Vol. 145, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1987 Pages 344-349

THE ADENOSINE RECEPTOR MEDIATED ACCUMULATION OF CYCLIC AMP IN JURKAT CELLS IS ENHANCED BY A LECTIN AND BY PHORBOL ESTERS

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Received December 4, 1986

SUMMARY. The accumulation of cyclic AMP in Jurkat cells was stimulated by adenosine and adenosine analogues. The accumulation of cyclic AMP induced by these agents was competetively antagonized by the adenosine receptor antagonist 8-p-sulphophenyl-theophylline (K_D appr 1.9 μM). The lectin PHA, the diacylglycerol OAG as well as tumor promoting phorbol esters enhanced the accumulation of cyclic AMP induced by the adenosine analogue NECA. The results suggest that activation of CD2/CD3 receptors by lectins could potentiate the endogenous cyclic AMP stimulator adenosine via activation of protein kinase C.

In the Jurkat cell, a human T-cell leukemia line, phytohemagglutinin (PHA) as well as anti-T3-antibody is able to stimulate the breakdown of phosphatidylinositol 4,5-bisphosphate (1), leading to i.a. an increase in cytosolic calcium (2,3). The two formed signals, diacylglycerol and inositol 1,4,5-trisphosphate ($InsP_3$), synergize to stimulate the activity of protein kinase C, which can also be activated by tumor promoting phorbol esters (4). It has been shown that in some instances phorbol esters, and compounds that activate protein kinase C via a receptor-mediated action, can enhance the effect of drugs or hormones that activate cyclic AMP formation (5-7). It is known that lymphocytes posses cell surface receptors for adenosine that are coupled to the formation of cyclic AMP (e.g. 8). Here we describe that Jurkat cells can be stimulated to form cyclic AMP by drugs that act on adenosine

Abbreviations: PHA - phytohemagglutinin; OAG - 1-oleyl-2-acetyl-glycerol; PDD-4-β-phorbol-12,13-didecanoate; PDiBu - phorbol-12,13-dibutyrate; TPA - 12-0-tetradecanoyl phorbol-13-acetate; NECA - 5'-N-ethylcarboxamido adenosine; $InsP_3$ - inositol 1,4,5-trisphosphate; 8-pst - 8-para sulphophenyl theophylline.

0006-291X/87 $1.50

receptors. This adenosine-receptor mediated cyclic AMP formation can be markedly enhanced either by PHA or by phorbol esters.

MATERIALS AND METHODS

5'-N-ethylcarboxamido adenosine (NECA) was from Boehringer, Mannheim, FRG, 12-0-tetradecanoyl phorbol-13-acetate (TPA), 4-α-phorbol-12,13-didecanoate and 4-β-phorbol-12,13-dibutyrate were from Bio Zac, Järfälla, Sweden. The phosphodiesterase inhibitor rolipram was from Schering AG., Berlin, FRG dipyridamole was from Boehringer, Ingelheim,FRG and 8-p-sulphophenyltheophylline was from Research Biochemicals, Wayland, Mass. Highly purified PHA was from Wellcome Laboratories, USA.

The Jurkat cells were maintained in RPMI 1640 medium supplemented with penicillin, streptomycin, L-glutamine (2 mM) and 7.5% fetal calf serum at 37° C in a humidified 5% CO_2, 95% air incubator. The cells were washed and resuspended in RPMI 1640 at a concentration of 10^6 cells/ml and preincubated for 40 min. Drugs were added and the incubation was continued for 10 min at 37° C in a water bath. The incubation was stopped by the addition of PCA to a final concentration of 0.4 M. The protein-free supernatant after centrifugation was neutralized with KOH and Tris and the cyclic AMP content was determined essentially as described before (8).

RESULTS

The basal level of cyclic AMP in the Jurkat cells varied between experiments from 1.3 to 6.3 pmol/10^6 cells. The reason for this variability is not known. In all experiments there was a clearcut stimulation of the cyclic AMP accumulation by the adenosine analogue NECA. The largest stimulatory effect was seen in the cells with the lowest initial content so that the difference in cyclic AMP content in the presence of 10 μmol/l NECA was smaller (between 20.7 and 53 pmol/10^6 cells). The effect of NECA was competetively antagonized by the adenosine receptor antagonist 8-p-sulphophenyl theophylline (8-pst, Fig. 1). The natural ligand adenosine at pmol/10^6 cells also, dose-dependently, raised the accumulation of cyclic AMP from 2.3 ± 0.5 to 11.4 ± 0.7 at 3 μmol/l and to 33.1 ± 1.2 pmol/10^6 cells at 30 μmol/l in the presence of the adenosine uptake inhibitor dipyridamole. This effect was also blocked by 8-pst (not shown).

PHA has no effect per se on the accumulation of cyclic AMP (Fig. 2), except at the very high concentration 100 μg/ml. However, there was a marked potentiation by PHA of the cyclic AMP accumulation induced by NECA, even though the concentration of NECA (10 μmol/l) was so high that the cyclic AMP accumulation was raised from 3.7 (n=21) to 32.8 (n=33) pmol/10^6 cells. The

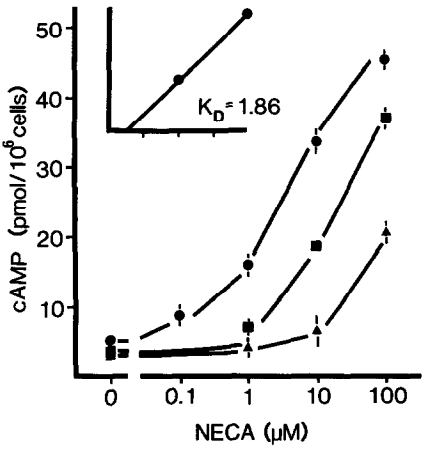
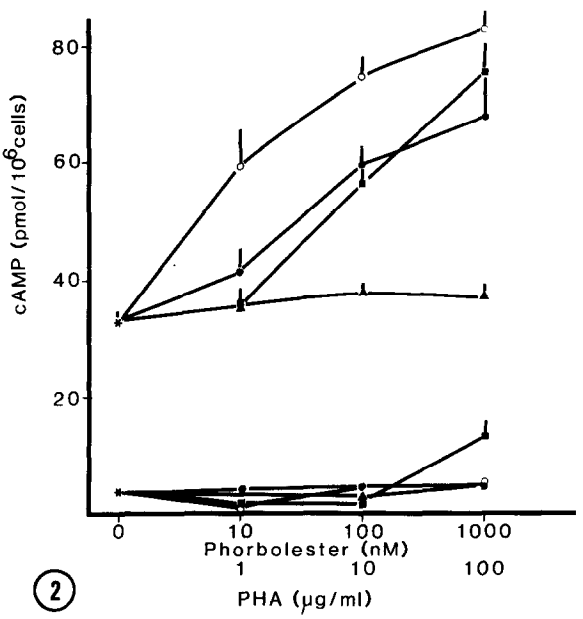


Fig 1. Adenosine receptor mediated accumulation of cyclic AMP in Jurkat cells. Jurkat cells were incubated with increasing concentrations of NECA ion the absence (filled circles) or presence of 8-p-sulpho phenyl theophylline (10 μM; squares or 100 μM; triangles). Results are mean and s.e.m. of triplicate determinations. The insert shows a Schild-plot of the data and the calculated K_D- (from pA_2) value for the antagonist expressed in the unit μmoles/l.



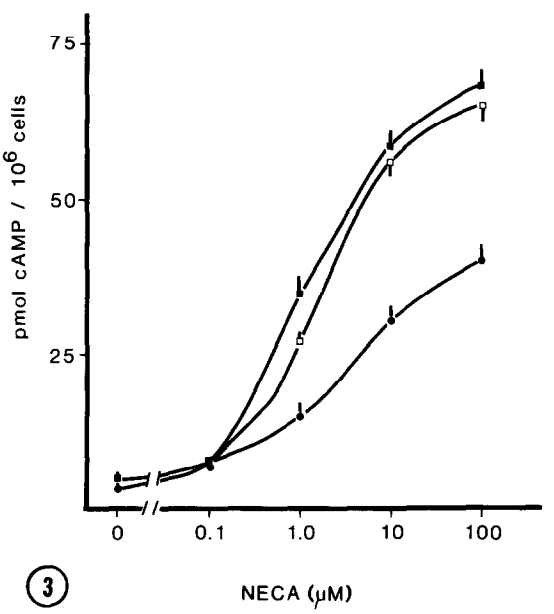


Fig 2. The effect of increasing concentrations of phorbolester or phytohemagglutinin on the accumulation of cyclic AMP in Jurkat cells. The experiments were carried out in the presence (upper four curves) and absence (lower four curves) of NECA 10 μmoles/l). Open symbols refer to experiments with PDiBu, closed circles to experiments with TPA, triangles to experiments with 4α-phorbol didecanoate and the squares to experiments with PHA. The results are given as mean and s.e.m. of 3-33 determinations.

Fig. 3. Potentiation of NECA-induced cyclic AMP accumulation by PHA (10 μg/ml, filled squares) and by TPA (100 nmol/l,unfilled squares). Jurkat cells were incubated at 0.5 x 10^6 cells/ml. TPA and PHA were added at the same time that NECA was added. Mean and s.e.m. of triplicate incubations in 2 - 3 separate experiments assayed in duplicate. Two other experiments with PDiBu gave similar results.

potentiation by PHA was dose-dependent and doses below 1 μg/ml were ineffective.

The stimulatory effect of PHA on NECA-induced cyclic AMP accumulation was mimicked by two tumor-promoting phorbol esters, TPA and PDiBu, but not by PDD (Fig. 2). It is known that the first two compounds are potent activators of protein kinase C, wheras the third one is not (4). OAG (50 μg/ml) was effective, but less so than TPA (not shown).

The effects of PHA and of the phorbol esters were observed over the entire dose-response curve for NECA (Fig. 3). Already at 0.1 μmol/l NECA there was a significant stimulation of the cyclic AMP accumulation by especially TPA (from 6.1 to 8.6 pmol/10^6cells). Phorboldibutyrate had a very similar effect whereas PDD was ineffective (not shown).

DISCUSSION

The present results show that Jurkat cells, as expected, can be stimulated to generate cyclic AMP by adding an adenosine analogue NECA and by adenosine itself. The fact that the effect of adenosine was enhanced by adding the uptake blocker dipyridamole (8, 11), and that the effect of adenosine as well as NECA was blocked by 8-pst, strongly implicates an adenosine receptor linked to adenylate cyclase. Indeed, adenosine receptors of the A_2-subtype, linked to adenylate cyclase, have been described previously in lymphocytes (14).

The major finding is that this adenosine receptor mediated increase in cyclic AMP accumulation was further increased by a lectin that acts on CD2/CD3 receptors (9, 10). The potentiation of the cyclic AMP accumulation by PHA is reminiscent of what is observed with α-receptor agonists in the CNS (5-7). There it is has been suggested that the synergism can be explained by the stimulation of protein kinase C secondary to elevation of intracellular calcium and accumulation of diacylglycerol. The findings presented here that the effect of PHA can be mimicked by tumor promoting phorbol esters and by

diacylglycerol derivatives support this contention. Furthermore, it has been found (1, 2, own unpublished observations) that PHA does lead to an accumulation of $InsP_3$ indicating an increased breakdown of phosphatidylinositolphosphate. To assume that PHA does lead to activation of protein kinase C therefore does not seem far fetched.

It is too early to ascribe biological relevance to our finding that adenosine effects on cyclic AMP may be enhanced by e.g. lectins. However, it should be remembered that adenosine is always present in tissues and body fluids in concentrations (0.1 - 2 μmol/l) that are sufficient to cause some degree of receptor stimulation, and that the concentration increases 10-fold or more upon hypoxia or physiological activation (11, 12). Therefore the possibility exists that the mechanism described here could be a means by which agents that activate phosphatidylinositol breakdown can raise cyclic AMP. Since cyclic AMP has a multitude of biological effects in the lymphocyte (13), this could add another dimension to the response. Interestingly, high concentrations of the T cell mitogen PHA actually suppresses proliferation. The finding that such high concentrations of the lectin, perhaps via protein kinase C- activation, increases cAMP might provide an explanation for this finding, since one of the effects of cAMP seems to be suppression of the immune response (cf 8, 13).

ACKNOWLEDGEMENTS

The present studies were supported by The Swedish Medical Research Council (proj. no. 2553), by Gustaf V 80 Years Fund and by Karolinska Institutet.

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