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Diol lipids are activators of protein kinase C

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Diol lipids (dioleoyl- and dioctanoylethylene glycol) at relatively low concentrations (~ 10 μM) were found to activate significantly protein kinase C in the presence of phosphatidylserine or phosphatidylinositol. Since diol lipids are widespread minor lipid constituents of many cells [(1974) Chem. Ind., 597–604], it has been suggested that they may be involved in the maintaining of basal protein kinase C activity in the absence of external stimuli.

Protein kinase C; Enzyme activation; Ethylene glycol diester; Diol lipid

1. INTRODUCTION

Fatty acyl or alkyl derivatives of ethylene glycol and other lower dihydric alcohols (diol lipids) are widespread minor constituents of animal, microbial and plant lipids (reviews [1–3]). Although the structure [4–8], biosynthetic pathways [9] and metabolism [10–12] of a number of diol lipids are known, their biological functions remain uncertain. Recently, dOcEG was found to inhibit diacylglycerol kinase, leading to a transient increase in diacylglycerol levels [13,14]. Since diacylglycerols function as intracellular second messengers activating PKC which plays a key role in several forms of signal transduction [15], it was of interest to study the effect of diacylethylene glycols on this enzyme. Here, we report the results of an investigation of the influence of dOcEG and dOlEG on the activity of rat brain PKC in the presence of PtdSer and PtdIns.

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Abbreviations: dOcEG, dioctanoylethylene glycol; dOlEG, dioleoylethylene glycol; dOlG, *rac*-1,2-dioleoylglycerol; PKC, protein kinase C; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine

2. MATERIALS AND METHODS

Diesters of ethylene glycol were synthesized by treatment of the latter with an excess of the appropriate acyl chloride and pyridine in chloroform. dOcEG and dOlEG were isolated by silica gel column chromatography, their authenticity being checked by IR spectroscopy and elemental analysis.

PtdIns, PtdSer, ATP, dithiothreitol, EDTA, EGTA, Tris and $CaCl_2$ were purchased from Serva; [γ-^{32}P]ATP was obtained from Amersham; histone H1 was isolated from calf thymus [16]. PKC was isolated from rat brain [17] and purified to near homogeneity on a TSK G-2000 SW gel (LKB) column (0.75 × 60 cm) in 20 mM Tris-acetate (pH 7.0), 0.2 M sodium acetate and 1 mM EDTA.

For assay of enzyme activity the modified method in [18] was followed; the reaction mixture (100 μl) contained 50 mM Tris-HCl (pH 7.0), 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM $CaCl_2$ or 1 mM EGTA, 8 kBq [γ-^{32}P]ATP and 20 μg histone H1; lipids were added as specified per experiment. Lipids for assay were prepared as in [18].

After enzyme addition the mixture was incubated for 10 min, then 40-μl aliquots were placed on carboxymethylcellulose filters (Whatman) and washed with distilled water. The ^{32}P radioactivity of histone was determined using an Intertechnique SL-40 counter.

3. RESULTS AND DISCUSSION

In contrast to previous data, according to which PKC from rat retina was not sensitive towards dOcEG [19], we found that in the absence of phospholipids both dOcEG and dOlEG stimulate

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rat brain PKC in a concentration-dependent and saturable fashion (fig.1). The effect of dOcEG was less pronounced than that of dOlEG whose action was quite similar to that of dOlG. EDTA greatly reduced the action of dOlG and completely abolished the effect of dOlEG (not shown). Activation of PKC was measurable at 2 μM dOlEG or dOlG and became maximal at ~10 μM neutral diacyl lipid.

Even lower concentrations of diol lipids markedly increased the affinity of PKC to PtdSer or PtdIns (figs 2,3, and table 1). In the case of PtdSer the apparent K_a value (10 ± 1 μg/ml) was shifted down to 3.2 ± 0.8 or 2.0 ± 0.2 μg/ml in the presence of 1 μM dOlEG or dOcEG, respectively. The effect of dOlEG on K_a coincided with that of dOlG (fig.2).

The enzyme appears to interact with PtdIns in a more complex manner. The Lineweaver-Burk plot (fig.3) may be interpreted as showing that PtdIns binds to two different sites of the PKC molecule which are characterized by the apparent activation constants $K_a^I = 17 \pm 1$ and $K_a^{II} = 2.4 \pm 0.3$ μg/ml. Alternatively, the break in the Lineweaver-Burk plot may be due to negative cooperativity or to heterogeneity of the enzyme preparation. The PKC sample used here was nearly homogeneous on SDS electrophoresis, however, earlier studies had demonstrated that rat brain PKC might exist in several isoforms [20].

In contrast to the PtdSer-PKC system, the effects of dOlEG or dOcEG on PKC activity differed from that of dOlG in the presence of PtdIns. At

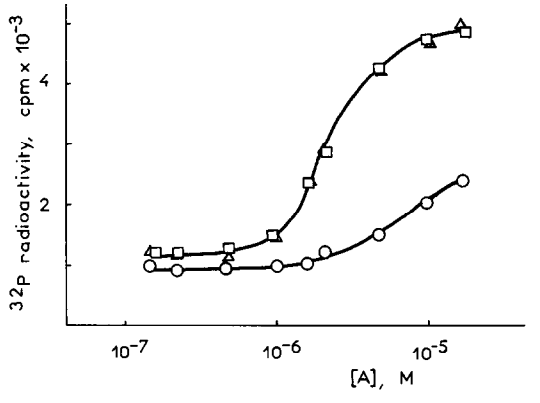

Fig.1. Dependence of PKC activity on concentration of diacylglycerol or diacylethylene glycols in the presence of 0.5 mM $CaCl_2$: dOlG (△); dOlEG (□); dOcEG (○).

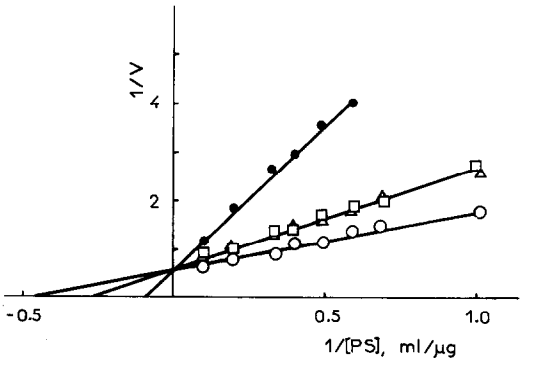

Fig.2. Lineweaver-Burk plots of the dependence of PKC activity on PtdSer concentration, [PS]: in the absence of diacyl lipid activator (●); 1 μM dOlG (△); 1 μM dOlEG (□); 1 μM dOcEG (○). The apparent K_a values are given in table 1. Means of three independent experiments.

1 μM, dOlG had no influence on the low activation constant K_a^I but caused an about 2-fold decrease in K_a^{II} (1.2 ± 0.3 μg/ml). At the same concentration dOlEG induced a 2-fold decrease in both constants ($K_a^I = 8.0 \pm 1.5$, $K_a^{II} = 1.2 \pm 0.4$ μg/ml). The effect of dOcEG was approximately the same as that of dOlEG with respect to the low activation constant K_a^I, however, it was much more pronounced for the high-affinity constant ($K_a^{II} = 0.5 \pm 0.05$ μg/ml) (fig.3).

These results may be biologically relevant in view of the relatively low concentrations of diol lipids used (dOlEG/phospholipid molar ratio

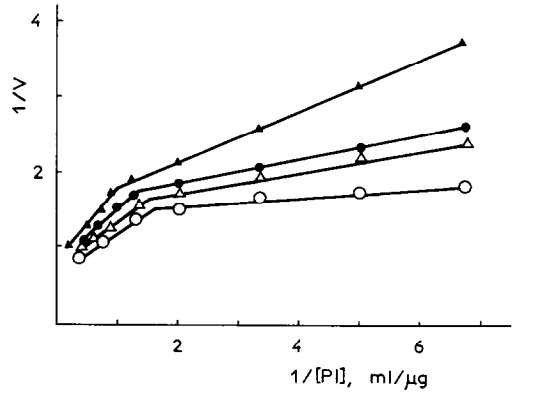

Fig.3. Lineweaver-Burk plots of the dependence of PKC activity on PtdIns concentration, [PI]: in the absence of diol lipid (▲); 1 μM dOlG (●); 1 μM dOlEG (△); 1 μM dOcEG (○). The apparent K_a values are given in table 1. Means of 5 independent experiments.

Table 1

Apparent activation constants (in μg/ml) of PKC by phospholipids in the presence of diacylglycerol and diacylethylene glycols

Diester	PtdSer[a] (K_a^I)	PtdIns[b]	
		(K_a^I)	(K_a^{II})
None (control)	10 ± 1.0	17 ± 1.0	2.4 ± 0.3
1 μM dOlG	3.1 ± 0.4	17 ± 1.4	1.2 ± 0.3
1 μM dOlEG	3.2 ± 0.8	8 ± 1.5	1.2 ± 0.4
1 μM dOcEG	2.0 ± 0.2	8 ± 0.8	0.5 ± 0.05

[a] Means of 3 independent experiments
[b] Means of 5 independent experiments

~1:40) corresponding to the diol lipid level in some cells [21], however their actual physiological significance still remains to be established. Diol lipids are metabolized much more slowly than diacylglycerols [12]. It thus seems possible that the relatively stable diol lipids may be involved in maintaining basal levels of PKC in resting cells whereas the short-lived diacylglycerols are second messengers responsible for PKC activation during receptor-mediated cell stimulation.

In this connection it may be of interest to note that the diol lipid level markedly increases in rapidly proliferating tissues [8,21,22] which are frequently characterized by enhanced basal PKC activity [23].

At present, the mechanism of interaction of ethylene glycol lipids with PKC is under investigation.

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