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