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

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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 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

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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

Glutathione Reductase

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Peroxynitrite, a potent cytotoxic oxidant formed by the reaction of nitric oxide(NO) with the superoxide anion radical(O2.-), reacts rapidly to cause of highly toxic oxidizing and nitrating process. The importance of the regulation of ONOO- has been recently recognized because of lack of specific endogenous anti-oxidative defense enzyme against ONOO-. This lack of defense necessitates search for the exogenous source of effective scavengers against ONOO-. Coffee is a complex mixture containing a variety of compounds. The mutagenic effects of instant coffee have been reported and its constituents are known free radical generators, damaging DNA, lipid and protein. In the present study, we report on the anti-oxidative effect of coffee ingredients. Our major effort was on the scavenging effect on peroxynitrite. At present, there is no information available on peroxynitrite scavenging activity of coffee ingredients. We investigated the protective effect of coffee ingredients against peroxynitrite using GSH reductase whose activity is dependent on the integrity of tyrosine. The effectiveness of protection was monitored by the prevention of protein nitration by peroxynitrite. In the study, we focussed on two major coffee components: hydroquinone and 3-methyl-1, 2-cyclopentadione. The scavenging activity of two coffee ingredients was assessed by following three methods: 1) the quantitation of the oxidation of dihydrorhodamine 123 to rhodamine 123. 2) inactivation of GSH reductase activity and 3) tyrosine nitration of GSH reductase by Western blot analysis.
Results showed that hydroquinone and 3-methyl-1, 2-cyclopentadione effectively suppressed peroxynitrite-mediated tyrosine nitration of glutathione reductase in a dose-dependent manner. The extent of prevention of nitration was reflected on GSH reductase activity, indicating the protection of tyrosine moiety in the enzyme. Data are further confirmed by level of tyrosine nitration in GSH reductase analyzed by Western blot analysis.
To our knowledge, this is the first report on the peroxynitrite scavenging action by hydroquinone and 3-methyl-1, 2-cyclopentadione in coffee. Reaction mechanisms of these active ingredients against peroxynitrite need further elucidation.

[PA2-2] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Hamamelitannin as a peroxynitrite scavenger

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Peroxynitrite (ONOO-), formed in vivo from the reaction of superoxide ('O2-) with nitric oxide ('NO), is a cytotoxic species that can oxidize several cellular components such as proteins, lipids, and DNA and nitrate many amino acids, including tyrosine. The purpose of our present study was to investigate the protective effect of hamamelitannin, the major active component isolated from witch hazel (Hamamelis virginiana L.) bark, against damages induced by ONOO-. Hamamelitannin (IC50=1.05μM) exhibited very potent ONOO- scavenging activity measured by oxidation of dihydrorhodamine 123 with fluorescence method. Our data suggest that hamamelitannin led to decrease ONOO--mediated nitration of tyrosine by its electron donation in spectrophotometric analysis. Using immunoassay, hamamelitannin showed the significant inhibition on nitration of bovine serum albumin (BSA) and low-density lipoprotein (LDL) by ONOO- in a dose dependent manner and the relative inhibitory effect on oxidation of BSA and LDL by ONOO-. Hamamelitannin also provided protection against cell damage mediated by ONOO-. In conclusion, our results suggest that hamamelitannin can be developed as an effective supplementary antioxidant in peroxynitrite toxicity.

[PA3-1] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]