THE PHOSPHORYLATION OF INTRACELLULAR PROTEINS BY TUMOUR-PROMOTING AND NON-PROMOTING PHORBOL ESTERS

S.F. Brooks[1], F.J. Evans[2], A. Drummond[3], A. Aitken[1], Dept. of Pharmaceutical Chemistry[1], Dept. of Pharmacognosy[2], Dept. of Pharmacology[3], the School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1 1AX

Phorbol esters have been reported to induce a variety of biological actions including tumour promotion, mitogenesis and platelet aggregation (Evans 1986). In response to many hormones and neurotransmitters phospholipid metabolism is stimulated, generating two second messengers, 1,2-diacylglycerol (DAG) and inositol trisphosphate. The latter is believed to be involved in the mobilisation of Ca^{2+} from internal stores, while DAG is the endogenous activator of the Ca^{2+} and phospholipid dependent protein kinase C (PKC). Following activation, this enzyme phosphorylates a number of proteins, a mechanism which has emerged as the most important in the covalent regulation of enzyme activity. Phorbol esters substitute for DAG as activators of this protein kinase.

We have studied a range of structurally related phorbols with diverse biological activities for their effects on protein phosphorylation in intact GH_3 cells. These cells are a rat pituitary cell line, which, in response to thyrotropin-releasing hormone, shows stimulation of phospholipid turnover and prolactin secretion. We are correlating the phosphorylation of particular proteins with the biological activity of the phorbol esters. Quiescent GH_3 cells were incubated for various times with ^{32}P-orthophosphate (100µCi/ml). 5 minutes prior to the termination of the labelling period, cells were stimulated with doses of the phorbols known to produce between 50 and 100% stimulation of PKC (Ellis et al). After washing, cells were homogenised and centrifuged at 100,000g to yield cytosolic and membrane fractions. Sodium dodecyl sulphate polyacrylamide gel electrophoresis was performed on both fractions to separate the proteins by their molecular weights, and subsequently, gels were autoradiographed at -70°C. ^{32}P incorporation into proteins was measured by densitometric scanning. We have shown that tetradecanoylphorbolacetate (TPA), a phorbol ester with a wide range of biological actions including tumour promotion, stimulated ^{32}P incorporation into many proteins (Fig.1). These results were largely mimicked by Sapintoxin A (SAP A) a pro-inflammatory phorbol which is a potent activator of PKC. However, 12-deoxyphorbolphenylacetate acetate (DOPPA), a non-promoting, non-platelet-aggregating phorbol, showed a marked reduction in the phosphorylation of proteins in the 120-130KDa range as well as the major band at 28-30KDa. Interestingly, DOPPA still activates PKC (Ellis et al).

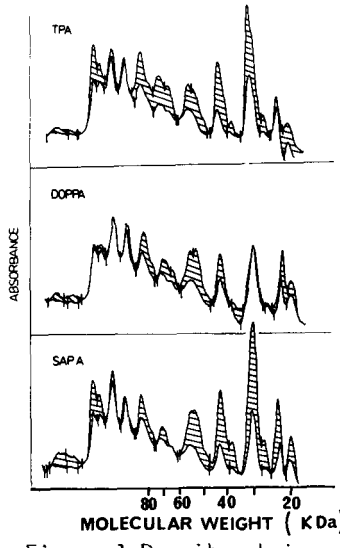

Figure 1 Densitometric scan of autoradiograph of GH_3 cell protein electrophoresis

From these results, one can conclude that phorbol esters with diverse biological activities differentially stimulate protein phosphorylation. This would imply that the phorbol esters do not exclusively activate PKC, or that they are acting at more than one site on the enzyme. Alternatively, variations in the uptake of the phorbol esters due to their different lipophilicities, or differential stimulation of the turnover induced by the phorbol esters within the cells could also lead to changes in protein phosphorylation.

Evans, F.J. (1986) Chap. 1 in "Naturally occurring phorbol esters" (ed. Evans, F.J.) CRC Press, Boca Raton

Ellis, C. et al (1986) Linnean Soc. Anniv. meeting, Kew, London.