isomerase related protein

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New signaling components of D3 dopamine receptor was searched using yeast two-hybrid system. The 3rd cytoplasmic loop of rat D3 dopamine receptor was used to screen the cDNA library of mouse brain, and protein disulfide isomerase related protein (PDIR) was found to interact with. The interaction in the yeast was observed specifcally with the 3rd cytoplasmic loop of D3 dopamine receptor but not with D2 or D4 dopamine receptor. The interaction between two proteins was also confirmed by co-immunoprecipitations from human embro kidney cells. When HEK 293 cells transiently transfected with D3 dopamine receptor were treated with 1 μM dopamine, PDIR was tyrosine phosphorylated. PDIR has the three CXXC-like motifs (Cys-Ser-Met-Cys, Cys-Gly-His-Cys and Cys-Pro-His-Cys), which are found in proteins within the PDI superfamily and are responsible for oxido-reductase activity. The functional significances of the interaction between these two proteins are under study.

[PA1-33] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Regulation of the M2 pyruvate kinase through direct interaction with the ITAM of the FcεRI gamma chain

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The downstream signaling components of high affinity IgE receptor (FcεRI) were studied using yeast two-hybrid screening of the cDNA library constructed from RBL-2H3 cells. The cytoplasmic part of the γ chain but not those of the β chain was found to interact with M2 type pyruvate kinase in the yeast, and this was further confirmed in RBL-2H3 cells by co-immunoprecipitation. Series of constructs were prepared for the cytoplasmic loop of γ chain, and tested for the interaction with pyruvate kinase in yeast. The Immunoreceptor Tyrosine based Activation Motif (ITAM) of γ chain was designated to be the domain required for the interaction of two proteins. Activation of FcεRI resulted in the phosphorylation of pyruvate kinase on tyrosine and serine residue, and decreased the affinity of the pyruvate kinase for the substrate without alteration in the maximum velocity of enzyme reaction. It was also demonstrated that both PI3 kinase and protein kinase C were involved in the regulation of the pyruvate kinase.

[PA1-34] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

G protein-mediated mitogen-activated protein kinase activation by dopamine D2, D3 receptors.

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The mitogen-activated protein kinase (MAPK) cascade is stimulated by both receptor tyrosine kinases and G protein-coupled receptors. Dopamine D2 and D3 receptors share similarities both in structural architecture and signaling pathway, and are known to activate MAPK. Using transiently transfected human embryo kidney cells (HEK 293), we further characterized the activation of MAPK by D2 and D3 dopamine receptors focusing on differences between two receptors. Stimulation of

D2 or D3 receptors with dopamine produced a rapid dose-dependent activation of MAPK. Activation was evident within 5 min, and showed maximum activation in 10 min, then gradually decreased by 30 min. The maximum activation was attained at 10μM of dopamine. However, D2 and D3 dopamine receptors showed some differences in MAPK activation such as ERK subtype specificity and pertussis toxicity sensitivity. Activation of MAPK mediated by both D2 or D3 dopamine receptors was not affected by coexpression of the C-terminus of beta-adrenergic receptor kinase (βARK), which selectively inhibits $G_{\beta\gamma}$-mediated signaling. Furthemore, co-expression of dominant-negative dynamin (K44A), an inhibitor of endocytic vesicle formation, did not affect MAPK activation mediated by both D2 or D3 dopamine receptors, suggesting that MAPK activation is not accompanied by the sequestration of D2 or D3 dopamine receptors.

[PA1-35] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

DMSO inhibits the degranulation of mast cells by affecting the signaling components of FcεRI

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DMSO, a non-polar solvent, is frequently used to dissolve the chemical compounds or natural products which are insoluble in water. However, DMSO is known to provokes various unwanted activites such as the stimulation of cell proliferation, also in our studies, over certain concentration, DMSO dose-dependently inhibited the antigen-stimulated degranulation of rat mast cells, RBL-2H3 cells. In accordance with this, we examined the effect of DMSO on the tyrosine phosphorylation of syk, PLCγ2, MAPK, and pyruvate kinase, the signal components of FcεRI (high affinity IgE receptor). At the concentration of 0.1 to 0.5%, DMSO did not have any effect on the tyrosine phosphorylation of Syk or PLCγ2. Interstingly, both pyruvate kinase and MAPK were tyrosine phosphorylated at concentration above 0.1 and 0.5%, respectively. Subsequent studies suggested that DMSO inhibits the degranulation of mast cells by modulating the pyruvate kinase activity.

[PA1-36] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

FcεRI negatively regulates of phospholipase C-γ2 through protein kinase C

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We have reported that the cytoplasmic part of the FcεRI-β chain interacts with PLC-γ2 and crosslinking of FcεRI causes the phosphorylation of PLC-γ2 both on tyrosine and serine residues. As subsequent studies we tested the roles of protein kinase C on the regulation of PLC-γ2 through FcεRI. When RBL-2H3 cells were treated with Go6983, a PKC subtype-specific inhibitor, the tyrosine phosphorylation of PLC-γ2 was potentiated. Meanwhile, the depletion of PKC by overnight incubation with PMA (0.1 μM) potentiated the tyrosine phosphorylation of PLC-γ2. It is well understood that the activation of PLCγ hydrolyses phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-triphosphate, which activates PKC and causes the release of Ca2+ from intracellular Ca2+ stores. Therefore, our observations suggest that PKC acts as a negative control over PLC-γ2.

[PA1-37] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]