E118 Increased ROS and lipid peroxidation in the cytochrome c oxidase incorporated liposome with cardiolipin

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Mitochondrial electron transport chain has been recognized as a major intracellular source of reactive oxygen species(ROS) and unsaturated fatty acids are particularly susceptible to ROS attack owing to the presence of double bonds. Generation of ROS during respiration increases dramatically in proportion to membrane potential($\triangle\Psi$)(Yu et al). On the basis of this report, we measured the ROS and lipid peroxidation produced by cytochrome c oxidase incorporated liposome under various conditions. The presence of cardiolipin in the liposome stimulated the ROS production and lipid peroxidation. Present result shows that ROS production was increased by inhibition of cytochrome c oxidase but not by cardiolipin peroxidation.

E119 Functional characterization of thioredoxin peroxidase in the phagocytosis of Amoeba proteus

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All organisms have self-defense mechanism to external hazard. In an aerobic circumstance reactive oxygen species (ROS) arise from the incomplete reduction of oxygen. These ROSs lead to damage which afflict all types of biological molecules. *Amoeba proteus* living on ciliates is very active in phagocytosis and endocellular digestion. Phagocytosis initiates respiratory burst and generates ROSs. In the study of survival mechanism of endosymbiotic X-bacteria entering the host through the phagocytic pathway, we cloned and characterized a cDNA for thioredoxin peroxidase (TPx) of *A. proteus*. The polypeptide encoded by the cDNA was 22.5-kDa, composed of 177 amino acid residues, and was homologous to the TPx of human. We produced a monoclonal antibody (AT 1-7) against the cloned protein. The mAb cross-reacted with 4 different proteins (22.5, 42, 45 and 60kDa) of amoeba. The immunofluorescence (IF) of AT 1-7 was very weak and hardly recognized in starving amoebae. When amoebae were treated with 2mM H_2O_2 for 15min, the IF of AT 1-7 was shown all over the cytoplasm. In a time-course study of phagocytosis, the IF of AT 1-7 was concentrated at the periphery phagocytic vacuole. In Northern blot analysis of mRNA in *A. proteus*, we confirmed that the TPx is overexpressed when amoebae were treated with 2mM H_2O_2. We suggest that *A. proteus* produce TPx as a host defense mechanism against H_2O_2 generated during the phagocytosis.

E120 Chronic Pentylenetetrazole-evoked Cell Death in Discrete Brain Regions.

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Pentylenetetrazole (PTZ) is a convulsant that blocks -aminobutyric acid-activated Cl^- channels. While application of PTZ commonly generates seizure behaviors, the no report thus far showing that PTZ causes neurodegeneration in the brain is available. In the present study, we daily administered PTZ at dose of 0, 35, 50, and 65 mg/kg for 14 days and examined behavioral changes before sacrifice. Although systemic injection of 35 or 65 mg/kg PTZ resulted in minor or severe kindled state, there was no cell death in any other brain regions. In contrast, rats being administered with 50

mg/kg PTZ showed progressive increase in seizure severity, and in particular, in the animals showing severe seizure responses and sudden loss of body weight, the serious brain damages were revealed by histological analysis such as Nissl staining. We found moribund cells in discrete brain regions, such as piriform, entorhinal, and parietal cortex, hippocampus, and substantia nigra (SN) using acid fuchsin and TdT-mediated dUTP nick end labeling (TUNEL) staining, respectively. Apoptotic bodies were observed in dying cells after TUNEL staining. These data are the first demonstration showing that the chronic, systemic PTZ administration induces cell death in discrete brain regions. These results suggest that the long-lasting blockage of inhibitory synaptic activity may lead to the neuronal cell death.

E121 Downregulation on Neuronal Calcium Signaling by ER stress in PC12 cells

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Neuronal activity facilitates neuronal survival and can lead to increases in synaptic strength. This phenomenon is mediated by the intracellular influx of calcium during membrane depolarization. The increase in calcium activates the transcription factor CREB that results in induction of multiple gene expressions including BDNF exon III. Since ER stress perturbs intracellular calcium signaling, we investigated the effects of ER stress on depolarization-induced CREB activation and BDNF exon III expression. Tunicamycin (TM) and -mercaptoethanol (ME), that induce ER stress by suppression on protein glycosylation or formation of disulfide bonds respectively, attenuated the depolarization-induced CREB activation, but not completely. ME also reduced the expression of BDNF exon III with a similar extent as that on the activation of CREB. On the other hand, TM completely abolished the expression of CREB in spite of a significant activation of CREB. These results suggest that ER stress can attenuate activity-dependent neuronal survival in part. In addition, TM appears to suppress the expression of BDNF exon III through multiple pathways.

E122 Insect Immunity - Purification and Some Properties of Immune Protein (Hemolin) from Hemolymph of Protaetia brevitarsis

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Hemolin is the 48 kDa protein induced in response to bactreial infection and binds to Lipopolysaccharide(LPS) identified in the hemolymph of two lepidopteran insect species, *Hyalophora cecripia* and *Manduca sexta*. Based on these informations, we found the protein was bacteria-induced and bound to LPS, and the molecular weight is about 48 kDa on the SDS-PAGE in the last larval hemolyph of *Protaetia brevitarsis* after bacteria injection. Hemolin-like protein has been partially purified by gel permeation chromatgraphy (Superdex) and resource Q (ion-exchange chromatography) using fast performance liquid chromatography (FPLC) system.

E123 Purification and Molecular Properties of the Ferritin from the Larval Hemlymph of Protaetia brevitarsis

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Ferritin, an iron storage protein, has been purified in the last larval hemolymph of *Protaetia brevitarsis* (coleopteran) by KBr