extracellular glycosylphosphatidylinositol-specific phospholipase C in porcine renal proximal tubules

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It was previously reported that human urinary dipeptidase was the released form of renal dipeptidase (RDPase) (EC 3.4.13.19), a glycosylphosphatidylinositol (GPI)-anchored glycoprotein to apical membrane of proximal tubules. This *in vivo* release was accomplished by GPI-PLC.
In *in vitro* model system of porcine proximal tubules, RDPase was also released by not only bacterial PI-PLC but also endogenous GPI-PLC, which did not follow the intracellular PLC signaling. In this study we assayed the activity of released RDPase using a direct NO donor, sodium nitroprusside (SNP), a nitric oxide synthase (NOS) substrate, L-arginine (Arg), an NOS inhibitor, Nω-Nitro-L-arginine-methyl ester (NAME) in order to investigate the relationship between NO and GPI-PLC activity. Addition of 5 mM Arg to porcine proximal tubules showed the increase of NO production and the decrease of RDPase release simultaneously in the time- and concentration-dependent manner. Treatment with 10 mM NAME inhibited the Arg effect on NO production and RDPase release. A concentration-dependent treatment with SNP also showed the inhibition of RDPase release and 0.5 mM SNP induced an immediate and continuous decrease in GPI-PLC activity. This effect was confirmed quantitatively by western blot using polyclonal antibody raised against porcine RDPase. These results suggest that NO down-regulates the RDPase release by inhibition of GPI-PLC, but not by direct inhibition.

[PC1-16] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Age-related up-regulation of NF-kappaB and modulation by calorie restriction

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Recent data strongly suggested that the calorie restriction (CR) may retard aging by its anti-oxidative action on the regulation of the intracellular redox status. Regulation of the redox-sensitive NF-κB during aging is important because oxidative stress increases during aging, and CR is an effective modulator against oxidative stress. In this present study, we investigated whether age affects the regulation of NF-κB, and how the age effect is modulated by CR. The kidney isolated from Fischer 344 rats at 6, 12, 18, and 24 months of age fed ad *libitum* (AL) and CR rats were used. Results show that the aging process strongly enhanced NF-κB DNA-binding activity that was in parallel with an increased generation of reactive oxygen species (ROS). Accompanied with changes in NF-κB was the decreased inhibitory IκBα protein in cytosol. At the same time, it was found that the nuclear p65 protein increased with age, affirming the increased translocation of NF-κB into nucleus. However, CR reversed the age-related activation and translocation of NF-κB. Our results further revealed that CR effectively blocked increased activation of NF-κB by suppressing the IκBα degradation. Based on these data, we concluded that the age-related increase in redox-sensitive NF-κB binding activity is associated with increased ROS, and CR modulates the NF-κB activation by suppressing oxidative stress.

[PC1-17] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

A 40 kDa Ca2+-dependent Cytoslic Phospholipase A2 Is Implicated in A23187-induced Release of Arachidonic Acid from Mammalian Red Blood Cells

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Red blood cells (RBCs) are known to modify platelet pathophysiology through the release of arachidonic acid (AA) and eicosanoid formation including thromboxane A_2 and thus influence thrombosis and hematosis. Treatment of RBC with a calcium ionophore A23187 could cause a marked enhancement in the release of arachidonic acid in a Ca^{2+}-dependent manner, suggesting that the agent may activate a Ca^{2+}-dependent phospholipase A_2 (PLA_2). A Ca^{2+}-dependent PLA_2 activity was detected in the cytosol of bovine RBC and purified to near homogeneity by sequential uses of chromatographies with ~7,000-fold increase in the specific activity. The purified enzyme migrated as a single band of a molecular weight of 40 kDa on a SDS-PAGE gel. Anti-40 kDa protein polyclonal antibody not only immunoprecipitated the enzymatic activity, but also reacted with the 40 kDa protein in a Western blot analysis, indicating that the 40 kDa protein is the RBC PLA_2. The 40 kDa RBC PLA_2 was characterized as a similar enzyme to Group IV $cPLA_2$, but different in the cross-reactivity with anti-porcine spleen Group IV $cPLA_2$ antibody and the sensitivity to methyl mercury and a newly synthesized quinolone derivative EA4, which has been developed as a selective inhibitor for the 40 kDa RBC PLA_2. Interestingly, pre-treatments of EA4 with human and bovine RBCs markedly attenuated A23187-induced release of AA. Together, our data strongly suggest that the 40 kDa cytosolic form of PLA_2 could be implicated in a Ca^{2+}-dependent physiological release of AA in mammalian RBCs and possibly in thrombotic process in concert with platelets.

[PC1-18] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Effects of endocrine disruptors using mouse mammary gland organ culture system on the formation of preneoplastic lesion

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Endocrine disruptors (EDs) are chemicals which interfere with endocrine system function. These EDs disturb normal endocrine mechanisms and have been observed in nearly all classes of vertebrates. The effects of these EDs are emerged as serious problems on human beings. Especially, as the effects of EDs are reported to be the main causes of hormone-related cancers such as breast cancer among women, we evaluated effects of EDs using mouse mammary gland organ culture (MMOC) model. Originally, the mouse mammary gland in whole-organ culture, an *in vitro* system that is capable of alveolar development differentiation, involution, and oncogenic transformation, has been used to examine the effects of the chemopreventive agents against breast carcinoma. Therefore, we examined that effects of EDs on the formation of preneoplastic lesion in MMOC. The MMOC provides a promising model system to study the mechanisms by which EDs initiate and promote the transformation *in vitro*. Furthermore, inhibition of EDs-induced precancerous lesions in MMOC will be used for evaluating the potential efficacy of chemopreventive agents against breast cancer.

[PC2-1] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

In vitro Inhibitory Effect of the fruits of Citrus aurantium on Rotavirus Infectivity

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