

flushing with Ringer's lactate. After that livers were immersed in Ringer's lactate at 37°C for 20min and reperused with Krebs Henselite bicarbonate buffer (KHBB, pH 7.4, 37°C). As the lactate dehydrogenase (LDH) and purine nucleotide phosphorylase (PNP) significantly increased after cold/warm ischemia and reperfusion. This increase was suppressed by GdCl<sub>3</sub> treatment. The rate of carbon uptake of Kupffer cell slightly increased but this increase was also inhibited by GdCl<sub>3</sub> 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<sup>o</sup>, 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 γ chain 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 γ chain of FcεRI was revealed to be M2 type. Specially, the pyruvate kinase interacted with the Immunoreceptor Tyrosine based Activation Motif (ITAM) of γ chain. 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-1β

Cho DI<sup>o</sup>, 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