E107 Protein Kinase C Isoenzymes by Growth Factors and Tetradecanoyl Phobol 13-Acetate (TPA) in Human Keratinocytes

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Growth control in many cell types has been linked to activation and subsequent down regulation of protein kinase C(PKC) isoenzymes. Subconfluent neonatal human keratinocytes were deprived of growth factors for 1 day, then treated with a concentration 200ng/ml of human recombinant epidermal growth factor(hrEGF), human recombinant insulin-like growth factor-1(hrIGF-1), with a combination of hrEGF and hrIGF-1, or with 50nM TPA. Cytosol and membrane proteins were extracted, separated by SDS-PAGE, and subjected to western blot analysis using PKC isoenzyme-specific antibodies. Treatment of cells for 1 day with EGF stimulated PKC-β II, -δ and -θ in the cytosol fraction, PKC -α, -β I, -δ, -ζ and -θ in the membrane fraction. IGF-1 stimulated PKC-β I, -ζ and -θ in the cytosol, PKC-α, -β I, -δ, -ζ, -ε and -θ in the membrane. In the cells treated with a combination of EGF and IGF-1, PKC-α, -β I, -ζ and -θ in the cytosol fraction, PKC-α, -δ, -ζ, -ε and -θ in the membrane fraction were stimulated. TPA treatment for 1 day caused down regulation of PKC-α, -β I and -θ in the cytosol, PKC-β I and -δ in the membrane but PKC-β II, -ζ and -ε remained essentially intact in both fraction.

E108 Some Properties of Chloragogenous Alkaline Phosphatase Isoenzymes from the Midgut of the Earthworm, *Eisenia andrei*

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Chloragogue tissue, the outer most layer of the earthworm intestine, is associated with various biochemical events such as glycogen-break down and bioactivation of xenobiotics. Because alkaline phosphatase(ALP) is thought to have important role in these biochemical processes, some biochemical properties were investigated. The native molecular weight of each isoenzyme appeared to be 200 kDa. The molecular weights of chloragogenous ALP isoenzymes determined by SDS-PAGE were 85 and 89 kDa, suggesting that these two forms are dimeric. Thermal stability of these two isoforms appeared to be very low. The pI of chloragogenous ALP was approximately 4.0-4.3. Each ALP isoenzymes appeared to be sensitive to levamisole. Zinc ion significantly inhibited all forms of ALPs to approximately 75% at the highest concentration tested. L-phenylalanine known as specific inhibitor for human and rat intestinal ALP, did not appear to be an effective inhibitor in this species. Imidazole and L-homoarginine were less effective than levamisole and zinc ion. The effect of L-tryptophan was marginal. The treatment of neuraminidase and PtdIns-PLC failed to change the migration rate of two isoforms, suggesting that sialic acid is not involved in carbohydrate side chain and phosphatidylinositol is not anchored. Also these forms of ALPs appeared to have the wide range of substrate specificity and among tested chemicals, glucose 1-phosphate was most effectively hydrolyzed by these forms.