Dept of Pharmacology, College of Pharmacy, Chung Ang University

It has been shown that SNP or SIN-1-induced decrease of the tone in cat LES is mediated via a NO/cGMP-dependent contraction. This study was performed using organ bath to define the participation of NO/cGMP signaling pathway on the relaxation in presence of contractile agent (carbachol). It was investigated the effect of soluble guanylate cyclase inhibitors, LY-83583 and 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ), on sodium nitroprusside (SNP), 3-morpholino-sydnomine (SIN-1), or forskolin-induced muscle relaxation. SNP caused dose-dependent relaxation of the contraction induced by carbachol. Preincubation with NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NNA) and LY-83583 had no influence on the relaxations induced by SNP. In contrast, the relaxation to SNP was blocked by ODQ. SIN-1 produced dose-dependent relaxation which was attenuated by L-NNA or ODQ, but not by LY-83583. Forskolin (0.1~10 mM) produced dose-dependent relaxation which was not inhibited by ODQ. These results suggest that SNP, or SIN-1-induced muscle relaxation in the presence of conteactile agent using cat LES is mediated by a cGMP/NO-dependent mechanism, which is similar to the relaxation to the resting tone

[PA1-35] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Effects of Ras farnesyltransferase inhibitor, SCH66336, on insulin actions in insulin-sensitive HIRc-B fibroblasts

Yi SJ°, Choi JE, Park SN, Ha JB, Pai JK # and Jhun BH

Laboratory of Cell Biology, Dept of Pharmacy, Pusan National University, Pusan, Korea and #Department of Tumor Biology, Schering-Plough Research Institute, New Jersey, USA

Ras has been shown to be a key regulator in the mitogenic signal transduction pathways of insulin. SCH66336, an orally bioavailable nonpeptide tricyclic farnesyltransferase inhibitor of Ras, is currently under clinical trials in cancer patients. In the present studies, we examined the effects of SCH66336 on the insulin signaling pathways in HIRc-B cells, Rat 1 fibroblast overexpressing human insulin receptors. The DNA synthesis, c-Jun expression and membrane ruffling induced by insulin were blocked by microinjection of GST-fusion dominant negative Ras, GST-RasN17, confirming that Ras protein is involved in the insulin mitogenic siganling pathways. The prenylation of an isoform of endogenous Ras in HIRc-B cells was inhibited by SCH66336 in dose-dependent manner. SCH66336 caused partial dose-dependent inhibition of DNA synthesis induced by insulin, while it did not affect cell viability, c-Jun expression, and membrane ruffling induced by insulin. These results indicate that 1) SCH66336 partly blocked insulin actions leading to mitogenesis of insulin and 2) isoform of Ras, probably K/N-Ras, which is unaffected by SCH66336, may be involved in insulin signaling pathways.

[PA1-36] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

The involvement of Kupffer cells in ischemia/reperfusion-induced hepatic injury

Lee YG°, Lee SH, Lee SM

College of Pharmacy, Sungkyunkwan University

Temporary interruption of hepatic blood flow is often required in the management of acute hepatic trauma and is obligatory during liver transplantation. Although growing evidences have been shown that Kupffer cells are involved in hepatic ischemic injury the mechanism of this injury still unclear. Therefore, these studies were designed to evaluate the role of Kupffer cells in hepatic ischemia/reperfusion in isolated perfused rat liver. Kupffer cells were destroyed selectively with gadolinium chloride treatment (GdCl3, 10mg/Kg) 2days prior to operation. Isolated rat livers from fasted 18 hours were preserved in UW (University of Wisconsin solution) at 4℃ for 24 hours after

flushing with Ringer's lactate. After that livers were immersed in Ringer's lactate at 37℃ for 20min and reperfused with Krebs Henselite bicarbonate buffer (KHBB, pH 7.4, 37℃). As the lactate dehydrogenase (LDH) and purine nucleotide phosphorylase (PNP) significantly increased after cold/warm ischemia and reperfusion. This increase was suppressed by GdCl3 treatment. The rate of carbon uptake of Kupffer cell slightly increased but this increase was also inhibited by GdCl3 treatment. In contrast, the oxygen consumption significantly decreased after cold/warm ischemia and reperfusion. Our findings suggest that Kupffer cells participate in the mechanism of injury of hepatic ischemia and reperfusion.

[PA1-37] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

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

Ryu H°, Kuo NY, Kim KM

Pharmacology Laboratory, College of Pharmacy, Chonnam National University

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 γchain but not those of the β chain was found to interact with pyruvate kinase in the yeast. A direct interaction between FcεRI and pyruvate kinase was also demonstrated by the co-immunoprecipitation in RBL-2H3 cells. The subtype of pyruvate kinase which interacts with γchain of FcεRI was revealed to be M2 type. Specially, the pyruvate kinase interacted with the Immunoreceptor Tyrosine based Activation Motif (ITAM) of γchain. Activation of FcεRI resulted in the decrease in the affinity for the substrate without alteration in the maximum velocity of enzyme reaction and the phosphorylation of pyruvate kinase on tyrosine and serine residue. Effects of wortmannin, genistein and protein kinase inhibitors in specific activity of pyruvate kinase were also determined.

[PA1-38] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Activation of D3 Dopamine Receptor Causes Phosphorylation and Intracellular Translocation of Elongation factor-1βγ

Cho DI°, Yang HJ, Kim KM

Pharmacology Laboratory, College of Pharmacy, Chonnam National University

The signaling pathway of D3 dopamine receptor was studied 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 the elongation factor-1 (EF-1) was found to interact with it. The interaction in the yeast was observed only with the 3rd cytoplasmic loop of D3 dopamine receptor but not with that of D2 or D4 dopamine receptor. EF-1, translated in vitro specifically interacted with the bacterially expressed GST fusion protein of the 3rd cytoplasmic loop of D3 dopamine receptor, and this interaction was further confirmed in mammalian cells, that is, EF-1 co-immunoprecipitated with D3 dopamine receptor in C6 glioma cells. The stimulation of D3 dopamine receptor with 20 nM of bromocriptine caused the intracellular translocation of EF-1 to the membrane fraction and phosphorylation of EF-1 at serine residues. The present study shows that D3 dopamine receptor interacts with EF-1 both in vitro and in vivo, and functionally linked to them. Thus D3 dopamine receptor may be involved with the regulation of the protein synthesis through direct interaction with EF-1.

[PA2-1] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Inhibition of Peroxynitrite-induced Nitration by Coffee Ingredients : Effect on