

## 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 specifically 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 embryo kidney cells. When HEK 293 cells transiently transfected with D3 dopamine receptor were treated with 1  $\mu$ M 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 Fc $\epsilon$ R1 gamma chain

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The downstream signaling components of high affinity IgE receptor (Fc $\epsilon$ R1) were studied using yeast two-hybrid screening of the cDNA library constructed from RBL-2H3 cells. The cytoplasmic part of the  $\gamma$  chain but not those of the  $\beta$  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  $\gamma$  chain, and tested for the interaction with pyruvate kinase in yeast. The Immunoreceptor Tyrosine based Activation Motif (ITAM) of  $\gamma$  chain was designated to be the domain required for the interaction of two proteins. Activation of Fc $\epsilon$ R1 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