations: cAMP, cyclic adenosine 3′,5′-monophosphate; PDiBu, β-phorbol-12,13-dibutyrate; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate; 4α-PDD, 4α-phorbol 12,13-didecanoate; NECA, *N*-ethyl-5′-carboxamidoadenosine; PGE_2, prostaglandin E_2; PK-C, protein kinase C; PCA, perchloric acid

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ference was due not to differences in cell line but to differences in which receptor was activated.

2. EXPERIMENTAL

2.1. *Cell culture*

The human T-cell leukemia cell line Jurkat was cultivated in RPMI-1640 supplemented with 7.5% fetal calf serum, 2 mM L-glutamine and antibiotics in a humidified incubator (37°C, 95% O_2/5% CO_2) at a cell density of 0.5×10^6/ml for 24 h before the assay.

2.2. *Assay conditions*

In all experiments the phosphodiesterase inhibitor rolipram was added to a final concentration of 30 μM, since it was found to block adequately the cAMP breakdown without interfering with adenosine receptors [13]. The incubations were performed in a shaking water bath (37°C) for 10 min in Hepes-buffered RPMI-1640 without fetal calf serum and terminated by the addition of PCA. Protein was removed by centrifugation and the cAMP content in the neutralized supernatants was determined with a protein binding assay [14].

3. RESULTS

Incubation of Jurkat cells with the adenosine analogue NECA induced a concentration-dependent accumulation of cAMP (fig.1). Maximal accumulation was obtained at 10–100 μM NECA. PGE_2 was more potent than NECA as a stimulator of cAMP formation (approx. 100-fold), but the maximal effect was smaller.

PDiBu alone had no effect on cAMP accumulation, but if added together with NECA the accumulation of cAMP was markedly potentiated. The highest concentration of NECA (100 μM), which alone produced a 9-fold increase of cAMP, increased the cAMP accumulation 16-fold in the presence of 100 nM PDiBu. The relative potentiation was 78% at 10 μM and 70% at 100 μM NECA.

The opposite effect was seen when PDiBu was added together with PGE_2. The effect of 1 μM PGE_2 was reduced from 29.8 pmol cAMP per 10^6 cells to 14.2, i.e. a reduction by 52%. The maximal effect of the phorbol ester was seen together with the second highest concentration of PGE_2 (100 nM) where the reduction was 69%.

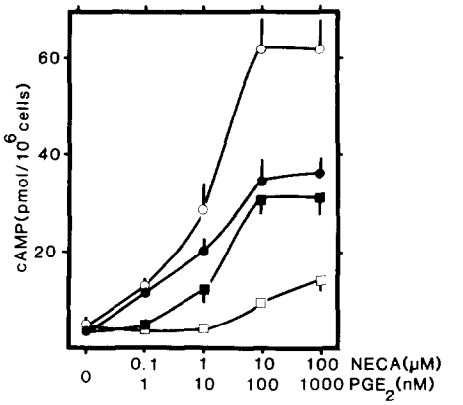

Fig.1. The effect of phorbol ester (PDiBu) on adenosine and prostaglandin receptor-mediated stimulation of cAMP accumulation in Jurkat cells. Jurkat cells were incubated for 10 min at 37°C in a shaking water bath with NECA (0–100 μM, circles) or PGE_2 (0–1000 nM, squares) in the presence of PDiBu (100 nM, open symbols) or vehicle (0.001% ethanol, closed symbols). Mean and SE from 6–9 determinations.

The potentiating and inhibiting effects of phorbol esters (fig.2) were further investigated by comparing different concentrations of the two

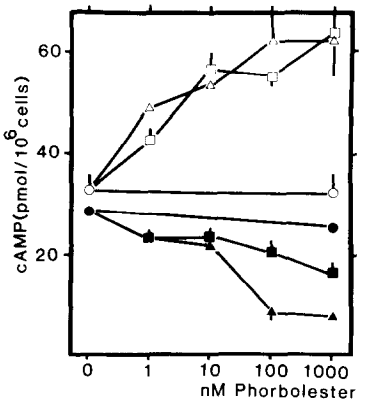

Fig.2. Dose-dependent effect of phorbol esters on PGE_2- and NECA-induced cAMP accumulation. Incubation conditions as described in the legend to fig.1. NECA 10 μM (open symbols), PGE_2 (filled symbols), PDiBu (triangles), TPA (squares), 4α-PDD (circles). Mean and SE from 3–9 determinations. The effects of TPA and PDiBu at concentrations above 1 nM on PGE_2- and NECA-induced cAMP accumulation were statistically significant ($p < 0.05$, or better) by Student's t-test.

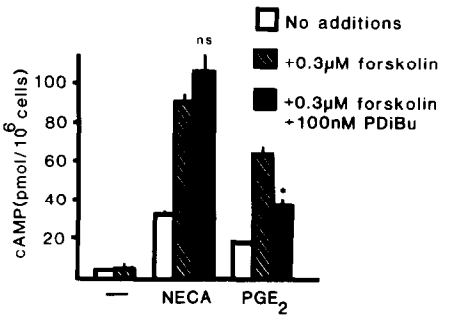

Fig.3. Effect of 100 nM PDiBu on the synergistic interaction between NECA (10 μM), PGE_2 (1 μM) and forskolin (0.3 μM). Mean and SE from 3 determinations. Statistical hypotheses were tested by Student's t-test.

tumor-promoting and PK-C-activating phorbol esters, PDiBu and TPA, with the inactive 4α-PDD [7]. Both PDiBu and TPA significantly increased NECA-stimulated cAMP accumulation and significantly decreased PGE_2-induced cAMP production already at 1 nM ($p < 0.05$), whereas 4α-PDD at 1000 nM was totally ineffective. The effect of TPA and PDiBu was also dose-dependent over the range 1–1000 nM. PDiBu appeared to be more effective than TPA as described earlier [3,12].

Forskolin at 0.3 μM had no effect on cAMP levels in the absence of added agonists, but if added together with NECA and PGE_2 a clear synergism was seen (fig.3) as described in other systems [15,16]. The effect of 10 μM NECA was increased by 178% and that of 1 μM PGE_2 by 241%. When 100 nM PDiBu was added together with 10 μM NECA and 0.3 μM forskolin, the cAMP response was 228% of that observed with NECA alone. Together with 1 μM PGE_2 and 0.3 μM forskolin the effect of 100 nM PDiBu was inhibitory, as in the absence of forskolin. The cAMP accumulation was clearly higher than with PGE_2 alone, but the potentiation was only 99%. Thus, the phorbol ester had reduced the response by more than 50%.

4. DISCUSSION

The important finding of the present study is that activation of PK-C by phorbol esters can modulate receptor-stimulated accumulation of cAMP in opposite ways depending on which receptor is being stimulated. Thus, the contradictory effects in the literature regarding the effect of PK-C activation on cAMP accumulation induced by adenosine analogues and PGE_2 [5,12,13,17] could be confirmed and extended by the present finding that even in the same cell the effect of PGE_2 was inhibited and that of adenosine was stimulated. Such differential receptor-receptor interactions are of potential biological significance.

The present data are also relevant for our understanding of how these interactions between phorbol esters and receptor-activated cAMP accumulation are brought about. Since the effects of PGE_2 and NECA were altered in opposite directions, the mechanism could not involve an interaction at a step that is common for the two receptor pathways. The potentiation of both the NECA and PGE_2 effects by a low dose of forskolin indicates that both types of receptors activate the adenylate cyclase in similar ways [15]. Thus, neither a direct effect on adenylate cyclase nor an effect of the interaction between $N_s\alpha$ ($G_s\alpha$) and adenylate cyclase can adequately explain the findings. It has been shown that phorbol esters stimulate the phosphorylation of transferrin, insulin, somatomedin C and α_1-adrenergic receptors [18–20] and that phorbol ester treatment significantly reduces the muscarinic receptor binding in cultured human neuroblastoma cells [21]. It is therefore an interesting possibility that the phorbol esters had stimulated the phosphorylation of both the adenosine and prostaglandin receptor protein and that this alters the way in which they interact with the GTP-binding protein(s) that affect adenylate cyclase.

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