[PC1-35] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

The GPI-specific PLC that releases renal dipeptidase from the GPI-anchor is present in detergent-insoluble GPI-rich membrane domains

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The activity of a glycosylphosphatidylinositol (GPI)-PLC that releases renal dipeptidase (RDPase, EC3.4.13.19) from the GPI-anchor was not modulated with the agents affecting the signal transduction via intracellular PI-PLC; carbachol (receptor agonist), AlF_4^- (G protein like activator) and 8-Br-cGMP (cGMP analogue) as well as U73122 (PI-PLC inhibitor), neomycin (PLC inhibitor), phorbol 12-myristate 13-acetate (protein kinase C activator) and staurosporin (protein kinase C inhibitor). These suggest that the GPI-PLC is distinct from intracellular PI-PLC. In contrast, the rapid release of RDPase with insulin, but no effect with epidermal growth factor, results from the hydrolysis of GPI-anchored RDPase by GPI-PLC forming the inositol 1,2-cyclic monophosphate cross reacting determinant. The GPI-PLC was inhibited with $HgCl_2$ and activated with $[Ca^{2+}]_i$ as was demonstrated in trypanosomal GPI-PLC. The monoclonal antibody raised against X domain of phospholipase Cδ4 reduced the GPI-PLC activity at the surface of proximal tubules indicating the cross-immunoreactivity and identified a single polypeptide of 54 kDa in the detergent (Triton X-100) insoluble GPI-rich membrane microdomain (DIG). These results strongly suggest that a GPI-PLC, RDPase-releasing-activity from the proximal tubules which is activated with insulin, is distinct from an intracellular PI-PLC, and that it is present in the DIG with RDPase.

[PC1-36] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Induction of inducible nitric oxide synthase by ceramide in human colon carcinoma HT-29 cell.

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Ceramide generated from sphingomyelin by hydrolysis has been shown to be a novel lipid second messenger for cellular functions ranging from proliferation and differentiation to growth arrest and apoptosis in various cell systems. To investigate the possible role of the sphingomyelin signaling pathway on nitric oxide (NO) production and expression of inducible nitric oxide synthase (iNOS), we studied the effect of synthetic ceramide (C6-ceramide) in human colon carcinoma HT29 cells. Ceramide increased NO synthesis in a concentration-dependent manner. N^G-methyl-L-arginine (NMA), a competitive inhibitor of iNOS blocked ceramide-dependent NO production. However, N^G-methyl-D-arginine (NMDA), an inactive form of NMA, did not show any significant change. Immunoblot analyses showed the expression of iNOS protein was stimulated by ceramide. Treatment of cells with bacterial sphingomyelinase (SMase) also enhanced iNOS expression. However, dihydroceramide, a biologically inactive ceramide, had no effect on iNOS induction. NMA suppressed induction of iNOS expression by ceramide. These results suggest that ceramide may modulate the cellular functions via increasing NO production and iNOS expression in human colon cancer cells.

[PC1-37] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Roles of ERK1/2 and p38 MAPK Signaling Pathways in Phorbol Ester -induced