cereus phosphoinositide-specific phospholipase C

Park EunMi[O]1, Yoon HyunJoong2, Park HaengSoon1

1 Department of Pharmacy, College of Pharmacy, 2 Department of Biology, College of Natural Science, Chonnam National University, Kwangju 500-757, Korea

Renal dipeptidase (RDPase, EC 3.4.13.19) and alkaline phosphatase (APase, EC 3.1.3.1) are known as glycosylphosphatidylinositol (GPI)-anchored proteins of renal proximal tubules. The bacterial PI-PLC, which was obtained from pure culture of Bacillus cereus, induced the release of RDPase and APase from porcine renal proximal tubules at 37℃ in a time- and protein concentration-dependent manners. Any effect of NO on the release of GPI-anchored RDPase and APase by the B. Cereus PI-PLC was examined. The bacterial culture was added directly to the proximal tubules in the presence and absence of sodium nitroprusside (SNP, direct NO donor) and incubated as a function of time. After incubation for 8 hours, it was observed that the RDPase release was decreased to 37.0 ±5.0% of the control in the presence of 0.1mM SNP, whereas APase release was not changed significantly. It was also confirmed with the result of partially purified bacterial PI-PLC from the bacteria cultured in the presence of SNP. RDPase and APase have slightly different structures at the lipid part of the GPI-anchor, RDPase having diacyl-glycerol whereas APase having alkylacyl-glycerol. The results suggest that there may be different isoforms of bacterial PI-PLC and NO may affect negatively the synthesis of the one responsible for RDPase release.

[PC1-32] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

The release of renal dipeptidase from proximal tubules in the presence of insulin is a [Ca2+]i-dependent process

Yoon HyunJoong2[O], Park EunMi1, Park HaengSoon1

1 Department of Pharmacy, College of Pharmacy, 2 Department of Biology, College of Natural Science, Chonnam National University, Kwangju 500-757, Korea

Renal dipeptidase (RDPase, EC 3.4.13.19), an ectoenzyme of renal proximal tubules, is covalently bound to outer leaflet of lipid bilayer via glycosylphosphatidylinositol (GPI)-anchor. *In vivo* release of RDPase was observed in urine of various animals including rat, rabbit, pig and human. Porcine and human RDPase were identified as a hydrophilic form as they were mostly partitioned into the aqueous layer by phase separation with Triton X-114. Insulin has been known to stimulate the release of several mammalian GPI-anchored cell surface proteins. *In vitro* release of RDPase was diminished by depletion of $[Ca^{2+}]i$ but restored by Ca^{2+} supply. EGTA (Ca^{2+} chelator), TMB-8 (inhibitor of Ca^{2+} release from intracellular Ca^{2+} stores) and nifedipine (L-type Ca^{2+} channel blocker) decreased RDPase release but ionomycin (Ca^{2+} ionophore) increased it. These $[Ca^{2+}]i$-regulating agents also synergistically controlled the releases of RDPase and other Cross-Reacting Determinant (CRD, inositol-1,2-cyclic monophosphate)-containing proteins such as porcine renal alkaline phosphatase (ALPase) and acetylcholinesterase (AChE) in the presence of insulin. These results demonstrate that the release of RDPase from its GPI-anchor in the presence or absence of insulin is a $[Ca^{2+}]i$-dependent process, different from the trypanosomal GPI-PLC which is independent of Ca^{2+}.

[PC1-33] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Modulation of Oxidative Status by Calorie Restriction in Mini rat

Lee Ji Hyeon[O], Kim Aera, Kim Ji Young, Kim Chul Hong, Park Dae-Ui, Han Suk Kyu, Shimokawa Isao, Chung Hae-Young

College of Pharmacy, Pusan National University, Pusan, Korea, Bioinformatics cooperative course, Pusan National University, Pusan, Korea, Department of Pathology, Nagasaki University, School of Medicine, Japan

Mini rat is a transgenic rat strain carrying antisense gene for rat growth hormone (GH), resulting in retarded growth. Mini rats have been used by several investigators as a GH deficiency model. In the present study, we assessed the redox status of mini rat liver to elucidate the effect of aging and calorie restriction (CR). Liver homogenates from mini rats at 31 and 100 weeks of age which were fed ad libitum (AL) and CR were used in the study. Total reactive oxygen species (ROS) generation was determined by dichlorofluorescin diacetate (DCFH-DA) method. We also investigated malonaldehyde (MDA) amounts using thiobarbituric acid (TBA) positive material assay. In addition, the mitochondrial membrane fluidity was measured by fluorescence polarization method. Results showed that total ROS generation of liver increased with age and was reduced by CR. Increased MDA levels might be due to increased ROS generation, which could cause a decrease of mitochondrial membrane fluidity. Moreover, other redox markers such as GSH/GSSG and total SH levels decreased during aging and was maintained by CR. The present findings are in agreement with our previous report showing age-dependent loss of the antioxidant potential and its modulation by CR.

[PC1-34] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Role of p38 MAPK on H-ras-Induced Invasion and Motility in MCF10A Human Breast Epithelial Cells

Lee Eun-Jung°, Kim Mi-Sung, Moon Aree

College of Pharmacy, Duksung Womens University, Seoul 132-714

One of the most frequent defects in human cancer is the uncontrolled activation of the ras-signaling pathways. We have previously shown that H-ras, but not N-ras, induces an invasiveness and motility in human breast epithelial cells (MCF10A), while both H-ras and N-ras induce transformed phenotype. Since migration plays a crucial role in invasive, we examined motility of MCF10A cells transformed with H-ras or N-ras. We show that cell motility was increased by H-ras, but N-ras suggesting that H-ras-induced invasive phenotype may be mainly due to enhanced cell motility. It has been recently shown that p38, a member of the mitogen activated protein (MAP) kinase family, is important for cell migration. We wished to investigate the functional role of p38 MAP kinase in H-ras-induced invasive phenotype. We show that p38 is prominently activated in H-ras MCF10A cells comparing to the parental MCF10A cells or N-ras MCF10A cells, while no significant difference was found in the activation of stress-activated protein kinase-1/c-Jun N-terminal protein kinase (SAPK-1/JNK). Extracellular signal-regulated protein kinase (ERK)-1,2 were activated in both H-ras and N-ras MCF10A cells. To assess the functional significance of H-ras-activated p38 in invasion and migration, we examined the effect of SB203580 and dominant-negative p38(DN p38). Treatment of SB203580, an inhibitor of p38, reduced invasive activity and motility of H-ras MCF10A cells. H-ras MCF10A cells were transfection with dominant-negative p38 but not dominant-negative JNK-1 inhibited cell migration. These results suggest a possible involvement of p38 in H-ras-induced invasiveness/motility.

[PC1-35] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Role of Phosphatidylinositol 3-kinase (PI3K) on H-ras-Induced Invasive phenotype

Shin Il-Chung°, Moon Aree

College of Pharmacy, Duksung Womens University, Seoul 132-714

We have previously shown that H-ras, but N-ras, induces an invasiveness and cell motility in human