ACTIVATION OF PROTEIN KINASE C BY TUMOUR-PROMOTING AND NON-PROMOTING PHORBOL ESTERS

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The effects of many neurotransmitters and hormones are mediated by the breakdown of phosphatidylinositol (Berridge 1984). The products of this hydrolysis, inositol 1,4,5-triphosphate and diacylglycerol are second messengers respectively for Ca^{2+} mobilisation from internal stores and protein kinase C activation. This protein kinase is phospholipid dependent and its activity is enhanced on binding diacylglycerol. The administration of phorbol-esters induces a number of biological effects (Williamson et al 1981; Edwards et al 1983a,b 1985). TPA which has a broad range of activities has been shown to substitute for diacylglycerol in protein kinase C activation (Aitken 1985). We have studied the effects of a range of structurally related phorbols of varying biological actions for their effects on protein kinase C activation (Table 1).

Table 1. Ability of phorbol-esters to stimulate protein kinase C

Compound	Pro-inflammatory	PGE_2 stimulation	Platelet aggregation	Lymphocyte mitogenesis	Tumour Promotion	Protein kinase C stimulation
TPA	+++	+++	+++	+++	+++	+++
12-DPPA	++	+++	+++	+++	++	++
12-DPPAA	+	++	-	+	-	++
Prostratin	+	n/a	+	n/a	+	+
α-SAP	-	+	-	-	-	+
Phorbol	-	-	-	-	-	-

n/a = information not available. TPA, tetradecanoylphorbolacetate; 12-DPPA, 12-deoxyphorbolphenylacetate; 12-DPPAA, 12-deoxyphorbolphenylacetate acetate; Prostratin, 12-deoxyphorbolacetate; α-SAP, 4-deoxy-4α-phorbol-2-methyl-amino-benzoate

Protein kinase C was assayed (Parker et al) at 30°C using histone III-S as substrate. To a total volume of 40µl containing 12.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 1.25 mg/ml histone, [γ^{32}P]ATP, 0.03 mg/ml phosphatidylserine and phorbol-ester (60 pM to 500 mM), 5µl of enzyme solution was added. The reaction was terminated with 10% trichloracetic acid.

Although kinase C is believed to be the phorbol-ester receptor site, this is the first report concerning the correlation of activation of this kinase with a variety of biological effects. In general we have found that activation of protein kinase C correlates well with biological activity of phorbols. However both promoting esters (TPA, 12-DPPA, Prostratin) and non-promoting esters (α-SAP, 12-DPPAA) stimulated protein kinase C. It is possible that phorbol-ester induced tumour-promotion is the result of more than one biochemical event, or possibly in intact cells differences in lipophilicity prevent uptake of non-promoting compounds to their site of action.

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This work is supported by an S.E.R.C. project grant.