[PA1-30] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

EGF antagonizes TGF-beta 1 induced collagen lattice contraction by human skin fibroblast

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Wound contraction plays an important role in healing, but in the extreme condition, it may lead to excessive scar formation and pathological wound contracture. To date, the key regulator of excessive contracture is known to be TGF-beta 1. In this study, we have evaluated EGF antagonism in fibroblast-populated collagen lattice (FPCL) gel contraction, which has been generally used as one of in vitro model thought to mimic wound contraction in vivo. As expected, TGF-beta 1 treatment enhanced normal fibroblast-induced collagen gel contraction in a dose-dependent manner. In contrast, EGF did not affect normal gel formation, but significantly antagonized TGF-beta 1-induced gel formation ($P < 0.05$ at 100 ng/ml), whereas the other growth factor, PDGF, did not altered both nor normal and TGF-beta 1-induced gel contraction. Similarly, EGF treatment but not PDGF also significantly suppressed TGF-b1 release that is autologously elicited by TGF-beta 1 treatment ($P < 0.01$ at 100 ng/ml). Therefore, the results suggest that EGF may negatively regulate the role of TGF-beta 1, through attenuating autologous release of TGF-beta 1.

[PA1-31] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

The Effect of Hydrogen Peroxide (H2O2) in the Relaxation of Cat Lower Esophagel Sphincter

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It has been shown that the relaxation in cat lower esophageal sphincter (LES) tone by acid-induced inflammation may occur in part because of depletion of Ca^{++} store caused by H_2O_2. This study was performed to investigate the influence of hydrogen peroxide (H_2O_2) on signal pathway in the relaxation of cat LES using organ bath.
Vasoactive intestinal peptide (VIP; 1-100nM) caused dose-dependent relaxation of the resting tone by inducing cyclic AMP-and NO/cyclic GMP-mediated response. Preincubation with H_2O_2 reduced the relaxation induced by VIP. Sodium nitroprusside (SNP), 3-morpholino sydnomine (SIN-1), cGMP analog (8-br cGMP) produced dose-dependent relaxation, which were not attenuated by H_2O_2. The relaxation caused by the adenylate cyclase activator (forskolin), or by the stable cAMP analog (dibutyryl-cAMP) were not reduced or reduced. This might indicate that H_2O_2 leads to a change in receptor-mediated cAMP production. Preincubation with PTX led to attenuation on VIP induced relaxation in cat LES. This result suggests that VIP induced relaxation may be mediated by G_i-dependent pathway. The attenuation by PTX was additive with H_2O_2. These findings suggest that H_2O_2 acts on other pathway rather than G_i-dependent pathway in cat LES.

[PA1-32] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Direct interaction between D3 dopamine receptor and protein disulfide

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