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ACTIVATION OF CALCIUM-ACTIVATED, PHOSPHOLIPID-DEPENDENT PROTEIN KINASE (PROTEIN KINASE C) BY NEW CLASSES OF TUMOR PROMOTERS:TELEOCIDIN AND DEBROMOAPLYSIATOXIN

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Summary: The new potent tumor promoters teleocidin and debromoaplysiatoxin, which are structurally unrelated to phorbol esters, activate Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C). The concentrations of 12-O-tetradecanoylphorbol-13-acetate, teleocidin and debromoaplysiatoxin for half-maximum activation of protein kinase C were found to be approximately 3 ng/ml, 40 ng/ml and 400 ng/ml, respectively. These three types of tumor promoters bind to protein kinase C, and appear to exhibit their pleiotropic actions through activation of this enzyme.

The pleiotropic actions of 12-O-tetradecanoylphorbol-13-acetate (TPA) appear to be mediated through activation of protein kinase C (1). This protein kinase is normally inactive, but a quaternary complex of protein kinase C, phorbol ester, Ca^{2+} and phospholipid is enzymatically fully active for protein phosphorylation (2). Under physiological conditions this protein kinase is activated by reversible association with membrane phospholipid directed by diacylglycerol, which is produced from inositol phospholipids in a signal-dependent manner (3,4). Tumor-promoting phorbol esters are intercalated into membranes, substitute for diacylglycerol, and activate protein kinase C directly without eliciting turnover of inositol phospholipids (1). New types of tumor-promoters structurally unrelated to TPA have been found in short term tests (5,6). These include teleocidin, a mixture of 93% teleocidin A and 7% teleocidin B, and debromoaplysiatoxin (Fig. 1), which are the potent tumor

Abbreviations used: TPA, 12-O-tetradecanoylphorbol-13-acetate; CREF cells, cloned rat embryo fibroblast cells; [^{3}H]PDPr, [20-^{3}H]phorbol-12,13-dipropionate; [^{3}H]PDBu, [^{3}H]phorbol-12,13-dibutyrate.

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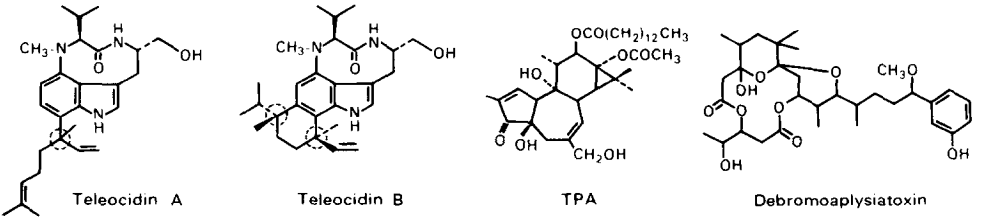

Fig. 1. Structures of tumor promoters.

promoters demonstrated in tests on mouse skin (7,8). Both teleocidin and debromoaplysiatoxin inhibit the specific binding of [^{3}H]PDBu to CREF cells (9,10) as well as the specific bindings of [^{3}H]PDPr and [^{3}H]TPA to a particulate fraction of mouse skin (11,12). These findings suggest that the three classes of tumor promoters, phorbol ester, teleocidin and debromoaplysiatoxin, may exert their tumor-promoting actions by interaction with a common receptor that is located on the surface membrane of cells (8). This paper briefly reports studies showing that teleocidin and debromoaplysiatoxin directly activate protein kinase C in the presence of Ca^{2+} and phospholipid.

Materials and Methods

Chemicals: TPA was purchased from P. Borchert, Eden Praire, MN. Teleocidin was isolated from mycelia of Streptomyces mediocidicus by a slight modification of a reported method (13,14). The teleocidin used for experiments was a mixture of teleocidin A and teleocidin B (14). Debromoaplysiatoxin, isolated from the blue-green alga Lyngbya majuscula (15), was kindly provided by Dr. Richard E. Moore, University of Hawaii. Calf thymus histone H1 was prepared as described previously (16).

Enzyme assay: A homogeneous preparation of protein kinase C was obtained from the soluble fraction of rat brain by the method described previously (17) Phosphatidylserine and other phospholipids were prepared as described previously (16). The assay was carried out in the presence of 20 μM $CaCl_2$, 20 μg/ml of phospholipid and various concentrations of each tumor promoter with about 0.1 μg of purified protein kinase C. Protein kinase C was assayed by measuring the incorporation of [^{32}P] into histone H1 from [γ-^{32}P]ATP as described previously (17).

Results and Discussion

Unlike tumor-promoting phorbol esters, neither teleocidin nor debromoaplysiatoxin have a diacylglycerol-like structure in their molecule, but nevertheless activate protein kinase C when added directly to purified cell-free systems. Maximum enzymatic activity was obtained in the presence of Ca^{2+} and phospholipid, and none of these tumor promoters alone activated the enyzyme. Kinetic analysis indicated that, as described for diacylglycerol (3,4) and TPA

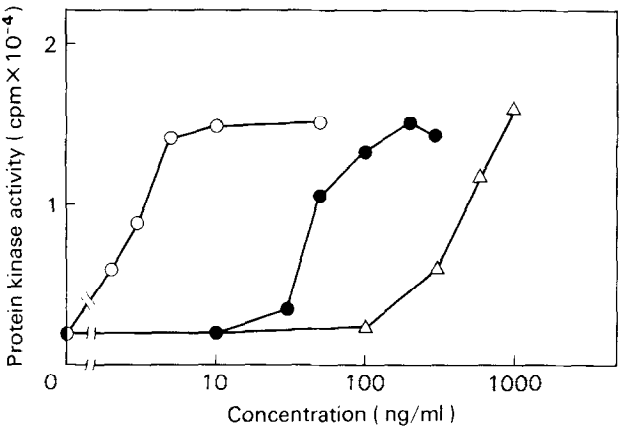

Fig. 2. Dose-dependent activations of protein kinase C by TPA (○-○), teleocidin (●-●) and debromoaplysiatoxin (△-△).

(1), these tumor promoters markedly increased the apparent affinity of protein kinase C for Ca^{2+} to less than the micromolar range, and thereby rendered the enzyme fully active without net increase in the Ca^{2+} concentration. The dose-response curves for these tumor-promoters are given in Fig. 2. Although both promoters had potent long-term effects, such as in tumor promotion (7,8), their abilities to activate protein kinase C *in vitro* appeared to be less than that of TPA; the concentrations needed for half maximum activation of protein kinase C were approximately 3 ng/ml for TPA, 40 ng/ml for teleocidin, and 400 ng/ml for debromoaplysiatoxin. The reason for this difference is not clear. Possibly the latter two tumor promoters are less lipophilic than the diterpene ester derivatives and may generate a lipid environment where the enzyme tends to be activated only when added at higher concentrations. In this connection it is noteworthy that Delclos *et al.* found using a phorbol ester photoaffinity probe that tumor-promoting phorbol esters interact primarily with phospholipids, not with protein (18), suggesting that they may activate protein kinase C by altering hydrophobic phospholipid protein interaction. Comparison of the stereochemical structures of TPA and dihydroteleocidin B by X-ray diffraction analysis has suggested that several functional groups of TPA overlap those of teleocidin B (19). Recently, Moore also found structural similarities between TPA and debromoaplysiatoxin by X-ray analyses (20). Presumably, the two classes

of tumor-promoters also modify the phospholipid bilayer structure in a similar manner to TPA or diacylglycerol.

Previously an approximate correlation was found between the activities of individual phorbol esters in tumor promotion and in activation of protein kinase C (1). Moreover the structural requirements of phorbol esters for tumor promotion in mouse skin were found to be roughly similar to those for binding to a specific cell surface receptor. Like these phorbol esters, both teleocidin and debromoaplysiatoxin were found to compete with [^{3}H]PDBu for binding to a receptor, presumably protein kinase C. With human platelets also it was demonstrated that TPA and teleocidin were intercalated into intact cell membranes and activated protein kinase C directly without change in other cellular constituents or mobilization of Ca^{2+}, as judged by the phosphorylation of its specific substrate protein, 40,000 dalton protein (21,22).

Protein kinase C is known to phosphorylate many endogenous substrate proteins at seryl and threonyl, but not tyrosyl, residues, and the potential roles of this enzyme in controlling cellular functions and proliferation have recently been suggested. For instance, vinculin (23), ribosomal S6 protein (24) and initiation factor 2 (ß-subunit) for protein synthesis (25) were found to act as its substrates. A possible cascade reaction from protein kinase C to tyrosine kinase has also been suggested, since the tyrosyl residues of some endogenous proteins may be phosphorylated when cells are treated with TPA or synthetic diacylglycerol (26). Presumably, protein kinase C lies on a common pathway eventually leading to tumor promotion. The actual target proteins of this unique protein kinase require further investigation.

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