Journal of Neurochemistry JNC

Monday Poster Session
MO01 Neurogenesis in embryonic and adult brain

MO01-01

GABAERGIC DIFFERENTIATION OF MURINE F9 EMBRYONIC CARCINOMA CELLS

Cha, Y. and Seo, H.

Division of Molecular & Life Science, Hanyang University, Ansan, South Korea

The murine F9 embryonic carcinoma cells are embryonic like stem cells and a good model system for endoderm cell lineage differentiation. We investigated the neuronal differentiation potentials of F9 cells to GABAergic neurons with various protocols which include endoderm stage differentiation exposed to 1uM retinoic acid (RA) and GABAergic neuron differentiation exposed to neurotrophic factors such as brain-derived neurotrophic factor (BDNF), Sonic Hedgehog (SHH), and retinoic acid (RA) for 2 weeks. After 1, 2, 4, 6 days of RA treatment, F9 cells showed differential morphological, immunocytochemical and biochemical evidences of the neuronal differentiation. We performed RT-PCR to detect the mRNA levels of embryonic markers, neuronal markers, and GABAergic markers. Differentiation, F9 cells showed selectively expressed nestin and neuronal molecules. RA treatment for 6 days decreased mRNA levels of embryonic markers such as Oct4, and increased the GABAergic markers such as GAD2, GABA B1 receptor. These data suggest that F9 cells can be used to study the cellular mechanism of GABAergic neuronal differentiation, and the GABAergic differentiation protocols used in this study can be applied to the studies of GABAergic differentiation from various kinds of stem cells.

MO01-02

KAP3 IS PREFERENTIALLY EXPRESSED IN GLUTAMATERGIC NEURONS AND CONTRIBUTES TO THE EXCITATORY CONTROL OF FEMALE PUBERTY

Choi, J.[1], Roh, G. S.[1], Kim, J. K.[1], Cho, G. J.[1], Choi, W. S.[1], Lee, B. J.[2] and Kang, S. S.[1]

[1]*Department of Anatomy and neurobiology, School of Medicine, Gyeongsang National University, Jinju*

[2]*Department of Biological Sciences, University of Ulsan, Ulsan, Korea*

It was earlier shown that expression of KAP3, a kinesin superfamily associated protein involved in the neuronal anterograde, microtubule-dependent transport of membrane organelles, increases in the hypothalamus of female rats during the juvenile phase of sexual development. KAP3 mRNA is abundant in the hypothalamus suggesting that it might be expressed in broadly disseminated neuronal systems controlling neuroendocrine function. The present study identifies one of these systems and provides evidence for an involvement of KAP3 in the excitatory control of female puberty. In situ hybridization and immunohistofluorescence studies revealed that the KAP3 gene is expressed in glutamatergic neurons, but not in GABAergic or GnRH neurons. Hypothalamic KAP3 mRNA levels increase during the juvenile period of female prepubertal development, remaining elevated throughout puberty. These changes appear to be estradiol-dependent because ovariectomy decreases and estradiol increases KAP3 mRNA abundance. Lowering hypothalamic KAP3 protein levels via i.c.v administration of an antisense oligodeoxynucleotide, resulted in reduced release of both glutamate and GnRH from the median eminence (ME), and delayed the onset of puberty. The ME content of vesicular glutamate transporter 2 and synaptophysin were also reduced, suggesting that the loss of KAP3 diminishes the anterograde transport of these proteins. Altogether, these results support the view that decreased KAP3 synthesis diminishes GnRH output and delays female sexual development by compromising hypothalamic release of glutamate.

MO01-03

AMYLOID PRECURSOR PROTEIN-BINDING PROTEIN 1 (APP-BP1) PLAYS AN IMPORTANT ROLE IN NEURAL STEM CELL CYCLE PROGRESSION.

Ha, S. J., Joo, Y., Hong, B. H., Suh, Y. H. and Kim, H. S.

Department of Pharmacology, College of Medicine, Seoul National University, Seoul, South Korea

Although the extract pathogenic mechanism of Alzheimer's disease (AD) remains to be elucidated up to now, the involvement of the amyloid precursor protein (APP) in some aspects of the etiology of the disease is suggested by the facts that mutations and overexpression of this gene can cause familial AD (FAD) and AD-like dementia symptom in Down's syndrome, respectively. Together with the study on the pathological roles for APP in AD pathogenesis, its normal physiological function has been intensively investigated. A number of proteins have been shown to interact with the cytosolic domain of APP. Among them, amyloid precursor protein -binding protein 1 (APP-BP1) was reported to be involved in cell cycle progression by serving as the bipartite activation enzyme for the ubiquitin-like protein NEDD8. Expression of APP-BP1 in dividing cells is known to drive the cell cycle through the S-M checkpoint; this function is mediated by the NEDD8 conjugation (neddylation) pathway. In this study, we focused on the normal physiological function of APP-BP1 in neural stem cell cycle progression, demonstrating that APP-BP1 knock-down with siRNA treatment arrests cell cycle progression at G1 phase in neural stem cells and reduced neuronal cell proliferation. In addition, APP-BP1 expression was found to be changed according to cell cycle phase, the highest at S-phase. In the brains of Tg2576 mice, the AD animal model, APP-BP1 expression was altered, compared with wt mice. These results suggest that APP-BP1 plays an important role in neural stem cell cycle progression, and that its upregulation observed in the brains of AD patients (Chen et al., 2003) and Tg2576 mice contribute to AD pathogenesis.

MO01-04

GENERATION OF OLIGODENDROCYTES IN THE POSTEMBRYONIC ZEBRAFISH FOREBRAIN

Ho, K., Suhyun, K., Ah-young, C. and Hae-Chul, P.

Graduate School of Medicine, Korea University, Ansan, South Korea

Oligodendrocytes have important roles for proper function of neurons by myelination of their axons. Olig2 is the basic helix-loop-helix transcription factor and olig2+ precursor cells produce primary motor neurons and migratory oligodendrocyte progenitor cells (OPCs) in the developing spinal cord. Several recent reports have shown that radial glia function as neural precursors to produce neurons during CNS development. Previously we have shown that subsets of GFAP+ radial glia express olig2 and olig2+ radial glia

generate oligodendrocytes in the developing spinal cord. Interestingly, like embryonic spinal cord, we found that olig2 is expressed continuously in the discrete population of radial glia, which have hallmarks of stem cells, in the postembryonic spinal cord and forebrain. Their divisions appear to be asymmetric to produce new oligodendrocytes. Here, we focused on oligodendrocyte generation in postembryonic forebrain and will show some evidence suggesting that olig2+ radial glial cells function as neural precursor/stem cells to produce oligodendrocytes in the postembryonic forebrain.

MO01-05

UNIDIRECTIONAL DIFFERENTIATION FROM HUMAN EMBRYONIC STEM CELLS INTO NEURONS

Inoue, N.[1], Otsu, M.[1,2], Kume, N.[1], Isono, M.[1], Shibata, M.[1], Sai, T.[1], Nakayama, T.[1,3], Momoki-Soga, T.[1] and Kasai, H.[1]

[1]*Laboratory of Regener. Neurosci., Tokyo Metropolitan University Grad. Sch. of Human Health Sciences*
[2]*Department of Chemistry, Kyorin University School. of Med., Tokyo*
[3]*Department Biochemistry, School of Med., Yokohama City University, Yokohama, Japan*

We have previously demonstrated that mouse and monkey embryonic stem (ES) cells can be efficiently differentiated into neurons by Neural Stem Sphere (NSS) method. Here, we applied the method to human ES cells. Cultured in astrocyte-conditioned medium (ACM) under free-floating conditions for 20 days, the colonies of ES cells gave rise to floating spheres, NSSs. Culturing the NSSs on adhesive dishes in ACM promoted neurogenesis, and many MAP2-positive neurons migrated from the NSSs. Changes in gene expression during the formation of the NSSs were analyzed by quantitative real-time RT-PCR. Gene expression of ES cell markers, Oct-3/4 and Nanog, was down-regulated during culture and became very low at day 7. In contrast, expression of a neuroectodermal marker Sox1 and a neural stem cell marker nestin became marked from day 12 and that of a neuronal marker MAP2 from day 20. However, expression of an astrocyte marker GFAP and an oligodendrocyte marker MBP did not increase. Expression of an early mesoderm marker Brachyury, a primitive endodermal marker GATA4 and an epidermal marker Cytokeratin-17 was very low and did not significantly increase throughout the culture. These results demonstrate that human ES cells can unidirectionally differentiate into neurons via neuroectodermal cells and neural stem cells by the NSS method as in the case with mouse and monkey ES cells. This work was partly supported by Grants-in-Aid for Scientific Research from JSPS, a grant of LRI by JCIA and Selective Research Fund of Tokyo Metropolitan University.

MO01-06

EFFECTS OF X-IRRADIATION ON EMBRYONIC STEM CELLS-DERIVED NEURAL STEM CELLS

Isono, M.[1], Otsu, M.[1,2], Konishi, T.[3], Nakayama, T.[4] and Inoue, N.[1]

[1]*Laboratory of Regenerative Neurosciences, Tokyo Metropolitan University Graduate School of Human Health Sciences*
[2]*Department of Chemistry, Kyorin University School of Medicine, Tokyo*
[3]*Deptartment of Technical Support and Development, Fundamental Technology center, National Institute of Radiological Sciences, Chiba*
[4]*Department of Biochemistry, Yokohama City University School of Medicine, Yokohama, Japan*

The neural stem cells increase in number and differentiate into neurons, astrocytes and oligodendrocytes in developmental brain during fetal period. Radiation exposure during the fetal period leads to disturbances in development of the brain and can induce microcephaly or anencephalia. Here we investigated biological effects of the X-irradiation on the neural stem cells, prepared from mouse embryonic stem (ES) cells by the Neural Stem Sphere (NSS) method. Cultured with mitogen fibroblast growth factor-2, the neural stem cells proliferated exponentially. When the neural stem cells were exposed to various doses of X-ray, the proliferation of the neural stem cells was dose-dependently inhibited by X-irradiation and almost completely stopped by 5 Gy-radiation. Morphology of the multipolar neural stem cells was affected by the radiation, and the surviving cells after radiation were bipolar and larger than the neural stem cells. However, immunofluorescent study showed that the surviving bipolar cells were positive for nestin, and gene expression of nestin and musashi-1 was also demonstrated in the cells by RT-PCR study. These results suggested that radiation inhibits proliferation of the neural stem cells but the surviving cells after radiation maintain basic properties of the neural stem cells. This work was partly supported by agrant of LRI by JCIA and Selective Research Fund of Tokyo Metropolitan University.

MO01-07

SUPPRESSION BY MYOSIN VI OF NEURAL PROGENITOR PROLIFERATION

Ito, Y., Tamaki, K., Takarada, T., Nakamichi, N. and Yoneda, Y.

Laboratory of Molecular Pharmacology, Kanazawa University Graduate School of Natural Science and Technology, Kanazawa, Japan

We have found that mRNA for Myosin VI (Myo6) is selectively expressed in the murine hippocampus within 24 h in response to the extremely traumatic experience, water-immersion restraint stress (WIRS), prior to a drastic but transient decrease in proliferation of progenitors in the dentate gyrus. Western blotting analysis clearly revealed a significant increase in the expression of Myo6 protein in the murine hippocampus within 24 h after the flashback experience by forced swimming in mice previously exposed to WIRS 9 days ago. Although Myo6 protein was ubiquitously distributed in discrete mouse brain regions with exceptional condensation in the olfactory bulb, Myo6 protein was also expressed in cultured rat astroglia and neurons, in addition to cultured progenitor cells isolated from embryonic mouse brains. In mouse embryonal carcinoma P19 cells endowed to proliferate for self-replication and differentiate into neurons and astroglia, Myo6 protein was highly expressed in consistency with the expression of a marker protein of astroglia but not of neurons. Transient overexpression of Myo6 led to a significant decrease in the size of clustered aggregates without affecting cell viability in P19 cells cultured with retinoic acid. In P19 cells with stable overexpression of Myo6, moreover, similarly significant inhibition was seen in the size of clustered spheres and MTT reduction compared with cells transfected with empty vector. These results suggest that Myo6 may play a pivotal role in the mechanism underlying the suppression of neurogenesis relevant to hippocampal atrophy seen in patients with posttraumatic stress disorder.

MO01-08

PRECOCIOUS DEPLETION OF RETINAL PROGENITOR POPULATION FROM THE IMPAIRED NOTCH SIGNALING IN PTEN-DEFICIENT RETINA

Jo, H. S.[1], Kang, K. H.[1], Lemke, G.[2] and Kim, J. W.[1]

[1]*Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Korea*

[2]*Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA, USA*

Developmental programs generating the repertoire of retinal neurons from the retinal progenitor cells (RPCs) are composed of the coordinated processes of differentiation, which is coupled to the tightly controlled cell proliferation. Since multiple developmental inputs act simultaneously to RPCs, the central signal integrators, by which the asymmetric fate acquisition between two daughter cells is conducted, must be required. Here, we propose the reciprocal antagonism between phosphoinositide 3-kinase (PI3K) and PTEN. as a pacemaker for mouse retinal neurogenesis. The embryonic RPCs lacking of PTEN proliferated faster to complete retinal neurogenesis earlier than their neighbors. The premature neurogenesis in PTEN-deficient mouse retina resulted in precocious depletion of RPC population, but didn't affect to overall composition of retinal neurons. It emphasized that the requirement of PTEN-supportive events for RPC maintenance rather than for the subtype specification of retinal neurons. We further found that the hyperactive Akt in PTEN-deficient RPCs suppressed the Notch-activated transcription. Together, the results suggested that the temporal elevation of Notch signaling following to the PTEN-dependent decay of Akt activity prevents RPCs from proceeding neurogenesis prematurely.

MO01-09

INVOLVEMENT OF NITRIC OXIDE IN PROLIFERATION OF NEURAL STEM/PROGENITOR CELLS DERIVED FROM THE HIPPOCAMPUS OF EMBRYONIC MOUSE

Kawada, K., Yoneyama, M. and Ogita, K.

Department of Pharmacology, Setsunan University, Osaka, Japan

To elucidate involvement of reactive oxygen species (ROS) and nitric oxide (NO) in proliferation of neural stem/progenitor cells (NPCs), in this study, we evaluated the effects of ROS scavengers and NO synthase inhibitor on proliferative activity in the NPCs of the hippocampus in embryonic mice. Neurosphere was prepared from the hippocampus of 15-days-old embryonic mice by primary culturing in DMEM/F12 medium with epidermal growth factor (EGF) and basic fibroblast growth factor for 9 days *in vitro* (DIV). After replating, the cells were cultured for 6 DIV under the same conditons in the absence or presence of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (tempol, radical capture agent), apocynin (NADPH oxidase inhibitor), Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, NO synthase inhibitor) or 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, NO-dependent soluble guanylyl cyclase inhibitor) for a period of 9-15 DIV. Treatments with tempol or L-NAME resulted in a marked reduction in ROS production in the cells. MTT assay and ELISA of 5'-bromo-2'-deoxyuridine revealed that a marked decrease in surviving neurospheres and the proliferative activity was seen by treatment with tempol, which produced a reduction in ROS level. Moreover, treatment with apocynin, L-NAME, and ODQ led to a decrease in the surviving neurospheres and the proliferative activity. However, no significant change in lactate dehydrogenase released in the culture medium was observed by treatment with any of drugs. In addition, L-NAME suppressed the activation of Akt, but not of EGF receptor and ERK. These results suggest that ROS and NO would positively regulate proliferative activity through the activation of Akt in NPCs of embryonic mouse hippocampus.

MO01-10

IDENTIFICATION AND CHARACTERIZATION OF A TRUNCATED ALTERNATIVE SPLICING VARIANT OF THE RAT NELL2

Kim, D., Hwang, E., Yoo, J., Kim, E., Park, N., Cho, M., Lee, Y., Kang,D., Han, J., Hong, S. and Park, J.

Department of Physiology, University of Gyeongsang National, BK21, Jinju, South Korea

NELL2 is a neuron-specific secreted glycoprotein containing thrombospondin I-like domain (TSPN) and six epidermal growth factor-like domains. NELL2 is highly expressed in the hippocampus and cortex, and has been reported to involve in survival and differentiation of neuron. In this study, we identified a novel variant of NELL2, derived from alternative splicing between exon 9 and exon 21. The splicing leads to the use of an additional stop codon and results from formation of a truncated splice variant of NELL2 (NELL2-Tsp), comprising of signal peptide and TSPN domain. NELL2-Tsp was specifically detected in the brain but not other tissues by RT-PCR. In addition, Western blot analysis confirmed NELL2-Tsp was secreted glycoprotein and oligomerized protein. When NELL2 and NELL2-Tsp were coexpressed in HEK cells, expression of NELL2-Tsp notably reduced secretion of NELL2. Treatment of NELL2 enhanced phosphorylation of extracellular signal-regulated kinase (ERK), but NELL2-Tsp was not affected. These results suggest that NELL2-Tsp was able to modulate neuronal survival by regulating NELL2 secretion.

MO01-11

FUNCTIONAL ANALYSIS OF LYSPHOSPHATIDIC ACID RECEPTOR-1 (LPA-1) GENE EXPRESSION IN NEOCORTICAL NEUROBLAST AND TRANSGENIC MICE

Kim, S. H., Hwang, J. S., Kim, N. H., Sun, Y. J., Oh, S. M. and Huh, S. O.

Department of Pharmacology, College of Medicine, Institute of Natural Medicine, Hallym University, Chuncheon, South Korea

Lysophosphatidic acid receptor-1 (LPA1) is a G protein-coupled seven transmembrane receptor with high affinity for the ligand, lysophosphatidic acid (LPA). The spatial and temporal regulation of LPA1 gene during gestation period is essential for normal development of the brain, as revealed by the loss-of-function mutation study. However, detailed information on the cis-acting elements involved in the transcription of LPA1 gene is not yet fully understood. Furthermore, the role of LPA1 gene in developing brain has not been assessed using transgenic mouse system. Here, using the 5' RACE (rapid amplification of cDNA ends) analysis, transcription initiation site of LPA1 gene was identified. Approximately 3500 bp of 5' flanking sequence of mouse LPA1 gene was characterized. In order to gain insight how the LPA overexpression affects cellular morphology of the forebrain area, mice with expression of bicistronic pBI-EGFP-LPA1 genes are being bred with mice expressing CamKII-promoter driven rtTA genes. Our experiments using both *in vitro* and *in vivo* animal model system

would lead us to novel insights for how LPA1 functions during brain morphogenesis and information on possible neurological diseases implicated with LPA1 gene dysfunction.

MO01-12

NOVEL ROLE OF PRONEURAL BHLHS FOR THE REGULATION OF NESTIN GENE

Kim, S. Y.[2], You, S. T.[2], Park, R. H.[2,5,6], Kwon, K. W.[2], Park, J. S.[2], Yoon, Y. S.[2], Kim, S. S.[1,2], Park, S. C.[3], Lee, Y. D.[1,2,4] and Suh-Kim, H. Y.[2,5,6]

[1]*Center for Cell death Regulating Biodrug, Ajou University School of medicie*
[2]*Department of Anatomy, Ajou University. School of medicine, Suwon*
[3]*Department of Life Science, Sookmyung University, Seoul*
[4]*Department of molecular science and technology, Ajou University*
[5]*BK21, Division of cell transformation and restoration, Ajou University, Shool of medicine*
[6]*Interdisciplinary course for neuroscience and technology, Ajou University, Suwon, Korea*

Neurogenin1 (Ngn1) is a basic helix-loop-helix (bHLH) transcription factor that is expressed in neuronal precursors during an early development of the nervous system. Nestin, which is expressed during the embryogenesis like Ngn1, is a characteristic intermediate filament protein expressed in neural stem cells. The posterior 637bp of the second intron of the rat Nestin gene has been known to sufficient for the CNS-specific Nestin expression. In this work, we found that Ngn1 can promote Nestin expression and majority of Ngn1 positive cells showed simultaneous expression with Nestin gene. Ngn1, Ngn2 and Mash1, proneural bHLHs, increased 2nd E-box in 637bp of the Nestin 2nd intron mediated reporter gene activity. Furthermore, direct interactions of Ngn1, Ngn2 and Mash1 proteins and E-box were observed by chromatin immunoprecipitation (ChIP) assay in E11.5 mouse embryo brain. Transgenic (Tg) mouse containing 637bp of Nestin 2nd intron containing wild type E-box and mutated E-box demonstrated the functional importance of the E-box (1407–1412) *in vivo*. These results indicate that proneural Ngn1 promotes Nestin expression via a binding of an E-box of the Nestin 2nd intron.

MO01-13

PREDOMINANT EXPRESSION OF IFRD1 BY NEURAL PROGENITOR CELLS IN ADULT MOUSE HIPPOCAMPUS

Konishi, S., Maeda, S., Watanabe, M., Nakamichi, N., Takarada, T. and Yoneda, Y.

Laboratory of Molecular Pharmacology, Kanazawa University Graduate School of Natural Science and Technology, Kanazawa, Japan

In our previous studies, neurospheres are similarly formed by clusters of neural progenitor cells under proliferation between hippocampi of adult and embryonic mice, whereas astroglial differentiation predominates over neuronal differentiation in neural progenitors of adult mouse hippocampus compared with those of embryonic mouse hippocampus. We have identified the gene differentially expressed in neurospheres between adult and embryonic mouse hippocampi as interferon-related developmental regulator-1 (Ifrd1). In this study, we have attempted to demonstrate the functionality of Ifrd1 in neural progenitor cells. In neurospheres of adult mouse hippocampus, more than 7-fold higher expression was seen for Ifrd1 mRNA than in embryonic mouse hippocampus on RT-PCR analysis. The mouse embryonic carcinoma P19 cells were cultured in αMEM containing 5% FBS with 0.5 μM ATRA for 4 days, followed by further culture in the absence of ATRA for an additional 12 days. Marked but transient expression was seen in MAP2 mRNA on Day 6 to 8, while expression of Ifrd1 mRNA was drastically increased in line with GFAP mRNA expression on Day 8 to 10. Cells were then cultured in the presence of ATRA for 4 days, followed by transfection with the Ifrd1 expression vector. In these P19 cells with transient overexpression of Ifrd1, a significant decrease was seen in MAP2 mRNA expression and numbers of cells immunoreactive for MAP2. These results suggest that spontaneous cellular differentiation would undergo into astroglia rather than neurons through a mechanism relevant to constitutive expression of Ifrd1 in hippocampal neural progenitor cells during adult neurogenesis.

MO01-14

INHIBITION OF NEURONAL DIFFERENTIATION BY AKT-DEPENDENT ID2 PHOSPHORYLATION

Kwon, I. S.[1], Ahn, S. J.[2], Lee, K. H.[2] and Ahn, J. Y.[1]

[1]*Department of Molecular Cell Biology*
[2]*Department of Anatomy, Sungkyunkwan University School of Medicine, Suwon, South Korea*

Inhibitor of differentiation (Id) family of helix-loop-helix (HLH) proteins act as negative regulators of bHLH transcription factors by forming transcriptionally inactive complex. These proteins are involved in the regulation of cell growth and differentiation, cell cycle progression, embryonic development, and cell death. In this study, we found Id2 protein as a kinase is one of substrates of Akt via *in vitro* kinase assay. Employing PC12 cells which are expressed with constitutively active Akt (CA-Akt) and kinase dead Akt (KD-Akt), we observed that overexpression of Id2 increases the cell number of in both G1 and S phase, compared with mock control of CA-Akt, whereas Id2 accumulates the cell number of G1 phase in KD-Akt cells. Therefore, Akt-dependent Id2 phosphorylation may promote the entry G1 to S phase of cell cycle, indicating that Id2 and Akt control a balance between proliferation and differentiation of neurons.

Kwon I.S. and Ahn S.J. contributed equally to this work.

MO01-15

PROMOTION OF ASTROGLIAL DIFFERENTIATION BY GROUP III METABOTROPIC GLUTAMATE RECEPTORS IN MURINE NEURAL PROGENITOR CELLS

Makanga, J. O., Yoshida, K., Fukui, M., Nakamichi, N., Takarada, T. and Yoneda, Y.

Kanazawa University Graduate School of Natural Science and Technology, Kanazawa, Japan

Our previous studies showed that activation of group III metabotropic glutamate receptors (group III mGluR) decreases proliferation via modulation of cellular cAMP levels and cyclinD1 expression in murine embryonic neural progenitor cells (NPCs). In this study, we evaluated the possible regulation of cellular differentiation by group III mGluR. Spheres were formed by clustered cells during the culture of NPCs isolated from embryonic mouse neocortex with EGF. Neurospheres were dispersed and cultured for an additional 4

days in the absence of EGF under adherent conditions. Sustained exposure to the group III mGluR agonist, L-AP4, significantly decreased the size of neurospheres formed during culture for 10 days. We next used the mouse embryonic carcinoma cell line P19, that is able to self-replicate and differentiate into neuronal and astroglial cells. Marked but transient expression was seen for MAP2 mRNA in P19 cells cultured with retinoic acid, followed by marked increase in GFAP mRNA expression. Forskolin significantly increased the luciferase activity in P19 cells transfected with the luciferase reporter plasmid linked to cyclinD1 promoter. L-AP4 was effective in significantly preventing the increase by forskolin in an antagonist-sensitive fashion. Prior exposure to L-AP4 led to a significant increase in GFAP-positive cells with decreased MAP2-positive cells upon spontaneous and induced differentiation in an antagonist sensitive manner.These results suggest that group III mGluR signals not only decrease proliferation but also promote differentiation into an astroglial lineage with concomitant suppressed differentiation into a neuronal lineage, in murine embryonic progenitor cells.

MO01-16

PROMOTION OF NEURONAL DIFFERENTIATION OF NEURAL PROGENITOR CELLS CULTURED UNDER STATIC MAGNETISM

Nakatani, E., Nakamichi, N., Ishioka, Y., Takarada, T. and Yoneda, Y.

Laboratory of Molecular Pharmacology, Kanazawa University Graduate School of Natural Science and Technology, Kanazawa, Japan

In this study, we investigated the effects of static magnetism on proliferation for self-replication and differentiation into neuronal, astroglial and oligodendroglial lineages in undifferentiated neural progenitor cells. Neural progenitor cells were isolated from embryonic rat neocortex and hippocampus, followed by culture under static magnetism at 100 mT for different periods and subsequent determination of the number of cells immunoreactive for a marker protein of particular progeny lineages. Static magnetism not only significantly decreased proliferation of neural progenitor cells without affecting cell viability, but also promoted differentiation into cells immunoreactive for MAP2 with a concomitant decrease in that for an astroglial marker irrespective of the presence of differentiation inducers. In neural progenitors cultured under static magnetism, a significant increase was seen in mRNA expression of several activator type proneural genes, such as Mash1, Math1 and Math3, together with decreased mRNA expression of the repressor type Hes5. These results suggest that sustained static magnetism could suppress proliferation for self-renewal and facilitate differentiation into neurons through expression of several proneural genes by progenitor cells in fetal rat brain. This study would lead to the innovative development of magnetic instruments clinically useful for the noninvasive treatment of patients with a variety of neurodegenerative and/or neuropsychiatric diseases relevant to neuronal dysfunctions in terms of promoted neuronal differentiation during embryonic neurogenesis.

MO01-17

ALZHEIMER'S DISEASE DRUG "MEMANTINE" PROMOTES NEUROGENESIS AND PROGENITOR CELL SELF-RENEWING IN ADULT MOUSE HIPPOCAMPUS

Namba, T.[1], Maekawa, M.[2], Yuasa, S.[2], Uchino, S.[1] and Kohsaka, S.[1]

[1]*Department of Neurochemistry*

[2]*Department of Ultrastructural Research, National Institute of Neuroscience, Tokyo, Japan*

New neurons are continuously generated in the hippocampus of the adult mammalian brain. Cumulative evidence has demonstrated that NMDA receptor antagonists increased the number of newly generated cells in the adult dentate gyrus (DG). In this study, we examined the effect of memantine, an NMDA receptor antagonist that is clinically used for the treatment of Alzheimer's disease, on the neurogenesis in the adult mouse DG. We injected 3-month-old mice with memantine (50 mg/kg body weight, intraperitoneally [i.p.]); 3 days later, we injected the mice with 5-bromo-2-deoxyuridine (BrdU; 75 mg/kg body weight, i.p.). The number of the BrdU-labeled cells was significantly increased 1, 7 and 28 days after the BrdU-injection. Immunohistochemical staining at 28 days after BrdU-injection showed that most of the BrdU-labeled cells were positive for neuronal marker proteins, suggesting that memantine increased the number of the newly-generated neurons. We next examined the effect of memantine on primary progenitor cells exhibiting a radial glia-like (RGL) morphology. We counted the number of BrdU-labeled RGL progenitor cells 1 or 7 days after the BrdU-injection. The number of BrdU-labeled RGL progenitor cells had increased by 5.1-fold on day 1 and by 13.7-fold on day 7 after BrdU-injection. We also found that memantine increased the ratio of horizontally aligned RGL progenitor cells, which are probably produced by symmetric division. These findings suggest that memantine increases the proliferation of primary progenitor cells and expands the primary progenitor cell pool in the adult DG by stimulating symmetric division.

MO01-18

FORMATION OF 4-HYDROXYNONENAL-ADDUCTED PROTEINS DURING NEUROREGENERATION AFTER NEURODEGENERATION IN THE HIPPOCAMPAL DENTATE GYRUS

Ogita, K., Nagashima, R. and Sano, S.

Department of Pharmacology, Setsunan University, Hirakata, Japan

Our previous study showed that trimethyltin chloride (TMT) causes neuronal loss in the hippocampal dentate gyrus selectively 2 days later, with recovery of the dentate gyrus 14 days afterward. Our previous reports have demonstrated that TMT-induced neuronal damage is caused by activation of cell death signals induced at least in part by oxidative stress. In this study, we evaluated if *in vivo* acute treatment with TMT produces formation of 4-hydroxynonenal (4-HNE), which is a major lipid peroxidation product, in the hippocampus of mice. A systemic injection of TMT (2.8 mg/kg, i.p.) produced a marked lipid peroxidation in the hippocampus on day 1 and afterward. Immunohistochemical studies revealed that formation of 4 HNE was seen selectively in the dentate gyrus on day 1 after TMT treatment. Immunoblot analysis revealed that TMT treatment produced a new 4-HNE-adducted protein, whose molecular weight is 48 kDa, in the dentate gyrus, but not in the CA subfield. The 4-HNE-adducted protein was found from days 2 to 21,

with a peak on day 10 after TMT injection. Taken together, our results suggest that 4-HNE may be involved in both neurodegeneration at the early time window and neuroregeneration at the late time window after TMT treatment.

MO01-19

EFFECTS OF HEAT SHOCK ON PROLIFERATION OF MOUSE ES CELL-DERIVED NEURAL STEM CELLS

Omori, H.[1], Otsu, M.[1,2], Yoshie, T.[1], Shibata, M.[1], Nakayama, T.[3] and Inoue, N.[1]

[1]*Laboratory.of Regener. Neurosci., Tokyo Metropolitan University Graduate School of Human Health Sci.*

[2]*Department of Chemistry, School of Medicine, Kyorin University, Tokyo*

[3]*Department of Biochemistry, School.of Medicine, Yokohama City University, Yokohama, Japan*

Heat shock due to thermotherapy has been frequently used for clinical treatment. It is important to examine the effects of heat shock on cultured neural cells to evaluate a clinical response of heat shock. In this study, we investigated effects of heat shock on proliferation of the neural stem cells prepared from embryonic stem (ES) cells. A large number of neural stem cells were prepared from mouse ES cells by Neural Stem Sphere (NSS) method. Cultured with fibroblast growth factor-2, the neural stem cells proliferated exponentially, and the cells were exposed to heat shock for 20 min and cultured for 4 days. After exposure to heat shock at not over 42°C, the cells could proliferate as well. However, when the cells were exposed to heat shock at 43°C and above, the proliferation was temperature-dependently inhibited. It suggests that heat shock may have effects on proliferation of the neural stem cells at high temperature. Gene expression of the neural stem cells cultured at not over 43°C was analyzed by real-time RT-PCR to evaluate effects of heat shock on differentiation of the cells. The gene expression of Nestin and Musashi-1, makers of neural stem cells, was demonstrated to be high and not affected by the heat-shock exposure. In contrast, the expression of a marker of neurons, astrocytes and oligodendrocytes was demonstrated to be low. These results suggest that the neural stem cells maintained its cellular properties after heat-shock exposure. This work was partly supported by Grants-in-Aid for Scientific Research from JSPS and Selective Research Fund of Tokyo Metropolitan University.

MO01-20

IDENTIFICATION OF A GENOMIC REGION IN THE EPHRIN-A5 AND EPHA7 LOCUS DIRECTING THEIR GENE EXPRESSION TO THE ANTERIOR NEURAL TUBE

Park, E., Kim, Y., Noh, H. and Park, S.

Department of Biological Science, Sookmyung Women's University, Seoul, South Korea

Ephrin ligand and Eph receptor mediate the communication between cells and their bidirectional signaling mechanism has emerged as a key determinant of various developmental processes. Previous studies revealed that both ephrin-A5 and EphA7 null mutation in mice resulted in neural tube defects. This is likely to be due to abnormal regulatory proliferation of cortical progenitors, suggesting that interaction between ephrin-A5 and EphA7 play an integral role in the formation of the neural tube. To further illustrate this mechanism, we have investigated on isolating the cis-acting DNAs involved in specifying ephrin-A5 or EphA7 gene expression to the forebrain and mesencephalon using a BAC transgenic approach. Various BAC clones have been selected to insert LacZ reporter gene using bacterial homologous recombination and then each recombinant BAC was injected to mouse embryo to generate BAC transgenic lines. These BAC transgenic embryos and mice are analyzed at various developmental stages using β-galactosidase assay. As a result, we identified that 57 kb for EphA7 and 46 kb genomic region for ephrin-A5 contain cis-acting elements specific for anterior neural tube, respectively.

MO01-21

COPINE1 TRIGGERS THE NEURONAL DIFFERENTIATION OF THE RAT HIPPOCAMPAL PROGENITOR CELL LINE, HIB5 CELLS

Park, N., Hwang, E., Kim, E., Kim, D., Kang, D., Yoo, J., Han, J., Hong, S. and Park, J.

Department of physilogy, BK21, University of Gyeongsang National, Jinju, Korea

C2 domains are protein modules found in numerous eukaryotic signaling proteins, where their function is to target the protein to cell membranes in response to a Ca^{2+} signal. We previously listed 175 human C2 domains of the whole genome from the NCBI gene bank data. RT-TCR was performed using total RNAs isolated from human tissues such as brain and placenta. Finally 150 human C2-domain master clones obtained through Gateway Cloning System, and then converted into GFP-tagged adenoviral vectors. Finally, 150 adenoviruses expressing the GFP-tagged C2 domains were infected into HiB5 cells under the unstimulated condition. In this condition, we screened 14 C2 domains, induced the neuronal differentiation of HiB5 cells. In detail, C2 domain of copine1 and full-length copine1 clearly triggered the neuronal differentiation of HiB5 cells.

MO01-22

SURVIVAL CONDITIONS FOR CONTINUED *IN VITRO* PROLIFERATION OF NEURAL STEM CELLS/NEURAL PRECURSOR CELLS ISOLATED BY FLOW CYTOMETRY

Sadakane, K., Toda, T. and Yamanoha, B.

Department of Environmental Engineering for Symbiosis, Faculty of Engineering, Soka University, Tokyo, JAPAN

Neural stem cells generate from the neuroepithelium where they originate in the neural tube of the rat forebrain. To culture cells from the dorsal region of the forebrain in suspension conditions, we have adopted a neurosphere method. Neurospheres are composed of neural stem cells / precursor cells (NSPCs) and can be generated in primary culture containing fibroblast growth factor (FGF-2). In primary culture, the sequential survival of neruospheres may be difficult at low density in serum-free medium, although neurosphere methods are useful to research the nature of NSPCs in vitro. It has been suggested that the deletion of cofactors might influence the survival of NSPCs.We have established a flow cytometry (FCM) method based on light scattering for rapid sorting of relatively highly purified NSPCs from the forebrain at embryonic day 12. Although sorted cells generated neurospheres in culture, the number of living neurospheres gradually decreased and their survival rate rapidly declined up to 10 DIV. Nevertheless, neurospheres generated in co-culture with sorted cells survived up to 10 DIV.

We therefore focused on sorted cells other than NSPCs from the forebrain. The results showed that adhesion-like cells in co-culture support the survival of NSPCs, whereas highly purified NSPC-like

cells were induced to cell death in a monoculture. Also, the adhesion-like cells were cultured preferentially and used to produce conditioned medium (CM). In this study, to analyze the target protein in the CM, we extracted proteins from Native PAGE and established a convenient bioassay using cell-suspensions prepared from the forebrain.

MO01-23

POSSIBLE INVOLVEMENT OF 5-AMP-ACTIVATED PROTEIN KINASE IN PROLIFERATION AND SURVIVAL OF NEURAL STEM/PROGENITOR CELLS

Shohei, S., Nobuyuki, K., Masanori, Y., Reiko, N., Koichi, K. and Kiyokazu, O.

Department of Pharmacology, Setsunan University Faculty of Pharmaceutical Sciences, Hirakata, Osaka, Japan

Neural stem/progenitor cells (NPCs), which are defined by their ability to self-renew and differentiate into the three major cell types, neurons, astrocytes, and oligodendrocytes, play a critical role in the development and maturation of the central nervous system. 5'-AMP-activated protein kinase (AMPK) acts as a metabolic sensor, which is potently activated by 5'-AMP. To elucidate roles of AMPK in proliferation of NPCs, in this study, we evaluated the effect of an activator and inhibitor for AMPK on proliferative activity in NPCs derived from the neocortex of embryonic mice. Neurospheres were prepared from the neocortex of 15-days-old embryonic mice by primary culturing in DMEM/F12 medium with EGF and bFGF for 9 days *in vitro* (DIV). After replating, the cells were further cultured for 6 DIV under the same condition in the absence or presence of in the presence of metformin (AMPK activator) and compound C (AMPK inhibitor). Immunoblot analysis revealed that the level of phospho-AMPK (p-AMPK), but not AMPK, was progressively increased in a culture time-dependent manner. MTT assay and ELISA of 5'-brom-2'-deoxyuridine revealed that treatment with metformin enhanced not only the expression of p-AMPK but also the proliferative activity and survival of neurospheres during culture. In contrast, treatments with compound C produced a decrease in the proliferative activity and survival of neurospheres during culture. In addition, compound C was the ability to facilitate cell damage during culture. These results suggest that the activation of AMPK may positively regulate the proliferation and survival in NPCs of embryonic mouse neocortex.

MO01-24

MELATONIN INCREASES THE PROLIFERATION OF NEURAL STEM CELLS IN ADULT MOUSE SUBVENTRICULAR ZONE

Sotthibundhu, A.[1], Phansuwan-Pujito, P.[4] and Govithapong, P.[1,2,3]

[1]*Center for Neuroscience, Faculty of Science*
[2]*Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok*
[3]*Neuro-Behavioural Biology Center, Institute of Science and Technology, Mahidol University, Nakornpathom*
[4]*Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*

Melatonin, a circadian rhythm hormone secreted mainly by the pineal gland, has shown a variety of biological functions and neuroprotective effects including control of sleep wake cycle, seasonal reproduction, and body temperature as well as preventing neuronal cell death induced by neurotoxic substances. A number of reports have indicated that melatonin can also modulate effects on neural stem cells (NSCs) functions including the proliferation and differentiation in the embryonic brain tissue. However, it is still unclear how the involvement of melatonin in adult neurogenesis. Here we report that adult subventricular zone (SVZ) of the lateral ventricle, the main neurogenic area of the adult brain, expresses melatonin receptor. In addition, treatment NSCs derived from this area with melatonin during proliferation period increases the total number of neurospheres. The properties of NSCs were determined by using passaging technique and immunocytochemistry method. As stem cell replacement is thought to play an important therapeutic role in neurodegenerative diseases, melatonin might be beneficially used for stimulating endogenous neural stem cells.

Acknowledgements: This study was supported by a Mahidol University Postdoctoral Fellowship to AS and a TRF-Senior Research Scholar Fellowship and a Mahidol University Research Grant to PG.

MO01-25

MYOSIN VI EXPRESSION IN RESPONSE TO TRAUMATIC STRESS IN MURINE HIPPOCAMPUS

Tachibana, M., Ito, Y., Tamaki, K., Takarada, T., Nakamichi, N. and Yoneda, Y.

Laboratory of Molecular Pharmacology, Kanazawa University Graduate School of Natural Science and Technology, Kanazawa, Japan

Posttraumatic stress disorder (PTSD) is characterized by recurrent recalls of bad memories, insomnia with nightmares and emotional withdrawal, all of which are long-lasting. In this study, we have evaluated the underlying mechanisms for PTSD from a viewpoint of adult neurogenesis in mice. Adult male mice were subjected to restraint stress in a metallic cage immersed in water up to the individual clavicle for 3 h as a traumatic experience (WIRS), followed by behavioral tests 14 days later. Stressed mice showed increased spontaneous locomotor activities, exhibiting freezing behaviors in response to a recall flashback signal on the tone fear-conditioning task and forced swimming test. A transient decrease was seen in the incorporation of the 5-bromo-2'-deoxyuridine (BrdU) into the hippocampal dentate gyrus 5 days after WIRS, while a significant decrease was seen in BrdU incorporation in the dentate gyrus 5 days after flashback signals given 9 days after WIRS. Chronic treatment with imipramine significantly improved those abnormalities in the animal behavior and neurogenesis. Moreover, we carried out differential display analysis to profile gene expression changes in the hippocampus obtained 1 day after WIRS. Myosin VI (Myo6) was identified as a gene upregulated in response to traumatic stress in the murine hippocampus. Real-time PCR and Western blot analyses revealed that WIRS led to a significant but transient increase in the expression of mRNA and protein for Myo6 in the hippocampus 1 day later. These results suggest that Myo6 could play a pivotal role in the mechanism underlying the decreased adult neurogenesis in the hippocampal dentate gyrus in mice with traumatic stress.

MO01-26

ROLES OF MOTONEURON-DERIVED NT-3 ON SENSORY NEURON DEVELOPMENT

Usui, N.[1,2], Watanabe, K.[3], Ono, K.[4], Tamamaki, N.[3], Ikenaka, K.[1,2] and Takebayashi, H.[3]

[1]*Department of Physiological Science, School of Life Science, The Graduate University for Advanced studies (SOKENDAI)*
[2]*Division of Neurobiology and Bioinformatics, National Institute for Physiological Sciences (NIPS), Okazaki*
[3]*Department of Morphological Neural Science, Kumamoto University, Kumamoto*
[4]*Department of Biology, Kyoto Prefectural University of Medicine, Kyoto, Japan*

Execution of motor and sensory behaviors depends on circuitries effectively integrating immediate sensory feedback to motor pathways controlling muscle activity. Motor and sensory circuitries are thought to interact during development, however, the molecular mechanisms are not fully understood. To investigate motor and sensory interaction, we analyzed DRG phenotype of Olig2 knockout (KO) embryos, which have no motoneuron in the spinal cord (SC). Olig2 KO mouse is a good tool for this study. In the E10.5 DRG of Olig2 KO mice, increased number of apoptotic cells was observed. Furthermore, abnormal axon projection of sensory neurons was also observed in Olig2 KO mice at E10.5 and E12.5. These data suggest that motoneuron-derived factor(s) modulate sensory neuron survival and axon projection. Among neurotrophic factors, we focused on neurotrophin-3 (NT-3), because NT-3 in known as a survival factor for sensory neuron and chemoattractant factor for sensory neuron axons *in vitro*. Using in situ hybridization, we confirmed NT-3 mRNA expression in motoneurons in wild type and Olig2 heterozygous SC at E10.5 and E13.5, but almost no expression in Olig2 KO mice SC at the same stages. NT-3 mRNA expression outside of SC became stronger at later stages. These data suggest that motoneuron-derivied NT-3 is one of the essential factors for survival and axon guidance of sensory neurons at an early developmental stage.

MO01-27

STUDY ON CELL LINEAGE OF THE NEUROSPHERES DERIVED FROM E12 RAT FOREBRAIN

Yamanoha, B., Nakazono, R. and Toda, T.

Department of Environmental Engineering for Symbiosis, Faculty of Engineering, Soka University, Tokyo, Japan

Neural stem cells and neural precursor cells (NSNPCs) were originally derived from the neural tube in early developmental stage of rat embryo. We focused on the cell lineage of NSNPCs and especially the differentiation of the various type of neurons. The NSNPCs separated by the flow cytometry(FCM) was derived from the forebrain of embryonic day 12, and stained with cell-surface antigens. The sorted cells in the suspensions were partially bound to anti-A2B5 and anti-cadherin cell-surface antibodies, however most of the cells were the nestin positive cells except the supporting cell as fibroblast. The positive cells expressed the surface antigens were able to separated in the FCM,respectively. Following experiments, the A2B5 bound cells were stained in anti-nestin antibody. The double positive cells were shown 10% in the total cells, however 7% in negative cells. Also, A2B5 bound cell give arise sequential frequency of the antigen on the cell surface. The other side, the cadherin bound cells were contained to the nestin positive cell populations. The A2B5 bound cell in primary culture were performed the neurosphere methods. The spheres were resulted the morphological change in primary culture, that floating spheres and adherent spheres to substratum. Results were shown that the floating spheres were presented at relatively high level of Tuji-1 and MAP-2 positive cells, however the adhesion spheres were GFAP and O4 positive cells.

MO01-28

ENHANCED SURVIVAL OF NEWLY GENERATED CELLS BY FREE RADICAL SCAVENGERS AFTER NEURONAL DAMAGE IN THE HIPPOCAMPAL DENTATE GYRUS

Yoneyama, M., Kawada, K. and Ogita, K.

Department of Pharmacology, Setsunan University Faculty of Pharmaceutical Sciences, Osaka, Japan

Our previous study indicated that trimethyltin chloride (TMT) causes neuronal degeneration in the hippocampal dentate gyrus selectively 2 days later, with regeneration of the dentate granule cell layer 14 days afterward. In this study, we evaluated the effect of the free radical scavenger edaravone on newly generated cells in the hippocampal dentate gyrus after the damage induced by TMT. Mice were given TMT (2.8 mg/kg, i.p.). Edaravone (50 mg/kg, i.p.) was injected for consecutive 2 or 7 days every 12 h from day 2 after TMT treatment. To label mitotic cells, we gave mice a single series of four injections of 5-bromo-2-deoxyuridine (BrdU, 50 mg/kg, i.p.) every 12 h from day 2 after TMT treatment. In addition, immuno-staining for proliferating nuclaer antigen (PCNA) was perfomed for determination of proliferating cells. marked increase in the number of BrdU- and PCNA-positive cells was seen in the hippocampal dentate gyrus on day 4 after TMT treatment. These positive cells dramatically disappeared on day 9 after TMT treatment in control animals. In edaravone-treated animals, the large number of BrdU-positive cells existed even on day 9 after TMT treatment., the number of PCNA-positive cells had no significant change between control and edaravon-treated animals. In the subvetricular zone, edaravone did not affect the number of BrdU- and PCNA-positive cells on days 4 and 9 after TMT treatment. These results suggest that the free radical scavenger contributes to survival of newly generated cells in the hippocampal dentate gyrus after the damage induced by TMT.

MO01-29

ENDOCYTOSIS OF EPHA-EPHRINA COMPLEXES IS ESSENTIAL FOR THE ESTABLISHMENT OF RETINOCOLLICULAR TOPOGRAPHY

Yoo, S., Kim, Y., Lee, H. and Park, S.

Department of Biological Science, Sookmyung Women's University, Seoul, Korea

Recent studies revealed that contact-mediated repulsion through high-affinity interaction between EphAs and ephrin-As underlie the establishment of retinocollicular topography. The paradoxical contact-mediated repulsion may be mediated by rapid endocytosis of Eph-ephrin complexes from the cell surface. Here we show that forward endocytosis was effectively induced by ephrinA5 ligand stimulation in both EphA8 expressing HEK293 cells and superior colliculus primary cells. Reverse endocytosis, however, occurred neither in ephrinA5 expressing HEK293 cells nor in primary RGC axons. Interestingly, a specific region in the juxtamembrane domain of EphA8 encoded by exon10 of ephA8 is absolutely required for the endocytosis of EphA8-ephrinA5 complexes. Importantly, it is

found that the EphA8 mutant lacking the exon10-encoded region is able to form dimer with other EphA receptor families and significantly reduce their internalization, suggesting its dominant negative function. To further illustrate the role of the endocytosis-defective EphA8 mutant for the retinocollicular topography, we generated BAC transgenic mice expressing this EphA8 mutant. Anterograde tracing experiments revealed that retinocollicular topographic mapping is disturbed in mice expressing the endocytois-defective EphA8 mutant. This topographic map error was also observed in mutant mice labeled nasal retinal cells with GFP. When cultured with mutant expressing cells, nasal ganglion cell axon became less sensitive to the repulsive signal. Taken together, these results suggest that the endocytosis mechanism is essential for the axonal repulsion and correct retinocollicular map formation.

MO01-30

OLFACTORY TRACT TRANSECTION ENHANCES ADULT NEUROGENESIS IN RAT PIRIFORM CORTEX

Yuan, T. F., Liang, Y. X., Tay, D. K., So, K. F. and Ellis-Behnke, R. G.

Department of Anatomy, University of Hong Kong, Hong Kong, Hong Kong

Adult neurogenesis happens continuously in olfactory bulb. Loss of sensory input to the olfactory bulb leads to decreased formation of new neurons in the bulb. Piriform cortex receives projection from ipsilateral olfactory bulb, and previous studies proved the existence of new neurons in adult piriform cortex under physiological conditions. However the effects of olfactory bulb input deprivation on adult neurogenesis in rat piriform cortex have not been investigated before. The existence of neuronal progenitor cells in adult rat piriform cortex were confirmed with Bromodeoxyuridine (BrdU) labeling and early neuronal markers such as doublecortin (DCX) and Polysialic Acid-NCAM (PSA-NCAM). Sensory deprivation to the piriform cortex achieved by transection of the olfactory tract at the caudal part of one olfactory bulb, before the frontal sinus, leads to a significant increase (~60%) of doublecortin positive cells in the ipsilateral piriform cortex compared with the contralateral (intact) side. Because deafferentation causes cell apoptosis, it is possible that deafferentation or local cell death can enhance the recruitment of new neurons in the piriform cortex.

MO01-31

EXPRESSION OF DRG2 IN THE ADULT MICE BRAIN

Yun, C. H., Kim, E. O. and Lee, B. J.

Department of Biological Sciences, University of Ulsan, Ulsan, South Korea

DRG2, a developmentally regulated GTP-binding protein 2, may play an important role in certain essential cellular or developmental functions. However, very little is known about DRG2 expression and functions in the adult nervous system. Here, we performed detailed characterization on the localization of DRG2 in the adult mice brain. We have investigated the expression pattern of DRG2 in the adult mice brains using immunohistochemistry and Western blot analysis. Our studies showed that DRG2 is widely expressed in major brain areas including olfactory bulb, cerebral cortex, hippocampus, caudate putamen, thalamus, hypothalamus, cerebellum and spinal cord. Moreover, DRG2 expressing cells also expressed NeuN, doublecortin, and calbindin D-28k. Expression profile of DRG2 may serve a helpful tool for understanding and studying functional characterization of DRG2 in the central nervous system.

MO02 Synaptic plasticity and neurodegeneration

MO02-01

L-LTP IN YOUNG RATS UNDER CONDITIONS OF LONG TIME APPLICATION OF PROTEIN SYNTHESIS INHIBITORS

Abbas, A. K., Huang, F. S., Li, R. and Wigström, H.

Institute of Neuroscience and Physiology, Gothenburg, Sweden

It has previously been shown that hippocampal long-term potentiation lasting longer than 2–3 h depends on synthesis of new proteins. This synthesis is believed to be triggered by the LTP-inducing stimulation and taking place during a critical window within about 30 min following induction of LTP. Constitutive proteins may also be important under conditions of reduced protein degradation or in transgenic animals with up-regulated machinery for constitutive synthesis. We have previously shown in 2–3-week-old rats that L-LTP is still possible when protein synthesis is blocked, suggesting that constitutive proteins are sufficient. Alternatively the critical time window may differ in young animals. Here, we have tested whether blockade of protein synthesis for longer times can influence LTP, using recording times of 5–10 h after induction. Several hours preapplication of protein synthesis inhibitor emetine to CA1 area still allowed L-LTP in cases where the effect on baseline was small. However, decaying baselines were generally associated with blockage of LTP. Normal L-LTP was observed in slices treated with cycloheximide from -30 min before induction and remaining in the bath for the rest of the experiment. Additionally, we used the same protocol but with a cocktail of inhibitors (anisomycin and cycloheximide in concentrations previously reported to be effective when used separately) without affecting LTP. Based on these results, where protein synthesis was blocked for long intervals both before and after LTP induction, we conclude that de novo protein synthesis is not needed for establishing L-LTP in this group of animals, implying that already available proteins are sufficient. The observed effect of preapplication of emetine may represent an effect on LTP induction.

MO02-02

IMMUNOCYTOCHEMICAL STUDY OF METHAMPHETAMINE INDUCED α-SYNUCLEIN IN SK-N-SH CELLS

Boontem, P.[1], Phansuwan-Pujito, P.[2], Ueda, K.[3] and Govitrapong, P.[1,4,5]

[1]*Center for Neuroscience, Faculty of Science, Mahidol University*
[2]*Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
[3]*Tokyo Institute of Psychiatry, Division of Psychobiology, Tokyo, Japan*
[4]*Neuro-Behavioural Biology Center, Institute of Science and Technology for Research and Development, Mahidol University, Nakornpathom*
[5]*Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand*

Methamphetamine (METH) is the extremely addictive drug that induced neurotoxicity. Our previous studies indicate that METH induces oxidative stress and stimulates α-synuclein protein expression in the dopamine neurons. Growing evidence indicated that oxidative stress could induce the fibrilization and aggregation of α-synuclein. In this study we examined the effect of METH on α-synuclein expression in SK-N-SH cell lines using immunocytochemistry analysis. The confocal microscopic analysis showed METH treatment group induces the translocation of α-synuclein from cytoplasm to nuclei of SK-N-SH cells when compared to the control group. This result suggesting that METH treatment enhances nuclear α-synuclein accumulation in SK-N-SH cells. This result may lead to understand the pathogenesis and cell death. However, the mechanism of METH-induced α-synuclein translocation remains to be further investigated.

Acknowledgement: This study was supported by a TRF-Senior Research Scholar Fellowship from the Thailand Research Fund and a Mahidol University Research Grant to PG.

MO02-03

INCREASED LIPID AND PROTEIN OXIDATION IN THE BRAIN OF AUTISTIC INDIVIDUALS

Chauhan, V., Muthaiyah, B., Essa, M. M., Wegiel, J., Brown, W. T. and Chauhan, A.

NYS Institute for Basic Research in Developmental Disabilities, New York, USA

Accumulating evidence suggests that oxidative stress may provide a link between susceptibility genes and pre- and post-natal environmental stressors in the pathophysiology of autism. Brain tissue is highly heterogeneous with different functions localized in specific areas. Studies on oxidative stress in relation to specific regions of the brain are lacking in autism. We compared the status of lipid peroxidation and protein oxidation in postmortem brain samples from the cerebellum and frontal, temporal, parietal and occipital cortex from autistic subjects with age range of 4 to 39 years (N = 7–10 for different tissues) and age-matched normal subjects (N = 9–10). Protein oxidation, assessed by quantitation of protein carbonyls, was significantly increased in autism by 123 % in frontal cortex, by 108 % in temporal cortex, and by 100 % in cerebellum as compared with controls. On the other hand, its levels in parietal and occipital cortex were similar between autism and control groups. In addition, the levels of malonyldialdehyde (MDA), a marker of lipid peroxidation, were significantly increased by 124 % in the cerebellum and by 256 % in the temporal cortex in autism as compared with control subjects. In contrast, no significant change in MDA levels was observed in frontal, occipital and parietal cortex between autism and control groups. These results suggest that oxidative stress differentially affects selective regions of the brain, i.e. cerebellum, frontal cortex and temporal cortex in autism.

MO02-04

DECIPHERING THE MECHANISM OF HYDROGEN SULFIDE-INDUCED NEURONAL DEATH IN CULTURED MURINE CORTICAL NEURONS

Chen, M. J.[1], Peng, Z. F.[2], Manikandan, J.[3], Moore, P. K.[4] and Cheung, N. S.[1]

[1]*Menzies Research Institute, Hobart, Australia*
[2]*Department of Biochemistry, National University of Singapore*
[3]*Department of Physiology, National University of Singapore, Singapore, Singapore*
[4]*King's College, Pharmaceutical Science Division, London, UK*

Hydrogen sulfide (H_2S), present in extremely high levels in the brain, was demonstrated to induce a dose-and time-dependent apoptotic-necrotic continuum in primary cortical neurons. Present study focus on deciphering N-methyl-D-aspartate (NMDA) receptor involve-

ment in H_2S-mediated neuronal death through time-course global gene profiling (5 h, 15 h and 24 h). Murine primary cortical neurons treated with 200 μM sodium hydrosulfide (NaHS) and NMDA demonstrated global differential gene expression profiles of 3395 and 2309 genes with significant regulation of at least ± 1.5 fold-change in a minimum of one out of three time-points respectively. Cross comparison of both profiles revealed a substantial overlap of 1911 genes. Among them were gene families related to cell death, endoplasmic reticulum stress and heat shock proteins and chaperones. Furthermore, an analysis of genes with significant transcriptional regulation exclusive to NaHS treatment showed ubiquitin-proteasome system (UPS) dysfunction. In conclusion, having 70% of NMDA global gene profile with ± 1.5 fold-change cutoff being present in that of H_2S strongly emphasizes the existence of a unique signal transduction pathway centered on NMDA receptors in H_2S-mediated neuronal death, in parallel with other exclusive H_2S-induced biological pathways such as UPS inhibition.

MO02-05

A NOVEL SYNTHETIC COMPOUND AS A CANDIDATE NEUROPROTECTIVE AGENT

Choi, J. H.[1], Shin, K. J.[2], Son, H. J.[1], Kim, S. T.[1], Kim, D. J.[2] and Hwang, O.[1]

[1]*Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine*

[2]*Medicinal Chemistry Research Center, Korea Institute of Science and Technology, Seoul, Korea*

Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with selective loss of the neurons containing dopamine (DA) in the substantia nigra pars compacta. Lines of evidence suggest oxidative stress, as a major factor contributing to the vulnerability of DA cells. DA quinone, produced by oxidation of DA, can generate oxidative stress, and removal of DA quinone by quinone reductase (NQO1) can provide protection. We have synthesized a novel compound KST007067 and determined its effect on NQO1 expression and capacity as a neuroprotective agent against PD. KST007067 was found to induce gene expression of NQO1, as determined by RT-PCR, and to increase the protein level of NQO1, as determined by Western blot analysis in the DArgic cell line CATH.a. This was accompanied by an increase in nuclear translocation of the transcription factor Nrf2. KST007067 also showed neuroprotective effect against the DArgic toxins tetrahydrobiopterin(BH4), MPP^+ and H_2O_2. *In vivo*, KST007067 was able to prevent the loss of tyrosine hydroxylase-immunoreactive DArgic neurons in the substantia nigra in MPTP-treated mice. This was accompanied by suppression of degeneration of the nigrostriatal pathway, as determined by silver staining and FluoroJade C staining. Acute toxicity test showed that KST007067 administration resulted in no lethality, apparent tissue toxicity or behavioral changes at 5000 mg/kg. KST007067 exhibited no significant inhibitory effect on hERG channel at 10 μM. Taken together, KST007067 is a candidate neuroprotective agent for Parkinson's disease.

MO02-06

THE FIRST SEROTONIN RECEPTOR POSITIVELY COUPLED TO ADENYLYL CYCLASE INVOLVED IN SYNAPTIC FACILITATION IN APLYSIA

Choi, S. L.[1], Lee, Y. S.[1], Lee, S. H.[1], Kim, H.[1], Park, H.[1], Lee, S.[1], Chae, Y. S.[1], Kandel, E. R.[2] and Kaang, B. K.[1]

[1]*Seoul National University, Seoul, Korea*

[2]*Columbia University, New York, USA*

Marine snail, Aplysia, is a fascinating model animal to study synaptic plasticity, because it has the biggest neuron ever known. In Aplysia, serotonin (5-HT) plays important roles in modulating synaptic plasticity. Signal cascade including PKA and PKC can be activated by 5-HT, probably via distinct types of G-protein coupled receptors in Aplysia sensory neurons. However, the molecular identity of these receptors has not yet been uncovered. We here report the cloning and functional characterization of the first 5-HT receptor positively coupled to adenylyl cyclase in Aplysia sensory neuron. 5-HTapAC1, the cloned receptor, stimulates the production of cAMP in HEK293T cell and Xenopus oocyte. Moreover, knock-down of 5-HTapAC1 expression blocked 5-HT induced membrane excitability and spike broadening. Moreover, knock-down of 5-HTapAC1 blocked short-term synaptic facilitation in non-depressed and partially depressed sensory-to-motor neuron synapses. This result suggests that 5-HTapAC1 plays as a major synaptic modulator in Aplysia sensory neurons.

MO02-07

EXPRESSION OF THE STRESS RESPONSE PROTEINS AFTER OPTIC NERVE INJURY

Fujikawa, C.[1], Nagashima, M.[1,2], Nishitani, M.[1], Mawatari, K.[1] and Kato, S.[2]

[1]*Division of Health Sciences, Graduate School of Medicine*

[2]*Deapartment of Molecular Neurobiology, Graduate School of Medicine, Kanazawa University, Kanazawa, Japan*

Heat shock proteins (HSPs) play an important role for cell protection against various environmental stresses. Following stresses, HSPs are rapidly induced to control cell survival and maintenance. Fish retinal ganglion cells (RGCs) can survive and regenerate their axons after optic nerve injury (ONI), which is the best model for CNS repair after injury. Although many factors are involved in the fish optic nerve regeneration process, there is no report about HSPs in the fish retina after ONI. In this study, we investigate the expression of HSP70 after ONI and heat shock condition (HS, 37 degree 30 min) in zebrafish (ZF) retina. HSP70 mRNA was hardly expressed in normal adult ZF retina. After HS, HSP70 mRNA drastically increased in all retinal layers at 0.5–1 h, and then returned to control value by 3 h. In contrast, after ONI, HSP70 mRNA rapidly increased in the RGCs during 0.5–24 h and then returned to the control level by 72 h. HSP mRNA is transactivated by heat shock factors (HSFs). HSFs normally exist in cytoplasm, and is translocated to nucleus with active phosphorylated form under stress condition. Phosphorylated HSF1 protein was detected after both HS and ONI. The levels of HSF1 and HSF2 mRNAs were significantly increased in ZF retina 0.5–24 h after ONI, but did not change after HS. These results indicate that the transient induction of HSP by HS is ascribed to elevated translocation of phosphrylated HSF1 without transcriptional activation, whereas the continuous induction of HSP by ONI is ascribed to elevated translocation of phosphrylated HSF1 with transcriptional activation.

MO02-08

HOMOCYSTEINE-INDUCED ERK PHOSPHORYLATION IN CULTURED CEREBELLAR GRANULE NEURON: DEPENDENCE ON SELF-SENSITIZATION VIA SYSTEM XC-

Gu, L., Hu, X., Xue, Z., Wan, L., Ran, Y., Hertz, L. and Peng, L.

China Medical University, Shenyang, China

Excess concentrations of homocysteine, a naturally occurring amino acid, exist during many pathological conditions, have adverse effects on the vasculature and the brain and may cause cognitive impairment. This study has shown that millimolar homocysteine concentrations cause phosphorylation of extracellular-signal regulated kinases 1 and 2 ($ERK_{1/2}$) in cerebellar granule neurons incubated in saline media without glutamine. A ~10-fold increase in potency occurred in the presence of the obligatory glutamate precursor glutamine. At the same time phosphorylation of $ERK_{1/2}$, like that evoked by glutamate, became inhibitable by either NMDA or non-NMDA antagonists and dependent upon transactivation of the epidermal growth factor (EGF) receptor following metalloproteinase-mediated release of an EGF agonist. However, there was no increased release of glutamate. Further enhancement of $ERK_{1/2}$ phosphorylation occurred in the additional presence of cystine, which by itself has no effect. These characteristics suggest a 'self-stimulation' similar to that described for quisqualate via system Xc^-, an antiporter accumulating extracellular cystine in exchange with intracellular glutamate. Like quisqualate, homocysteine interacts with this transporter so that it initially can be taken up in exchange with glutamate and later released to the extracellular space at high local concentrations in exchange with its own, lower extracellular concentration and/or added cystine. In support of this concept, pharmacological inhibition of system Xc^- prevented the micromolar, transactivation-dependent ERK phosphorylation. Accordingly inhibitors of this system might be neuroprotective during hyperhomocysteinemia.

MO02-09

VERTICAL GRID TEST IS A SENSITIVE METHOD FOR EVALUATING MOTOR DYSFUNCTIONS IN THE MPTP MOUSE MODEL OF PD

JI, I. J., Kim, S. T., Son, H. J., Choi, J. H. and Hwang, O.

Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul, Korea

Parkinson's disease (PD) is caused by selective degeneration of the nigral dopamine(DA)rgic neurons and is accompanied by motor dysfunctions such as tremor, akinesia and rigidity. Changes in the degree of motor deficit can be utilized as a non-invasive way of assessing alterations in the number of DArgic neurons and/or the amount of DA in animal models of PD, such as mice systemically administrated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this study, in order to develop sensitive methods to detect DA-associated motor deficits, we designed a new test called vertical grid test and modified the existing horizontal grid test. After acute MPTP treatment, decreases in the levels of striatal DA (17.4% of vehicle-treated control), dihydroxyphenylacetic acid (33.3%), and homovanillic acid (40.5%) were observed. On the modified horizontal grid test, the MPTP-administered mice exhibited average forelimb step distance that was lower than control (82.58%) and correlated with the striatal DA levels. Vertical grid test has been designed so that the animal is placed facing upward at the top of a vertically positioned grid and the time taken for it to turn around and climb down are measured. The MPTP-treated mice took dramatically longer total time to climb down (220.94%) and time to make the turn (339.29%) compared to control, and this correlated well with the degree of striatal DA depletion. These results show that these parameters on vertical and horizontal grid tests can provide sensitive measures of motor impairments following MPTP administration.

MO02-10

MATRIX METALLOPROTEINASE-3 IS INCREASED AND MEDIATES THE CASPASE-12-INDUCED APOPTOSIS IN RESPONSE TO ER STRESS

Kim, E. M.[1], Choi, J. H.[1], Park, I. S.[2], Choi, D. H.[1], Joh, T. H.[3], Kim, Y. S.[3] and Hwang, O.[1]

[1]*Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul*

[2]*Research Center for Proteineous Materials and Department of Bio-Materials Engineering, School of Medicine, Chosun University, Gwangju, Korea*

[3]*Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, USA*

While ER stress-induced apoptosis has been associated with the pathogenesis of neurodegenerative diseases, the cellular components involved have not been well delineated. We have previously observed that matrix metalloproteinase (MMP)-3 enzyme activity is involved in the apoptotic signaling, acting upstream of caspase-3. This study shows that matrix metalloproteinase (MMP)-3 plays a critical role in the ER stress-induced apoptosis. Specifically, ER stress induced by brefeldin A (BFA) or tunicamycin (TM) causes gene induction and cleavage to active form of MMP-3, and this occurs selectively among MMP subtypes in brain-derived CATH.a cells. Both pharmacological inhibition and gene knockout of MMP-3 lead to protection against ER stress. MMP-3 acts downstream of caspase-12 because 1) pharmacological inhibition of caspase-12 attenuates MMP-3 generation, but inhibition or knockout of MMP-3 does not affect caspase-12 production; and 2) overexpression of active caspase-12 causes production of cleaved caspase-3 in wild type but not in MMP-3 knockout cells. Taken together, the activation of apoptotic signaling in response to ER stress appears to take place via induction of gene expression and an increase in the catalytic activity of MMP-3, acting downstream of caspase-12 and upstream of caspase-3. MMP-3 may therefore serve as a cellular target for therapy against neurodegenerative diseases.

MO02-11

NEURITIN-INDUCED POSTSYNAPTIC PLASTICITY AND MATURATION ASSOCIATED WITH INCREASED SPONTANEOUS MINI-EPSCS FREQUENCY, BUT NOT AMPLIT

Kim, H. G., Jeong, A. Y., Jung, S., Kim, J., An, K. M., Jung, J. H., Ryeo, S. E., Lee, J. H., Song, J. Y. and Kim, J. H.

Department of Life Science,Pohang University of Science & Technology(POSTECH), KOR

Neuritin (CPG15), an activity-regulated gene that has been known to promote growth of neurites encodes a small, highly conserved protein from Xenopus to human. In a view of its proposed neuritogenic activity in neuromuscular junctions of Xenopus, it is

conceivable that neuritin is involved in synapse formation and also in regulation of synaptic transmission of mammalian neurons. To this end, we examined whether neuritin has any physiological effects on synaptogenesis and synaptic transmission. Interestingly, overexpression of neuritin in cultured hippocampal neurons led to an increase in frequency, but not in amplitude of spontaneous mini-EPSCs. We also observed significant enhancement of axonal and dendritic arborization when neurons were transfected with neuritin (cpg15-IRES-EGFP). Our co-culture experiment (mixed culture of neurons and neuritin-overexpressing HEK293 cells) substantiated synaptogenetic activity of neuritin by showing an increase in the number and intensity of PSD95 puncta in the contacting area with HEK293 cells. Collectively these results suggested that neuritin plays a role in regulating basal synaptic transmission through controlling the number of functional synapses in mammalian neurons especially by recruitment of postsynaptic components.

MO02-12

TRANSCRIPTOME ANALYSIS, IDENTIFICATION OF REGULATORS INVOLVED IN LONG-TERM SYNAPTIC PLASTICITY IN *APLYSIA KURODAI*

Kim, J. I.[1], Choi, S. L.[1], Lee, Y. S.[1], Kim, T. H.[3], Bhak, J.[3] and Kaang, B. K.[1,2]

[1]*Department of Biological Sciences, Seoul National University*
[2]*Department of Brain and Cognitive Science, Seoul National University, Seoul*
[3]*Korean Bio Information Center, Daejeon, Korea*

Molecular studies of Aplysia, well-known animal model to study the mechanism of synaptic plasticity, have been hindered by the lack of genomic information so far. Lately, a large scale characterization of neuronal transcripts was performed in *A. californica*. Here, we report the analysis of a parallel set of neuronal transcripts from *A. kurodai*, a closely related species found in the northwestern Pacific. From the nervous tissue of *A. kurodai*, we collected 4859 nonredundant sequences. Using microarray and real-time PCR analyses, we found that ApC/EBP, matrilin, antistasin, eIF3e, and a BAT1 homologous clone were significantly up or down-regulated by *in vivo* 5-HT treatment. Moreover, we found that ApeIF3e might play a pivotal role in long-term facilitation (LTF).

MO02-13

PTEN-SUPPORTED STRUCTURAL HOMEOSTASIS IN RETINAL PIGMENT EPITHELIUM

Kim, N. S.[1], Kang, K. H.[1], Burrola, P.[2], Lemke, G.[2] and Kim, J. W.[1]

[1]*Department of Biological Sciences, Korea Advanced Institute of science and Technology, Daejeon, South Korea*
[2]*Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, USA*

Intercellular adhesions among epithelial cells are not only necessary to obtain the polarized features, but also correlated with the tight control of their proliferation and survival. The defects in epithelial cell adhesions, therefore, are associated with many abnormalities of tissues from degeneration to cancer. The intercellular adhesions in retinal pigment epithelium (RPE), of which defects cause many retinal degenerative diseases, enable them to provide structural and functional supports for the retina. The specific deletion of PTEN in mouse RPEs resulted in a progressive degeneration of photoreceptors following to the loss of mutant RPEs. The PTEN-deficient RPEs failed to preserve the basolateral intercellular adhesions, and underwent epithelial-to-mesenchymal transition (EMT). The C-terminal PSD-95/Dlg/ZO-1 homology (PDZ)-binding domain was turned out to be essential for PTEN-supported intercellular adhesion, implicating that PTEN facilitates junctional integrity through the direct interaction with PDZ domain proteins. The failure in junctional maintenance and phosphorylational inactivation of PTEN, which also interferes with the association of PTEN with PDZ domain proteins, in RPEs from ccr2-/- mice suffering from age-related macular degeneration (AMD) as well as in RPEs exposed to oxidative damages further emphasized a key role of PTEN in retinal homeostasis against physiological stresses.

MO02-14

REGULATION OF DENDRITIC SPINE MORPHOLOGY AND FUNCTION BY SPIN90, A NOVEL SHANK BINDING PARTNER

Kim, S. M.[1], Choi, K. Y.[2], Cho, I. H.[1], Ryu, J. H.[1], Kim, S. H.[3], Park, C. S.[1], Kim, E.[4] and Song, W. K.[1]

[1]*Department. of Life Science, Cell Dynamics Research Center and Bioimaging Research Center, Gwangju Institute of Science and Technology, Gwangju, Korea*
[2]*National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892*
[3]*Department of Biochemistry, Weill Medical College of Cornell University, New York NY 10021, USA*
[4]*National Creative Research Initiative Center for Synaptogenesis, Korea Advanced Institute of Science and Technology , Daejeon 305-701, Korea*

Dendritic spine morphogenesis is modulated by synaptic activity and regulated by molecules within the postsynaptic density (PSD), including various scaffold, cytoskeletal and signaling proteins. Although it is becoming increasingly clear that PSD scaffolding protein is critical for dendritic spine morphogenesis, the molecular signal that regulates spine morphogenesis and synapse formation is not well characterized. Here we show that SPIN90, which is highly enriched in the PSD, is a novel binding partner for Shank proteins that mediate the formation of multiple protein complexes. Overexpression of both SPIN90 and Shank promotes greater enlargement of dendritic spines than overexpression of Shank alone. Knockdown of SPIN90 inhibits postsynaptic clustering. Furthermore, we generated mice with targeted disruption of the SPIN90 gene to investigate the function of SPIN90 *in vivo*. SPIN90-/- mice showed altered protein composition of the PSD; reduced size of dendritic spines; altered PSDs. The knockout mice also showed altered significant abnormalities in spine morphology. These results demonstrate that SPIN90 is important *in vivo* for regulating dendritic spine morphogenesis and brain function.

MO02-15

NUCLEAR FACTOR OF ACTIVATED T CELLS (NFAT) COMPLEX MEDIATES NEURONAL ACTIVITY-DEPENDENT TRANSCRIPTION OF PROTOCADHERIN 8

Kim, S. Y., Mo, J. W., Han, S. B., Woong, S. and Kim, H.

Korea University College of Medicine, Seoul, Korea

Cadherin family members are important mediators of numerous developmental events, including activity-dependent brain development. Protocadherin 8 (PCDH8), one of such activity-regulated

cadherin members, is implicated in the induction of long-term potentiation (LTP). However, the molecular mechanisms underlying transcriptional upregulation of PCDH8 in neurons have not been investigated. In this study, we observed that PCDH8 transcription was highly induced in cortical neurons by depolarization. Deletion assay of PCDH8 promoter region demonstrated that the -390/-155 region is enough for the transcriptional activity of PCDH8 promoter. This region contains putative binding sites for cAMP response element-binding protein (CREB) /activating transcription factor-2 (ATF-2), activator protein-1 (AP-1) and nuclear factor of activated T cells (NFAT). NFAT signal blockade completely inhibited the induced PCDH8 promoter activity and endogenous PCDH8 transcription activity, whereas dominant-negative mutants of Jun or CREB did not inhibit the PCDH8 promoter activity. These results suggest that NFAT pathway plays an essential role in the induction of PCDH8 gene expression by neural activity, and NFAT-PCDH8 cascades may be involved in the regulation of activity-dependent long-term changes in the synaptic connections.

MO02-16

AMYOTROPHIC LATERAL SCLEROSIS-ASSOCIATED METABOLITE BIOMARKER PATTERN REVEALED BY (1) H NMR SPECTROSCOPY

Kumar, A. and Babu, G. N.

Department of Neurology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

In amyotrophic Lateral Sclerosis (ALS), an invariably fatal motor neuron disorder, increasing weakness and atrophy of limbs, facial muscles, and diaphragm is seen, while sensory functions remain intact. Neurological diseases may produce characteristic perturbations of the metabolome, the collection of small-molecules (metabolites) present in a cell, tissue, or organism. To test this hypothesis, we have investigated metabolite profile in the blood serum of 30 patients with ALS and 25 healthy controls by using (1)H NMR. Patients with ALS had significantly higher median concentrations (microM) of creatine/creatinine, glutamate, beta-hydroxybutyrate, acetate, acetone, and formate than healthy controls, and lower concentrations of glutamine, histidine and N-acetyl derivatives. Furthermore, we found that serum glutamate, formate and histidine showed significant correlation with the duration of the disease in ALS. Such (1)H NMR study of serum provides an insight into aberrant biochemical pathways which may have the potential to serve as surrogate markers for monitoring ALS disease progression.

MO02-17

IN VITRO MODELING OF PERINEURONAL NET: THE ROLE OF CARTILAGE LINK PROTEIN 1 AND HYALURONAN SYNTHASE IN ITS FORMATION AND INTEGRITY

Kwok, J. C., Carulli, D. and Fawcett, J. W.

Cambridge Centre for Brain Repair, University of Cambridge, Cambridge, UK

Perineuronal nets (PNNs) are dense extracellular matrix which enwraps the surface of neuronal cell bodies and proximal dendrites late in development. Hyaluronan, link proteins, tenascin-R, together with various chondroitin sulfate proteoglycans (CSPGs) are identified in the PNNs in cerebellum. Their temporal appearance correlates with the ending of critical period in ocular dominance plasticity, suggests their role in the process. Immunostaining in adult rat spinal cords showed similar molecular compositions as in the cerebellum. Of the CSPGs, aggrecan was present in all PNNs, while neurocan, versican and phosphacan were only found in some of the PNNs. Examination of the PNNs distribution showed that perineuronal-like pattern was first observed between postnatal day 7 to 14 in the developing spinal cords. In situ hybridization suggested that aggrecan, cartilage link protein (CTRL 1) and brain link protein-2 (BRAL2) are produced by neurons. Unlike cerebellum, the PNN-bearing neurons in the spinal cords expressed hyaluronan synthase (HAS) 1 and 3. RT-PCR showed that the temporal expression of link proteins, HASs and aggrecan mRNA was correlated with the PNN formation during postnatal development. *In vitro* modeling of the PNNs showed that CRTL1 and HAS are critical for the PNN formation. Expression of CRTL1 and HAS in cells was able to induce the formation of organised pericellular matrix similar to that on neurons, which retain aggrecan on the surface. Cells lacking any one of these showed impaired integrity of the PNNs. The results suggested that link protein and HAS are important in triggering the formation of the PNNs which finishes with the incorporation of aggrecan.

MO02-18

EFFECT OF CHRONIC VALPROIC ACID TREATMENT ON NEURO-GLIAL PLASTICITY IN FEMALE RATS

Lakhanpal, D. and Kaur, G.

Department of Biotechnology, Guru Nanak Dev University, Amritsar, India

Gonadotropin releasing hormone (GnRH) system in the brain constitutes the final common pathway for the central regulation of gonadotropin secretion. Our previous study shows pulsatile release of GnRH into perivascular space during estrous cycle overlaps with the expression of polysialylated form of neural cell adhesion molecule (PSA-NCAM), the molecular marker of neuronal plasticity.It has also been shown that cell-to-cell communication processes involving non-neuronal cells, such as glial cells, are important in regulating the secretory activity of those hypothalamic neurons that secrete GnRH. We are further testing a hypothesis whether GABAergic anti-epileptic drugs (AEDs) have their inhibitory effects on GnRH synthesis and/or release and thereby affect reproductive health. Valproic acid (VPA)is one of the most frequently prescribed AEDs and is associated with hyperandrogenism and polycystic ovaries in women with epilepsy (WWE) suggesting change in normal levels of estrogens - the gonadal steroids in females. Three month old cycling wistar strain female rats were given VPA (i.p.) at a dose of 300 mg/Kg once a day for 12 weeks; control group received an equivalent volume of vehicle. ELISA showed decrease in serum estradiol in VPA treated group. Using fluorescent in-situ hybridization (FISH) we observed that VPA reduced expression of polysialyl transferase (PST) and glial fibrillary acidic protein (GFAP) mRNA in the ME-ARC region of the hypothalamus, semi quantitative RT-PCR further confirmed FISH results. These results supports our hypothesis that VPA disrupts normal neuronal-glial plasticity in ME-ARC region of the hypothalamus and may be an important factor to cause reproductive neuroendocrine disorders in patients undergoing medication for epilepsy, bipolar disorders or migraine.

MO02-19

INCREASED CAMP LEVEL IN FOREBRAIN ENHANCES SYNAPTIC PLASTICITY AND COGNITIVE MEMORY IN APLYSIA OCTOPAMINE RECEPTOR TRANSGENIC MICE

Lee, H. R.[1], Kim, J. I.[1], Isiegas, C.[2], Wu, L.[3], Zhuo, M.[3], Abel, T.[2] and Kaang, B. K.[1]

[1]*National Creative Research Initiative Center for Memory, School of Biological Sciences, Seoul National University, Seoul, Korea*
[2]*Department of Biology, University of Pennsylvania, Philadelphia, USA*
[3]*Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Canada*

The cAMP-protein kinase A (PKA) signaling pathway is the important neuronal signaling pathways. Until now, many researches have been shown to elucidate the specific role of cAMP-PKA signaling *in vivo* and vast knowledges regarding its physiological role in synaptic plasticity and memory formation and storage have been accumulated. Nevertheless, few studies have been successful in illuminating the roles played by this signaling pathway in high temporal resolution because of current technical difficulties; in contrast, relatively non-specific genetic or pharmacological approaches have been more successful. For this study, we employed Aplysia octopamine receptor transgenic mice in whom heterologous Aplysia octopamine receptors were expressed only in the forebrain neurons by using the CaMKII-alpha promoter. Here, we found that a transient application of octopamine activated the octopamine receptors and greatly enhanced the potentiation of hippocampal synaptic plasticity. Furthermore, once potentiated by octopamine treatment, these synapses became resistant to depotentiation, which is a characteristic of late-phase LTP. Moreover, systemic injection of octopamine improved fear memory and recognition memory. Thus, this novel system may greatly heighten our understanding of the physiological importance of the cAMP-PKA pathway *in vivo* and its targets in synaptic plasticity and memory storage.

MO02-20

THE CHANGES OF MICROTUBULE-ASSOCIATED PROTEIN 2 IN CA1 OF HIPPOCAMPUS IN RAPIDLY HIPPOCAMPUS KINDLING EPILEPTIC MODEL

Li, Y. and Lu, C. H.

Institute of Neuroscience, Chongqing Medical University, Chongqing , China

Objective: To investigate the changes of microtubule-associated protein 2 (MAP2) in CA1 of hippocampus in rapidly hippocampus kindling epileptic model and explore the significance in epilepsy.
Methods: Epileptic models were established by rapid electrical hippocampus kindling method. Alterations of MAP2 in CA1 of hippocampus in the kindling model of epilepsy were investigated by immunohistochemistry method in rats with survival times ranging from three to thirty days. Nissl staining was used to check the degeneration of neurons and electron microscopic studies were applied to observe ultrastructure of neurons.
Results: The expression of MAP2 was obviously increased in CA1 of hippocampus three days after kindling and remained enhanced up to fourteen days ($P < 0.01$), then decreased to control level twenty to thirty days after kindling ($P < 0.01$). Nissl staining showed the degeneration and loss of CA1 neurons was time-dependent after kindling ($P < 0.01$). Electron microscopic results showed that microtubules of CA1 of hippocampus was increased three days after kindling and obviously increased fourteen days after kindling, but it was decreased thirty days later. Axons sprouting of neurons in CA1 and synaptic contacts between nerve terminal and dendrite were apparent after kindling.
Conclusions: It was suggested that the overexpression of MAP2 via the formation of microtubules may play an important role in the sprouting of mossy fibers in epileptic rats, and the extensive denervation of inhibitory cells by many damaged CA1 neurons may trigger a process that leads to overexpression of MAP2.

MO02-21

THE PHOSPHORYLATION OF PTPRT ATTENUATED THE SYNAPSE FORMATION IN THE HIPPOCAMPAL NEURONS

Lim, S. H.[1,2] and Lee, J. R.[1]

[1]*Brain Research Center, Korea Research Institute of Bioscience and Biotechnology*
[2]*College of Pharmacy, Chungnam National University, Daejeon, Korea*

PTPRT is the brain-specific expressed protein tyrosine phosphatase and was known to regulate the synapse formation of the hippocampal neurons. When PTPRT was phosphorylated by neuronal tyrosine kinase Fyn, the induction of synapse formation was attenuated. The dimerization/multimerization of PTPRT was enhanced and the catalytic activity was inhibited by phosphorylation. The key residue of phosphorylation was suggested as 912 tyrosine on the N-terminus of PTPRT catalytic domain. According to the structural modeling of PTPRT catalytic domains the phosphorylation of 912 tyrosine residue seems to make the flexible wedge enter the active pocket of reciprocal catalytic domain and as a result the catalytic activity was inhibited by the strengthened dimeric interaction. This novel regulation mechanism of PTPRT activity by phosphorylation could be applied to the functions of many protein tyrosine phosphatases on the synapse formation.

MO02-22

β2-ADRENERGIC AGONISTS IMPROVE SPATIAL LEARNING AND MEMORY VIA JNK-DEPENDENT CREB SIGNALING WITH INCREASED BDNF EXPRESSION

Moon, S. A., Kim, H. G., Jung, W. R., Shin, M. K., Lee, B. J., Park, D. I. and Kim, K. L.

Department of Biological Science, University of Sungkyunkwan, Suwon, Republic of Korea

A number of recent studies have documented that β2-adrenergic receptor agonists have effect on the memory formation and cognitive function of the hippocampus. However, the signaling and molecular mechanisms that underlie these effects remain poorly understood. Thus, in this study, we investigated the signaling and molecular mechanisms using β2-adrenergic receptor agonists (salbutamol, clenbuterol) and antagonist (ICI118.551). Administration of β2 agonists have been shown to improve, while antagonist impairs spatial learning and memory performance as measured by Y-maze and Morris water maze test. The β2 agonists injected group showed significantly increased levels of phosphorylated form of JNK which is involved with long-term potentiation (LTP) underlying learning and memory in the hippocampus region. Furthermore, JNK downstream signaling molecules such as c-Jun and CREB were also activated by β2 adrenergic receptor agonists. Activation of CREB can regulate the expression of several proteins especially BDNF.

Therefore, we next examined the expression level of BDNF using β2 agonists and antagonist injected rat hippocampus. Injection of β2 agonists significantly stimulated BDNF expression, while β2 antagonist reduced BDNF expression compared with untreated group. These effects of β2-agonists on activation of JNK, c-Jun, CREB and upregulation of BDNF expression were blocked by JNK inhibitor (SP600125) in rat hippocampal neuronal cell line, H19-7. These results demonstrated that JNK-dependent CREB signaling with increased BDNF expression played an important role in the memory enhancement by β2 agonists.

MO02-23

MELATONIN ATTENUATES AMPHETAMINE-INDUCED DECREASE IN VESICULAR MONOAMINE TRANSPORTER-2 IN POSTNATAL RAT STRIATUM

Mukda, S.[1] and Govitrapong, P.[1,2,3]

[1]*Center for Neuroscience, Faculty of Science, Mahidol University*
[2]*Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok*
[3]*Neuro-Behavioral Biology Center, Institute of Science and Technology for Research and Development, Nakornpathom, Thailand*

The vesicular monoamine transporter-2 (VMAT-2) is responsible for packaging intraneuronal dopamine into synaptic vesicles in preparation for synaptic release therefore a critical regulator of cytoplasmic dopamine levels and dopaminergic function. It has long been recognized that the VMAT-2 is a critical mediator of amphetamine-induced dopamine release. Amphetamine-induced lesions or changes in transmitter levels during development have the potential to produce numerous, permanent abnormalities in neural circuitry and function that do not occur following drug exposure in the mature nervous system. Therefore, in this study we investigated the effect of amphetamine on developing VMAT-2 and other dopaminergic content in striatum of neonatal rats. We found that chronic amphetamine administration in postnatal rat produces dopaminergic deficits in striatum on both a decrease in VMAT-2 immunoreactivity and a decrease in phosphorylated tyrosine hydroxylase expression. In addition, we observed an increase in α-synuclein immunoreactivity in striatum of postnatal rat following chronic amphetamine treatment. Moreover, we identified a role of melatonin, a hormone releases from pineal gland, in attenuating the alterations of dopamine homeostasis produced by amphetamine. However, the precise mechanism remains unclear.

Acknowledgements: This study was supported by a Postdoctoral Fellowship from the Commission on Higher Education to SM, a Senior Research Scholar Fellowship from the Thailand Research Fund and a Mahidol University Research Grant to PG.

MO02-24

TRANSCRIPTIONAL MECHANISM OF PURPURIN GENE IN FISH RETINA

Nagashima, M.[1], Mawatari, K.[1] and Kato, S.[2]

[1]*Division of Health Sciences, Graduate School of Medicine*
[2]*Department of Molecular Neurobiology, Graduate School of Medicine, Kanazawa University, Kanazawa, Japan*

Purpurin is a retina specific secretory protein. After zebrafish optic nerve injury, purpurin rapidly increases and secretes as a trigger molecule for nerve regeneration from photoreceptors. To elucidate of the regulatory mechanism of purpurin is essential for understanding initial event for CNS regeneration. In this study, we cloned 1.4 kbp 5'-upstream region of purpurin genomic DNA. To confirm promoter activity, we injected purpurin promoter-GFP repoter vector into zebrafish embryos. In the transgenic zebrafish, GFP expression was strictly detected in the outer nuclear layer. This result indicates the 1.4 kbp 5'-upstream region contains regulatory elements for purpurin gene expression. This region contained several photoreceptor specific transcriptional factor binding motifs. In particular, we focused on cone-rod homeobox (crx) gene. During zebrafish retinal development, crx mRNA was expressed in the ventral retina at 32 h post fertilization (hpf) and the expression was limited to the outer nuclear and outer most inner retinal layers at 72 hpf. This expression pattern was very similar to purpurin mRNA expression, which was firstly detected in ventral retina at 40 hpf and was limited in outer nuclear layer at 72 hpf. Knockdown of purpurin expression using morpholino disrupted formation of retinal lamination as observed in crx knockdown embryos. Knockdown of crx expression completely blocked purpurin expression, but not vice versa. Exogenous expression of purpurin mRNA rescued the formation of retinal lamination in crx knockdown embryos. These results strongly suggest that crx is one of the possible transcriptional factors for purpurin expression.

MO02-25

MUSCARINIC CHOLINERGIC NEUROTRANSMISSION IN CA1 SYNAPSES OF RAT HIPPOCAMPUS

Oberholzer, M. V.[1,2], Cerveñansky, C.[3], Urbano, F.[2], Kornisiuk, E.[1] and Jerusalinsky, D.[1,4]

[1]*LaN&N, IBCN, BsAs*
[2]*FCEN, UBA, BsAs, Argentina*
[3]*Inst Pasteur, Montevideo, Uruguay*
[4]*CBC, UBA, BsAs, Argentina*

Muscarinic antagonists produce amnesia while agonists facilitate memory when injected into the dorsal hippocampus of rats immediately after training in an inhibitory avoidance task. Muscarinic toxin MT2 from Green Mamba venom acts as selective M1 agonist and M4 antagonist, improving performance in an inhibitory avoidance task. MT3 from the same venom, a highly selective M4 antagonist, inhibited long-term potentiation (LTP) induced by high frequency stimulation, in field potential and in whole-cell configuration recordings of CA1 synapses in rat hippocampal slices. Our goal was to characterize the participation of muscarinic receptors in those synapses, either in basal conditions or after potentiation by theta-burst stimulation (TBS). Field excitatory postsynaptic potentials (fEPSP) were obtained by stimulating Schaffer Collaterals and recording at glutamatergic synapses on pyramidal cell dendrites in the presence of scopolamine, atropine (specific non-selective muscarinic antagonists), pirenzepine (selective antagonist for M1 and M4 receptors) and MT2. fEPSP maximum slopes were analysed and compared with control recordings. Both scopolamine and pirenzepine 25 uM blocked LTP induction, while atropine 10 uM did not. None of them affected basal transmission in the concentrations used. On the other hand, 1 uM MT2 showed a facilitatory, though no lasting effect (+ 50 ± 7%), which was blocked by pirenzepine. This facilitation by MT2 was not blocked with scopolamine, but was suppressed by atropine. Altogether, our results show that both M1 and M4 appear positively involved in LTP induction by TBS, while only M1 receptors appear to positively modulate basal transmission in CA1 synapses.

MO02-26

INTERLEUKIN-1β ENHANCES SYNAPTIC STABILITY OF MOUSE PRIMARY CORTICAL NEURONS VIA UP-REGULATION OF THE P2Y2 RECEPTOR

Peterson, T. S.[1], Baker, O. J.[2], Camden, J. M.[2], Seye, C. I.[2], Simonyi, A.[2], Sun, G. Y.[1,2] and Weisman, G. A.[1,2]

[1]*Interdisciplinary Neuroscience Program*
[2]*Department of Biochemistry, University of Missouri, Columbia, USA*

In the mammalian nervous system, P2 nucleotide receptors are known to mediate neurotransmission, release of proinflammatory cytokines, and reactive astrogliosis. Extracellular nucleotides activate multiple P2 receptor subtypes in neurons and glial cells, including G protein-coupled P2Y receptors and P2X receptors, which are ligand-gated ion channels. It has been demonstrated that adenosine triphosphate (ATP) released from the leading edge of the cell surface amplifies chemotactic signals and directs cell orientation by feedback through $P2Y_2$ nucleotide receptors ($P2Y_2Rs$). Previous results show that under inflammatory conditions, interleukin-1β (IL-1β) release causes functional upregulation of $P2Y_2Rs$ in neurons, increasing sensitivity to extracellular nucleotides. Additionally, stabilization of dendritic spines has been shown to be mediated by regulation of the actin-depolymerization factor cofilin, which is phosphorylated by $P2Y_2R$ activation. Stability of dendritic spines has been shown to depend upon cofilin phosphorylation and local F-actin expansion. Through unknown mechanisms, F-actin formation increases postsynaptic spine density (PSD) and stabilizes long-term potentiation. Here, we show that overnight treatment of primary cortical neurons with the pro-inflammatory cytokine IL-1β upregulates both $P2Y_2R$ mRNA expression and that subsequent $P2Y_2R$ activation by extracellular nucleotides enhances the expression of markers of synaptic stabilization.

This work was supported by NIH grant PO1 AG18357.

MO02-27

NEURONAL NOGO-A MODULATES GROWTH AND FASCICULATION OF NEURITES IN THE DEVELOPING NERVOUS SYSTEM

Petrinovic, M. M.[1], Duncan, C.[1], Bourikas, D.[2], Weinmann, O.[1], Stoeckli, E.[2] and Schwab, M. E.[1]

[1]*ETH and University of Zurich, Brain Research Institute*
[2]*Institute of Zoology, University of Zurich, Zurich, Switzerland*

In addition to their expression by oligodendrocytes, Nogo-A mRNA and protein are also expressed by neurons in the developing and adult CNS which suggests that, in addition to its well-known function as a myelin-associated axon-growth inhibitor, Nogo-A has additional role(s) in developing neurons. In this study, we aimed to investigate whether Nogo-A ontributes to axonal guidance during CNS development.Results of this study shows that suppression of Nogo-A, either by genetic ablation or by neutralization with function blocking Nogo-A antibody, leads to increased neurite outgrowth, increased fasciculation, and decreased branching in cultured DRG and hippocampal neurons. Thus, Nogo-A acts as a negative regulator of axon-axon adhesion and a facilitator of branching during neuronal development. In addition, time lapse analysis of outgrowing neurites showed that increased lateral adhesion in the absence of Nogo-A function is involved in bundle formation.Blocking the Nogo receptor subunit NgR or Rho kinase, a known key intracellular mediator of Nogo-A, produced similar changes of outgrowth like Nogo-A neutralization or knockout, which points to the NgR-Rho/ROCK mechanism of signaling.In the chicken embryo, injection of antibodies against Nogo-A led to aberrant innervation of the hindlimbs. Genetic ablation of Nogo-A causes increased fasciculation and reduced branching of peripheral nerves in Nogo-A knockout mouse embryos. In conclusion, our data support the hypothesis that neuronal Nogo-A acts as a negative regulator of neurite-neurite adhesion and as a repulsive/inhibitory regulator of neurite growth in the developing nervous system.

MO02-28

ANTICONVULSANT ACTIVITY OF ACONITUM VIOLACEUM SEIZURE MODEL IN MICE

Raza, M. L., Zeeshan, M., Ahmad, M., Shaheen, F. and Simjee, S. U.

University of Karachi, HEJ, ICCBS, Karachi, Pakistan

Aconitum violaceum which is commonly found in Asia, is selected for bioassay-guided isolation of natural products. Different fraction isolated from the Aconitum violaceum is tested for anticonvulsant activity against MES Test.

Method: Animals were divided in seven groups with six animals in each group. The saline (vehicle control), phenytoin (20 mg/kg), AV EtAc (50 mg/kg), AV MtAc (100 mg/kg) and AV Ia, AV Ib and AV Ic (50 mg/kg, 85 mg/kg and 100 mg/kg) were administered interaperitoneally to Group 1 Group II, Group III, Group IV, Groups V-VII respectively. However, a current of 80 mA for 1-2 s via ear electrode is given to animals in each group. The drugs and AV fractions were administered 30 min before given the current.

Results: All the fractions isolated from Aconitum violaceum have retarded the hind limb tonic extension and the results are comparable with phenytoin group. Whereas saline group is failed to abolish the HLTE induced by MES test.

Conclusion: Based on the obtained results we may conclude that Aconitum violaceum possesses the anticonvulsant constituent that may serve as future antiepileptic drug.

MO02-29

CAMKII BINDING PARTNERS VARY WITH CELL TYPE AND PHOSPHORYLATION STATE

Rostas, J. A.[1], Skelding, K. A.[1], Verrills, N. M.[1], Suzuki, P. W.[2] and Dickson, T.[1]

[1]*School of Biomedical Sciences and Hunter Medical Research Institute, Faculty of Health, University of Newcastle, Callaghan, Australia*
[2]*Department of Neuroplasticity, Shinshu University Graduate School of Medicine, Matsumoto, Japan*

CaMKII is an important mediator of synaptic function that is regulated by multisite phosphorylation and targeting to cellular locations via interactions with proteins. CaMKII phosphorylation at T286 (pT286) is well characterised. Recently, we identified T253 as a new phosphorylation site *in vivo* (pT253) that enhances CaMKII binding to post-synaptic densities. We hypothesise that pT253 or pT286 differentially regulates CaMKII function *in vivo* by altering the binding of CaMKII interacting proteins. To test this we used an overlay binding assay using recombinant CaMKII (wildtype and phosphomimic mutants T253D or T286D), and examined the CaMKII binding profile for subcellular fractions from rat brain, as well as a panel of known CaMKII interacting proteins. We have shown that either pT253 or pT286 can enhance, inhibit, or have no effect on the binding of CaMKII to specific proteins, and that the effects of CaMKII phosphorylation at these sites on binding to

proteins are independent of each other. Furthermore, the binding interaction with CaMKII is also sensitive to changes in phosphorylation of the binding protein. By comparing CaMKII binding profiles of the same subcellular fractions prepared from different brain regions, we have also shown that the expression pattern of CaMKII binding proteins varies with cell type. This data highlights the importance of the microenvironment in regulating CaMKII function *in vivo*. Thus, the complement of CaMKII binding proteins expressed by a cell and their subcellular location could determine the functional consequences of CaMKII activation in that cell.

MO02-30

IMMUNOHISTOCHEMICAL STUDY ON ALPHA-SYNUCLEIN EXPRESSION WITHIN NIGROSTRIATAL PATHWAY IN AMPHETAMINE-TREATED POSTNATAL RATS

Sae-ung, K.[1], Govitrapong, P.[2,3], Ueda, K.[4] and Phansuwan-Pujito, P.[1]

[1]*Department of Anatomy and Neuroscience Research Center, Srinakharinwirot University*
[2]*Center for Neuroscience and Department of Pharmacology, Mahidol University, Bangkok*
[3]*Neuro-Behavioural Biology Center, Institute of Science and Technology for Research and Development, Mahidol University, Nakornpathom, Thailand*
[4]*Tokyo Institute of Psychiatry, Division of Psychobiology, Tokyo, Japan*

Alpha-synuclein (a-syn) is an abundant presynaptic protein that has been implicated in several neurodegenerative diseases, such as Parkinson's disease (PD). It has been shown to play a central role in the pathogenesis of PD include interacts with numerous proteins at the axon terminal that function to regulate dopamine (DA) homeostasis in nigrostriatal pathway. Amphetamine (AMPH) has been known to damage the nigrostriatal DA pathway that may cause long-lasting changes in the central dopaminergic pathway. Recently, AMPH significantly increased the level of a-syn while reducing tyrosine hydroxylase (TH) levels in dopaminergic neuroblastoma cell culture (SK-N-SH cells). The present study, therefore, has been performed to detect a-syn expression within nigrostriatal pathway in AMPH-treated postnatal rats by using the immunofluorescence method. The postnatal rats at age 4 (P4) were injected with D-AMPH daily for 7 days and sacrified on P10. The substantia nigra and striatum were, then, double-stained with antibodies raised against a-syn and TH. In substantia nigra, a-syn located in nucleus and nerve fibers of dopaminergic neurons both control and AMPH-treated groups. In addition, there were a lot of a-syn-immunoreactive nuclei in the neurons of the striatum together with dopaminergic fibers in both groups. Therefore, the exact localization of a-syn should be further investigated to clarify these results.

MO02-31

NMDA-INDUCED NUCLEAR TRANSLOCATION OF JACOB REQUIRES CALPAIN CLEAVAGE

Sahin, J., Karpova, A., Dieterich, D., Mikhaylova, M., Gundelfinger, E. D. and Kreutz, M. R.

Leibniz Institute for Neurobiology, Mageburg, Germany

Jacob, a newly identified Caldendrin binding partner, is a novel protein present in the PSD and neuronal nuclei. Strictly depending upon activation of NMDA-type glutamate receptors, Jacob is recruited to neuronal nuclei, resulting in a rapid stripping of synaptic contacts and in a drastically altered morphology of the dendritic tree. In a yeast two-hybrid screen we have identified α-Internexin as a Jacob-binding partner. Neurofilament triplet proteins (NFTPs) are obligate heteropolymers, but α-Internexin can form homopolymers, and this suggests that α-Internexin and the NFTPs form separate filament systems. Our data suggest that α-Internexin might serve as a docking site for Jacob in the dendrite. It has been previously speculated that α-Internexin could be involved in the maintenance or the formation of dendritic spines and by yet unknown mechanisms Jacob might dissociate from α-Internexin and might be recruited either to the nucleus or the synapse. Most interestingly, Jacob binds at the carboxy-terminal tail region of α-Internexin. In general intermediate filaments, like vimentin, are highly susceptible to cleavage of a cysteine protease Calpain. Similarly, α-Internexin has two potential Calpain cleavage sites located at the tail region. We also found that Jacob is highly sensitive to Ca^{2+}-dependent proteolysis and that the subcellular localization of Jacob seems to be controlled by, Calpain. Calpain appears to cleave an N-myristolation site in Jacob that is crucial for its extranuclear localization and that has to be removed prior to retrograde transport of Jacob to the nucleus in response to NMDA-receptor activation.

MO02-32

MITOCHONDRIAL INJURY AND PROGRAMMED CELL DEATH INCLUDING APOPTOSIS, AUTOPHAGY AND ENDOPLASMIC RETICULUM STRESS IN NEURONAL INJURY

Shin, Y. S.[1,2], Chu, P. W.[1], Mercer, L. D.[1], Higgins, G. C.[3], O'Shea, R. D.[1], Nagley, P.[3] and Beart, P. M.[1,2]

[1]*Florey Neuroscience Institutes*
[2]*Centre for Neuroscience, University of Melbourne*
[3]*Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia*

Various forms of programmed cell death (PCD) including apoptosis, autophagy and endoplasmic reticulum (ER) stress may contribute to neurodegeneration. The aim of this study was to investigate how inhibition of mitochondrial respiratory chain complexes produced PCD and how differential recruitment of these forms of PCD influenced the resultant signature of neuronal injury. Primary cultures of cerebellar granule cells (CGCs; d7 C57Bl6/swiss mice) were exposed for 1-48 h on 7 div to insults targeting mitochondrial respiratory chain complexes I-IV (IC_{50} measured by MTT cell viability assay) - rotenone (0.35 μM), 3-nitropropionic acid (13 μM), antimycin A (23 nM) and KCN (7.9 μM), respectively. Cellular morphology examined by phase contrast microscopy revealed breakdown of neuritic networks. Furthermore, Annexin V and propidium iodide labelling showed a time- and insult-dependent continuum of PCD in insulted CGCs. Further work focused on how components of autophagy and ER stress contributed to PCD by comparing injury profiles to those induced by staurosporine (apoptosis), rapamycin, (autophagy) and tunicamycin (ER stress). An immunocytochemical strategy employing specific antibodies (caspase-9, microtubule-associated protein light chain-3 and protein disulphide isomerase) was developed to examine contributions to PCD and to examine crosstalk between these 'death' processes.

MO02-33

CAMKII PHOSPHORYLATION AT T253 ALTERS NEURONAL GROWTH RATES AND MORPHOLOGY

Skelding, K. A.[1], Liao, X.[2], Verrills, N. M.[1], Fluechter, L.[1], Dickson, P. W.[1] and Rostas, J. A.[1]

[1]*School of Biomedical Sciences and Hunter Medical Research Institute, Faculty of Health, University of Newcastle, Callaghan, Australia*

[2]*School of Life Sciences, Huazhong Normal University, Wuhan, People's Republic of China*

CaMKII is an important regulator of synaptic function, and its biological properties are regulated by phosphorylation and targeting to cellular domains via protein interactions. The roles of two phosphorylation sites, T286 and T305/6, have been well characterised. We have identified a new site at T253 whose phosphorylation *in vivo* (pT253) is regulated independently of T286 phosphorylation (pT286) following physiological stimuli. This study aims to identify functional effects following pT253 using neuronal cells transfected with phosphomimic mutants of CaMKII. Following transfection of a neuroblastoma cell line with recombinant wildtype CaMKII (WT), T286D-CaMKII (mimics pT286), or T253D-CaMKII (mimics pT253), changes were observed in cell morphology and growth rate. Transfection with WT doubled the growth rate without any change in morphology. The effect of transfection with T286D was identical to that produced by WT showing that pT286 had no effect. By contrast, transfection with T253D reduced cell growth and altered morphology. This data demonstrates that functional consequences of pT253 and pT286 are independent of each other. As the presence of binding proteins, as well as their subcellular location, can determine the functional consequences of CaMKII activation in that cell, the binding profiles of different neuronal cell lines were examined. Binding patterns varied with cell type. These results strongly suggest that pT253 is involved in regulating neuronal cell growth and morphology independently of pT286, and clearly identifies functional consequences following pT253.

MO02-34

FACTOR XIII EXPRESSION AND ITS IMPLICATION IN FISH OPTIC NERVE REGENERATION

Sugitani, K.[1], Oogai, K.[1], Shintani, T.[2], Noda, M.[2] and Kato, S.[3]

[1]*Division of Health Sciences, University of Kanazawa, Kanazawa*

[2]*Division of Molecular Neurobiology, National Institute for Basic Biology, Okazaki*

[3]*Department of Molecular Neurobiology, University of Kanazawa, Kanazawa, Japan*

Unlike mammals, the fish CNS neurons can repair their axons following nerve injury. After fish optic nerve transection, the regenerating optic axons can reinnervate the tectum by 3–5 weeks. We have used this regenerating fish optic nerve system to search involving factors in this process. Factor XIII is one of enzymes which are induced after nerve injury. It is well known as a plasma transglutaminase that catalyzes the formation of covalent cross-linking reactions in polymerized fibrin clots. In addition, the important role of Factor XIII in wound healing has been recently reported. Using the well-established fish model, the present study has investigated the role of Factor XIII on optic nerve regeneration after injury. Using molecular cloning techniques, we identified the cDNA of goldfish Factor XIII. Analysis by RT-PCR showed that level of Factor XIII mRNA started to increase within a short time in damaged optic nerves. In situ hybridization revealed that a lot of Factor XIII positive cells could be detected in the injured site of optic nerve and retinal ganglion cells. To elucidate molecular involvement of Factor XIII, we estimated the effect of recombinant Factor XIII protein on neurite outgrowth using an retinal explant culture system. Recombinant Factor XIII protein clearly induced neurite outgrowth from adult goldfish retina. These effects were observed only both in the naïve retina and in the retina with 1–2 days post conditioning lesion. These results suggest that Factor XIII activation is necessary for fish optic nerve regrowth in an early stage of regeneration.

MO02–35

OKRA (*ABELMOSCHUS ESCULENTUS* LINN), QUERCETIN AND RUTIN ATTENUATE DEX-INDUCED INCREASE IN HIPPOCAMPAL NMDA RECEPTOR IN MICE

Tongjaroenbuangam, W.[1], Tuayjun, A.[1], Inpao, D.[1], Mungkung, R.[1] and Govitrapong, P.[2,3]

[1]*Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham*

[2]*Neuro-Behavioural Biology Center, Institute of Science and Technology for R&D, Mahidol University, Nakornpathom*

[3]*Neuroscience Center, Faculty of Science, Mahidol University, Bangkok, Thailand*

Chronic stress exposure has been shown to affect neuronal death in hippocampus. This study was aimed to investigate the effect of okra (*Abelmoschus esculentus* Linn.) quercetin and rutin on dexamethasone-induced stress mice. The mice were induced to be stress with dexamethasone (DEX) 60 mg/kg (i.p.) for 21 days. The okra, quercetin and rutin (60 mg/kg, p.o.) were administered 3 h before treated with DEX. Thereafter, the mice were perfused with 4%paraformaldehyde and 0.1% glutaraldehyde. Following perfusions, brains were free floating coronal sectioned and stained with 0.1% cresyl violet. The results revealed that prolonged treatment with DEX alone showed morphological changes in the hippocampus neurons especially in the CA3 region. Pretreatment with okra, quercetin or rutin prevented these morphological changes. Furthermore, the hippcampal NMDA receptors were determined by the immunohistochemistry technique. The results showed that the DEX-treated mice increased expression of NMDA receptor immunoreactivity in the CA3 region, pretreatment of okra, quercetin or rutin attenuated these changes. The present results indicated that the effects of okra, quercetin and rutin were contributed to protect the neuronal death in the dexamethasone-induced hippocampus damage.

This study was supported by a TRF Grant for Young Researcher from the Thailand Research Fund to WT and a TRF-Senior Research Scholar Fellowship from the Thailand Research Fund to PG.

MO02-36

ROLE OF THE RAC-GAP BCR IN DENDRITIC AND SYNASE DEVELOPMENT

Um, K., Niu, S., Liu, F. and Tolias, K. R.

Baylor College of Medicine, Department of Neuroscience, Houston, USA

Dendritic spines are essential for the proper functioning of the nervous system since they serve as the primary sites of excitatory synaptic transmission in the brain. Spine morphology is known to

reflect its function, and morphological abnormalities are frequently associated with brain disorders including mental retardation. While proper spine development appears to be critical for normal cognitive function, the precise mechanisms that regulate the formation and remodeling of spines are poorly understood. We previously identified the Rac-specific GEF Tiam1 as a critical positive regulator of NMDA receptor- and EphB receptor-dependent spine developments. To further characterize the mechanism by which Tiam1 regulates dendritic development, we conducted a yeast two-hybrid screen and identified the Rac-specific GAP Bcr as a Tiam1-interacting protein. Bcr is a multi-domain protein best known for its involvement in chronic myelogenous leukemia. However, Bcr is also highly expressed in the brain, and mutant mice lacking Bcr and the highly homologous protein Abr exhibit cerebellar developmental defects, indicating a role for these proteins in nervous system development. We have confirmed that Bcr interacts with Tiam1 in neurons and can block Tiam1-induced Rac1 activation and actin polymerization. Furthermore, we found that overexpressing Bcr, but not a GAP-dead Bcr mutant, robustly decreases dendritic arbor complexity and dendritic spine density in hippocampal neurons. In contrast, Bcr knockout mice display an increase in spine size and dendritic arbor complexity *in vitro* as well as *in vivo*. Taken together, these data suggest that Bcr opposes Tiam1 function in neurons by inactivating Rac, thereby restricting dendritic growth and spine development.

MO02-37

DFMO, ORNITHINE DECARBOXYLASE INHIBITOR, PREVENTS 6-HYDROXYDOPAMINE-INDUCED NEUROTOXICITY IN RAT MESENCEPHALIC DOPAMINERGIC NEURON

Won, S. Y.[1,2] and Jin, B. K.[3,4,5]

[1]*Brain Disease Research Center*
[2]*Neuroscience Graduate Program, and Division of Cell Transformation and Restoration, Ajou University School of Medicine, Suwon*
[3]*Age-related & Brain Diseases Research Center,Kyung Hee University School of Medicine*
[4]*Neurodegeneration Control Research Center, Kyung Hee University School of Medicine*
[5]*Department of Neuroscience, Kyung Hee University School of Medicine, Seoul, South Korea*

The present study examined whether polyamines, endogenous ligand for transient receptor potential vanilloid 1 (TRPV1), could mediate 6-hydroxydopamine (6-OHDA)-induced dopaminergic (DA) neuronal cell death in rat mesencephalic cultures. Tyrosine hydroxylase (TH) immunostaining indicated that treatment with 6-OHDA or polyamines produced a significant loss of (DA) neurons. By contrast, pretreatment with difluoromethylornithine (DFMO), an inhibitor of polyamines biosynthesizing enzyme ornithine decarboxylase (ODC) and/or TRPV1 antagonist capsazepine (CZP), reversed 6-OHDA- or polyamines-induced loss of DA neurons in neuron-enriched mesencephalic cultures, indicating TRPV1-mediated neurotoxicity. Western blot analysis also showed that pretreatment with difluoromethylornithine (DFMO) and TRPV1 antagonist capsazepine (CZP) reversed 6-OHDA-induced downregulation of phosphorylated Akt and upregulation of caspase-3 activity in mesencephalic cultures. To our knowledge, this study is the first to show that 6-OHDA-polyamines-TRPV1 signaling pathway exerts neurotoxicity on dopaminergic neurons in mesencephalic cultures.

MO02-38

NOVEL ROLE OF M-CHANNELS IN REGULATING NEURONAL CELL VIABILITY

Zhou, X.[1,2], Wei, J. F.[2], Wei, L.[1] and Yu, S. P.[1,2]

[1]*Emory University, Atlanta*
[2]*Med Univ South Carolina, Charleston, USA*

Voltage-gated K^+ channels play a key role in regulating membrane excitability of excitable cells. Overactivation of certain K^+ channels, especially the delayed rectifier channels, can mediate excessive K^+ efflux that is an essential step in apoptosis. M-channels (KCNQ channels) are also non-inactivating K^+ channels. The kinetics and activation characteristics of M-channels lead us to hypothesize that, in addition to affecting membrane excitability, M-channels may have broader physiology and pathological effects. Using whole-cell patch clamp recording, we confirmed existence of M-currents in cultured hippocampal neurons, but much smaller M-currents were seen in cortical neurons. The M-channel opener N-Ethylmaleimide (NEM) caused dose-dependent cell death in hippocampal neurons, 30 and 50 uM NEM (24 h) caused 52 and 74% cell death, respectively. However, only 14 and 19% cell death was induced by the same concentrations of NEM in cortical neurons. The cell death caused by NEM was antagonized by M-channel inhibitor XE991 or by high potassium external solution. Application of NEM (20 uM, 8 h) in hippocampal cultures increased caspase-3 activity. NEM also increased the expression of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2. Blocking ERK1/2 attenuated NEM-induced neuronal death. NEM treatment induced mitochondrial membrane depolarization, triggered cytochrome c release, formation of apoptosome, and AIF translocation from mitochondria to nucleus after 4 hrs. The nuclear translocation of cytochrome c and AIF were blocked by XE991 or UO126. These data suggest that NEM induced apoptosis involves caspase-dependent and caspase-independent pathways. It is suggested that M-channels may be an important regulator in neuronal apoptosis.

MO03 Neurotransmitter transporters

MO03-01

MOLECULAR CLONING AND PHYSIOLOGICAL CHARACTERIZATION OF ANOCTAMIN 2

Cho, Y. G., Yang, Y., Cho, H., Kim, H., Tak, M., Jang, Y., Lee, J., Lee, B., Chun, H., Jung, S., Kim, D., Kim, H. S. and Oh, U.

The Sensory Research Center, Seoul national university, Department of Pharmacology, Seoul, Republic of Korea

Chloride channels perform many fundamental functions in cell physiology including ionic homeostasis, cell volume regulation, transepithelial transport and regulation of electrical excitability. Despite this important function, chloride channels are not well characterized at the protein and molecular level. In previous study, it was reported that the anotamine1 (ano1, also called TMEM16A) is a Ca^{2+} activated Cl^- channel (CaCC). ANO1 belongs to a large family that includes other membrane proteins that may also have an ion channel function. ANO2 is most similar to ANO1 with 62% identity at the amino acid level.In this study, in order to identify the function and role of the anoctamine family, we cloned anoctamine2 (ANO2, also called TMEM16B).ANO2 gene was from mouse eye, followed by sub-cloned to a mammalian vector and an oocyte vector. When ANO2-GFP fusion protein was expressed in HEK 293T cells, ANO2 was observed at the plasma membranes, lysosome, and mitochondria. We analyzed the expression of ANO2 in various mouse tissues by real-time quantitative PCR. ANO2 mRNA was widely expressed in various tissues, especially high in eye, heart, thymus, brain and lung. Futher studies on the functions of this protein will help to understand its functions and diseases associated ion channels.

MO03-02

DEVELOPMENT OF A STABLE CELL LINE EXPRESSING V5-TAGGED EAAT2 TO ANALYSE CELL-SURFACE EXPRESSION OF A GLIAL GLUTAMATE TRANSPORTER

Lau, C. L.[1], Beart, P. M.[1,2], Rattray, M.[3], Peacey, E.[4] and O'Shea, R. D.[1,2]

[1]*Florey Neuroscience Institutes*
[2]*University of Melbourne, Dept of Pharmacology, Parkville, Australia*
[3]*University of Reading, Whiteknights*
[4]*King's College London, London, UK*

Excitatory amino acid transporters (EAATs) are responsible for homeostasis of extracellular L-glutamate, and EAAT expression or function is altered in acute and chronic neurological conditions. The glial transporter EAAT2 is responsible for the bulk of glutamate uptake in the brain, but little is known about the factors regulating its cell-surface expression. Current studies were undertaken to determine the cellular mechanisms regulating EAAT2 trafficking, by establishing a cell line stably expressing V5-tagged EAAT2. HEK-293T cells were grown in DMEM containing 10% fetal bovine serum, 1% penicillin/streptomycin and 2 mM L-glutamine. Cells were plated on 24- and 6-well plates at 10^5 cells/ml and transfected with 0.8 – 4 μg cDNA using Lipofectamine™ 2000. Pharmacological and kinetic properties of the transfected cells were determined by studies of [^{3}H]D-aspartate uptake with K_m 20 μM and V_{max} 0.87 pmol/mg/min. Sensitivity of uptake to the EAAT2 blockers, dihydrokainate, SOS, L-CCG-III and TBOA revealed K_m 121 μM, 130 μM, 8 μM and 2 μM respectively. Immunoblotting revealed EAAT2 immunoreactivity as a monomer of 66 kDa and as higher molecular weight bands similar to those found in primary astrocytic culture. Cytochemistry for V5-EAAT2 was undertaken to examine the expression and localization of the V5-EAAT2 following transfection. This cell line will allow us to determine the rates of EAAT synthesis and internalization, as well as the involvement of the intracellular organelles in the targeting and trafficking of EAATs, providing data on EAAT function in living cells.

MO03-03

THE NA^+-DEPENDENT GLUTAMATE TRANSPORTER GLAST (EAAT1) INTERACTS WITH NA^+/K^+ ATPASE α1

Lee, A.[1], Poronnik, P.[2] and Pow, D. V.[1]

[1]*Centre for Clinical Research, University of Queensland, Brisbane*
[2]*School of Medical Sciences, RMIT University, Melbourne, Australia*

Previous studies have shown that activation of glutamate transport (by addition of L-aspartate/glutamate) increased Na^+/K^+ ATPase (NKA) activity in cultured astrocytes, suggesting that glutamate transporters and NKA activity are tightly linked (Pellerin and Magistretti, J. Neurochem. 1997) In this study, we have shown by co-immunoprecipitation experiments that the glial glutamate transporter GLAST (EAAT1) associates with NKA α 1 in the rat brain. GST-pulldown assays demonstrate that the cytoplasmic loop region (residue 350–775) of NKA α1 pulls down GLAST from brain lysate. Similarly, the extreme C-terminal tail (residue 513–543) of GLAST pulls down NKA α1 from brain lysate. Confocal immunofluorescence studies revealed that GLAST co-localised with NKA α1 in astrocytes. We have utilised *in vitro* D-aspartate uptake in transfected Cos-7 cells to study the effects of co-expression of GLAST and a construct encoding the cytoplasmic loop region (residue 350–775) of NKA α1. The construct was predicted to block normal interactions between GLAST and NKA α1. Co-expression led to a significant decrease (~20%) in D-aspartate uptake when compared with the control (expression of GLAST alone). These findings indicate that GLAST is capable of forming a functional complex with NKA α1. This association is mediated via the cytoplasmic loop region of NKA α1 and the C-terminal tail of GLAST, is likely to involve other accessory proteins and provides a biochemical substrate for the functional interactions between glutamate transport and NKA.

MO03-04

ENHANCED 3-O-METHYL-D-GLUCOSE UPTAKE BY CURCUMIN IN CONDITIONALLY IMMORTALIZED RAT BRAIN CAPILLARY ENDOTHELIAL CELL LINE (TR-BBB)

Park, H. J. and Kang, Y. S.

College of Pharmacy, Sookmyung Women's University, Seoul, Korea

Curcumin is a major component of Curcumae rhizome with antioxidant, anti-inflammatory properties. It is known to induce apoptosis in various cancer cells and prevent from Alzheimer's disease (AD). Recently, it is reported that curcumin lowers blood glucose levels in diabetic mice and rats. Glucose plays a major source to produce energy in brain as metabolic fuel. Low glucose level in brain can induce diverse diseases including brain coma and hypoglycemia, etc. Thus, transport of glucose across the blood-brain barrier (BBB) is very important. In this study, we investigated the characteristics of glucose transport across the BBB and the effect of

curcumin on glucose uptake in conditionally immortalized rat brain capillary endothelial cell line (TR-BBB) *in vitro*. We measured uptake of [3H] 3-O-methyl-D-glucose ([3H] 3-OMG) in TR-BBB in order to know change of glucose permeability across BBB by curcumin. As a result, it was found that [3H] 3-OMG uptake activity which was concentration-dependent with a Michaelis–Menten constant of 20.6 mM and the maximum rate of uptake (Vmax) was 97.60 nmol/ (30 sec mg protein) in TR-BBB. The uptake of [3H]-3-OMG in TR-BBB was increased by increasing the concentration of curcumin pretreatment. In conclusion, curcumin can modulate glucose transport across the blood-brain barrier (BBB). These results suggested that curcumin may help glucose uptake across the BBB.

MO04 Nitric oxide synthase

MO04-01

NITRIC OXIDE AND OXIDATIVE STRESS IN THE NEUROCHEMICAL MECHANISMS OF BRAIN ISCHEMIA: EXPERIMENTAL AND CLINICAL STUDY

Bashkatova, V.[1], Koshelev, V.[2], Fedyukova, O.[2] and Skvortsova, V.[2,3]

[1]*Anokhin Institute of Normal Physiology*
[2]*Lomonosov Moscow State University*
[3]*Institute of Molecular Genetics, Moscow, Russia*

Nitric oxide (NO) has been proposed as a key pathophysiological factor in neurological disorders which involve neurotoxic effect of glutamate. The aim of our study was to determine whether NO and lipid peroxidation (LPO) processes contributes to the mechanisms of brain ischemia and elucidate whether novel synthetic analogue of ACTH 4-10 Pro-Gly-Pro (Semax) is neuroprotective via a mechanism involving the regulation of NO and/or LPO. NO generation was directly measured using Electron Paramagnetic Resonance spectroscopy. LPO intensity in brain tissue was determined as thiobarbituric acid reactive substances (TBARS). Incomplete global ischemia was induced by clamping (4 h) the bilateral carotid arteries. In addition, we sought to clarify whether TBARS levels may alter in cerebrospinal fluid (CSF) of the patients with acute ischemic stroke. Both NO generation and TBARS formation were elevated in the ischemic brain cortex of rats. An increase in TBARS content in CSF of the patients with ischemic stroke beginning with the first 6 h of the disease onset was observed. Semax proved to be highly effective in restoring neurological functioning and abating the rise in NO levels in experimental study. A reduction of TBARS levels in CSF of 53 patients treated with Semax by day 3 in comparison to that of group receive placebo was found. The trial confirmed the safety profile of the Semax treatment. While, the molecular mechanism underlying Semax's action is unclear it is likely that its effect on NO and LPO production is contributing to its neuroprotective action.

MO04-02

ENOS-DERIVED NO MEDIATES BLOOD-BRAIN BARRIER ALTERATIONS IN THIAMINE DEFICIENCY ENCEPHALOPATHY

Beauchesne, É., Desjardins, P., Hazell, A. S. and Butterworth, R. F.

Neuroscience Research Unit, CHUM, Saint-Luc Hospital, Montreal, Canada

Wernicke's Encephalopathy (WE) is a cerebral metabolic disorder caused by thiamine deficiency (TD) and is characterized by region-selective neuronal cell death and the presence of petechial hemorrhagic lesions. Increased expression of the endothelial isoform of nitric oxide synthase (eNOS) occurs selectively in vulnerable regions of the brain in TD. We hypothetize that region-selective eNOS induction in TD leads to tight junction (TJ) protein alterations and BBB breakdown. TD was induced in C57BL6 (WT) and eNOS-/- mice by feeding with a thiamine-deficient diet and treatment with the thiamine antagonist pyrithiamine. Pair-fed control (PFC) mice were fed the same diet with additional thiamine. In medial thalamus of TD-WT mice (vulnerable area), increased heme oxygenase-1 (HO-1) and S-nitrosocysteines immunostaining was observed in vessels, compared with PFC-WT mice. A concomitant increase in IgG extravasation, a decrease in expression of the TJ proteins occludin and zonula occludens (ZO-1, ZO-2), as well as an up-regulation of matrix metalloproteinase-9 (MMP-9) were observed. eNOS gene deletion led to an attenuation of neuronal cell death and hemorrhagic lesions in TD. Moreover, eNOS gene deletion prevented the increases in oxidative/nitrosative stress markers in vessels, and prevented BBB breakdown as shown by absence of IgG extravasation, normalization of TJ protein expression and absence of MMP-9 up-regulation, compared with WT mice. These data demonstrate that eNOS-derived nitric oxide (NO) is a major factor leading to alterations of the cerebrovascular endothelial cells in TD. [Funded by Canadian Institutes of Health Research (CIHR)].

MO04-03

EXCITOTOXIC CELL DEATH MEDIATED BY A PROTEIN INTERACTION CASCADE DOWNSTREAM OF PSD95

Courtney, M. J.[1,2], Parviainen, L. E.[1], Ginet, V.[3], Tuittila, M. T.[2], Li, L. L.[1] and Truttmann, A. C.[3]

[1]*Department of Neurobiology, A. I. Virtanen Institute, Kuopio*
[2]*Turku Centre for Biotechnology, Åbo Akademi University and University of Turku, Turku, Finland*
[3]*Department of Pediatrics and Pediatric Surgery, Division of Neonatology, University Hospital Center and University of Lausanne, Lausanne, Switzerland*

PSD95 assembles a ternary complex with the Ca-permeable NMDA receptor (NR) and Ca - activated neuronal nitric oxide synthase (nNOS). Peptides mimicking the NR ligand for PSD95 PDZ domains prevent excitotoxic neuronal death (1) and are in clinical trials for stroke. We found that rapid activation of p38MAPK by NR-gated Ca requires nNOS catalytic activity and early p38 activity is required for cell death. As a central node of cellular regulation, p38MAPK may be of limited therapeutic value. We found that sequences mimicking the PSD95-binding domain of nNOS inhibited excitotoxic p38 activation and were neuroprotective (2), suggesting an intact NR-PSD95-nNOS complex is required for Ca influx to generate NO, activate p38 and cause cell death. We now identified a NO-regulated component of this pathway whose recruitment is also required for p38 activation and cell death. Our results suggest we have identified a downstream sequence of protein interactions that efficiently couple the receptor-gated Ca influx and nitric oxide generation to activation of p38 and neuronal cell death. To evaluate the *in vivo* significance of this protein complex, we designed a cell-permeable inhibitor of effector recruitment. We found this caused as much reduction in lesion size as a p38 inhibitor in an animal model of neonatal hypoxia-ischaemia. As protein interactions have previously been revealed as potential drug targets suitable for clinical trials, the protein interaction interfaces revealed by our study may have value in future therapeutic approaches.

References:

1. Aarts et al. (2002) Science 298, 846–850
2. Cao et al. (2005) J. Cell Biol. 168, 117–126

MO04-04

ANTI-INFLAMMATORY MECHANISM OF GINSENOSIDE RH1 IN LPS-STIMULATED MICROGLIA: INVOLVEMENT OF HEMEOXYGENASE-1 AND PKA PATHWAY

Jung, J. S.[1], Shin, J. A.[2], Park, E. M.[2] and Kim, H. S.[1]

[1]*Department of Molecular Medicine*
[2]*Department of Pharmacology, School of Medicine, Ewha Womans University, Seoul, Korea*

In this study, we investigated the effect of ginsenoside Rh1 on microglial activation. Rh1 inhibited the expressons of iNOS,

COX-2, and proinflammatory cytokines, while it increased IL-10 and hemeoxygenase-1 (HO-1) expression in LPS-stimulated BV2 cells and rat primary microglia. Suppression of microglial activation by Rh1 was also observed in the mouse brain inflammed by LPS. Further mechanistic studies revealed that Rh1 inhibited LPS-induced MAPK phosphorylation and NF-κB-mediated transcription without affecting NF-κB DNA binding activity. Since pCREB is known to compete with p65 subunit of NF-κB for limited amount of CBP and thus inhibit NF-κB-mediated transcription instead of directly affecting NF-κB DNA binding, we examined the effect of Rh1 on pCREB levels. As expected, Rh1 increased CREB phosphorylation in LPS-stimulated microglia. PKA inhibitors significantly attenuated Rh1-mediated inhibition of iNOS and upregulation of IL-10 and HO-1, suggesting that PKA pathway may be involved in anti-inflammatory mechanism of Rh1. Furthermore, HO-1 siRNA attenuated the inhibition of NO and ROS by Rh1. Since CREB binding site is resided in the promoter region of HO-1, the effect of Rh1 on CREB and HO-1 appears to be closely related. In conclusion, the present study indicates that PKA pathway and HO-1 are involved in anti-inflammatory effects of Rh1 in microglia, which may be good therapeutic targets for various neurodegenerative diseases caused by neuroinflammation.

MO04-05

ALPHA-SYNUCLEIN ALTERS NO-MEDIATED SIGNAL TRANSDUCTION. RELATIONSHIP TO POLY (ADP-RIBOSE) POLYMERASE-1 ALTERATION

Kazmierczak, A., Adamczyk, A., Czapski, G. A. and Strosznajder, J. B.

Department of Cellular Signalling, Medical Research Center, Polish Academy of Sciences, Pawińskiego 5, Warsaw, Poland

Alteration of α-synuclein (ASN) is implicated in the pathogenesis of various neurodegenerative diseases. Our previous data indicated that ASN enhances oxidative stress and that poly ADP-ribose polymerase (PARP-1) is a nuclear target for free radical-dependent signalling. The aim of the present study was to investigate the time-dependent effect of ASN on nitric oxide synthase (NOS) as well as on PARP-1 activity and protein level. The studies were carried out in rat brain slices and homogenate and also in hippocampal cell line (HT22) using radiochemical, spectrophotometrical and Western blot methods. Our results obtained in the rat brain show that short time (30 min) treatment with ASN (10 μM) stimulated NOS by 60% and decreased PARP-1 activity by 27% with no effect on its protein level. The inhibition of NOS by the NG-nitro-L-arginine (NNLA, 100 μM) partially reversed the effect of ASN on PARP-1 activity, suggesting that NO may be in part responsible for the alteration of PARP-1. In addition, in HT22 cells extracellular ASN increased NOS activity by 62%. Moreover, in the same model, ASN induced time-dependent significant decrease of PARP-1 protein level. Concomitantly ASN enhanced the activity of caspase-3 through NO-mediated mechanism. The inhibitors of NOS (NNLA, 100 μM) and caspase-3 (Z-DEQD-FMK, 40 μM) completely reversed ASN-evoked PARP-1 degradation. In conclusion, the results demonstrated that NO pool liberated by ASN during short period of action is involved in the inhibition of PARP-1 activity. However, prolonged treatment induces PARP-1 cleavage by NO-mediated caspase-3 activation.

Financial support was provided by the MSHE Grants 2PO5A4129 and NN401024236.

MO04-06

7-NITROINDAZOLE EFFECTS ON L-DOPA-INDUCED DYSKINESIAS AND ON STRIATAL NITRIC OXIDE PRODUCTION

Padovan-Neto, F. E., Romano, A. C., Ferreira, F. and Del Bel, E. A.

University of São Paulo, Ribeirão Preto, SP, Brazil

Introduction: Rats with 6-hydroxydopamine (6-OHDA) lesion of dopaminergic neurons chronically treated with L-3, 4-dihydroxyphenylalanine (L-DOPA) develop a rodent analog of human dyskinesia. We hypnotized that these effects may involve the nitric oxide (NO) system.

Background: In this study we further investigate the selective NO synthase inhibitor (NOS) 7-Nitroindazole (7-NI) effect on dyskinesias in rats with unilateral 6-OHDA-lesion and quantified striatal NO metabolites.

Methods: Chronic L-DOPA treatment was administered to 6-OHDA-lesioned rats during 22 days (30 mg/kg/day; oral-gavage) to induced diverse amount of dyskinesia changes. 7-NI (1-30 mg/kg i.p.) was given 30 min before L-DOPA on day 22. Groups of parkinsonian and dyskinetic rats had their brain removed to measure tissue levels of NO metabolites NO(x)(-) (nitrite plus nitrate) in the striatum using the Griess reaction.

Results: 7-NI (1–30 mg/kg) attenuated the dyskinesias ($P < 0.05$) improving motor performance in the rota-rod test ($P < 0.05$). NOx levels increased in the striatum ipsi- and contralateral to lesion ($P < 0.05$). 7-NI pretreatment reduced NOx in the striatum ipsilateral to lesion (ANOVA, $F(1.18) = 4.58$; $P < 0.05$). L-DOPA chronic treatment per se reduced the levels of NOx in the striatum ipsilateral to lesion (ANOVA, $F(1.28) = 4.41$; $P < 0.05$). 7-nitroindazole pretreatment to dyskinetic rats induced no further NOx reduction ($P > 0.05$).

Conclusion: These results points to the involvement of NO on dyskinesias and suggest the possibility that NOS inhibitors may be valuable to L-DOPA-induced dyskinesia treatment.

MO04-07

CHANGES IN CYCLIC GUANOSINE MONOPHOSPHATE IN RAT BRAIN REGIONS DURING MORPHINE WITHDRAWAL CORRELATES WITH ABSTINENCE SEVERITY

Peregud, D. I.[1,3], Yakovlev, A. A.[2,3], Stepanichev, M. Y.[2], Onufriev, M. V.[2], Panchenko, L. F.[1,3] and Gulyaeva, N. V.[2]

[1]*National Research Center on Addictions*
[2]*Institute of Higher Nervous Activity and Neurophysiology RAS*
[3]*Institute of general pathology and pathophysiology RAMS, Moscow, Russia*

The aim of this study was to explore whether a correlation exists between morphine dependence severity and biochemical indices of the nitric oxide (NO) / cyclic guanosine monophosphate (cGMP) signaling cascade in rat brain regions. We have found that cGMP level was changed in the frontal cortex, midbrain, and striatum after spontaneous morphine withdrawal, these changes correlating with severity of abstinence manifestation. In particular, cGMP concentrations in frontal cortex of rats with mild morphine withdrawal were increased, while in midbrain they decreased as compared with control values. However, in rats with severe morphine withdrawal cGMP concentrations were decreased in striatum only. In contrast to cGMP level, NO-synthase activity did not change during abstinence in the brain regions studied. We also checked whether cGMP

alterations in brain regions were accompanied by changes in mRNA and/or protein content of cGMP producing enzyme soluble guanylate cyclase (sGC). Neither mRNA nor protein level of sGC subunits α1 and β1 changed during opiate withdrawal suggesting that cGMP content may depend on GC or phosphodiesterase enzymatic activity rather than on quantitative changes of sGC on transcriptional or translational level. We suggest that individual features of opiate dependence development can be related to specific alterations of regional cGMP levels. This work was supported by RFBR grant # 07-04-00829a.

MO04-08

ARGININE METABOLISING ENZYMES: THERAPEUTIC TOOLS FOR NEURODEGENERATIVE DISEASES AND CATALYSTS IN FIBRILLOGENESIS

Whiteley, C. G. and Padayachee, E.

Rhodes University, Grahamstown, South Africa

The accumulation of arginine in the brains of patients suffering from acute neurodegenerative diseases like Alzheimer disease, points to defects in the metabolic pathways involving this amino acid. Neural nitric oxide synthase (nNOS) is an enzymes that uses arginine as a substrate. The deposits of neurofibrillary tangles and senile plaques perhaps as a consequence of fibrillogenesis of amyloid peptides has also been shown to be a hallmark in the etiology of certain neurodegenerative diseases. We now show that nNOS is not only inhibited by the two peptides AB(1–40) and AB(17–28) but assists in the proteolytic degradation of these peptides with the concomitant formation of insoluble fibrils. The formation of amyloid fibrils was rate limiting involving an initial lag time that was dependent on the initial concentration of amyloid peptides. In view of the fact that AB(17–28) peptide also inhibited the enzymes reflected that the C-terminal region of these peptides was responsible for binding to the enzymes. The enzyme was satisfactorally purified from bovine brain by standard biochemical processes. Michaelis Menten kinetics was used to establish inhibitor kinetics of the peptides; congo red binding was used to establish the aggregation kinetics of amyloid peptides; fluorimetry and electron microscopy were used to obtain images of the peptides as a function of aggregation. Identification of binding domains within each of the peptides was studied using suitable computational structural software.

MO04-09

EFFECTS OF BARAKOL AND NITRIC OXIDE SYNTHASE INHIBITOR, L-NAME ON THE OPEN FIELD BEHAVIOR OF STRESS RATS

Wongwitdecha, N.[1], Soo-ampon, S.[2], Rittilert, P.[2] and Verawatnapakul, V.[3]

[1]*School of Medicine, Walailak University, Nakhon Si Thammarat*
[2]*Department of Pharmacology, Faculty of Science*
[3]*Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand*

Objective: The aim of the present study was to investigate the effects of barakol and nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) on the open field behavior of stress rats.
Methods: Male Wistar rats were raised from weaning either alone (isolation rearing) or groups of five rats/cage (social rearing). After five weeks, both socially and isolation reared rats were placed individually into an open field arena, under either normal light (50 lux) or high light condition (500 lux) for a 5 min test.
Results: Under normal light (50 lux), pretreatment with barakol (5, 10 and 25 mg/kg, i.p.) did not significantly induce the anxiolytic effect in both isolation and socially reared rats compared with the saline treated control rats. However, under high light (500 lux) this compound significantly produced a dose-related anxiolytic effect in socially reared rats, as indicated by decrease the number of entry and time spent on the inner zone ($P < 0.05$), but the anxiolytic-like effect of barakol (5, 10 and 25 mg/kg, i.p.) was not observed in isolation reared rats. Under high light condition (500 lux), pretreatment with L-NAME (10, 25 and 50 mg/kg i.p.) decreased locomotor activity in socially reared rats, in a dose-related manner, but had no effect on the isolation reared rats. L-NAME significantly reduced the number of rears and produced the anxiolytic-like effect in both socially and isolation reared rats, however, these effects were more pronounced in isolation reared rats.
Conclusion: These results indicate that early life stress e.g. rearing rats in social isolation from weaning modifies the effects of both barakol and L-NAME in the adult rats.

MO05 Neurotoxins in neurochemistry

MO05-06

EFFECTS OF 1-PENTANOL ON ROTATIONAL MOBILITY OF N-9-ANTHROYLOXYSTEARIC ACID IN NEURONAL AND MODEL MEMBRANES

Jang, H. O., Pyun, J. H., Bae, J. H., Jeong, J. H., Lee, K. S., Kwon, I. H., Kim, W. S., Choi, C. S., Jeon, Y. C., Chung, I. K., Bae, M. K. and Yun, I.

College of Dentistry and Research Institute for Oral Biotechnology, Pusan National University, Yangsan, Republic of Korea

To provide a basis for studying the molecular mechanism of pharmacological action of 1-pentanol, we carried out a study of the membrane action of the general anesthetics. The set of n-(9-anthroyloxy)stearic or palmitic acd (n-AS) probes (n = 2, 6, 9, 12 and 16) have been used to examine gradients in fluorescence polarization. In a does-dependent manner, 1-pentanol decreased the anisotropies of 6-AS, 9-AS, 12-AS and 16-AP in the hydrocarbon interior of the synaptosomal plasma membrane vesicles isolated from bovine cerebral cortex (SPMV), and liposomes of total lipids (SPMVTL) and phospholipids (SPMVPL) extracted from the SPMV, but the 1-pentanol increased the anisotropy of 2-AS in the membrane interface. The magnitude of rotational mobility in accordance with the carbon atom of phospholipids comprising SPMV, SPMVTL and SPMVPL was in the order at the 16, 12, 9, 6 and 2 position of aliphatic chain present in phospholipids. The sensitivity of increasing or decreasing effect of rotational mobility of the hydrocarbon interior or surface region by 1-pentanol differed depending on the carbon atom numbers in the descending order of 16-AP, 12-AS, 9-AS, 6-AS and 2-AS. Furthermore, the sensitivity of increasing or decreasing effect of rotational mobility of the hydrocarbon interior or surface region by the 1-pentanol differed depending on the neuronal and model membranes in the descending order the SPMV, SPMVPL and SPMVTL.

MO05-01

OXIDATION OF MITOCHONDRIAL IRON-SULFUR CLUSTER CONTRIBUTES TO ENHANCED MPTP/METH-INDUCED OXIDATIVE DAMAGE IN AGED MICE: AN *EX VIVO*

Ali, S.[4], Karunakaran, C.[1], Kanthasamy, A.[2], Kanthasamy, A.[4], Antholine, W.[1], Kalyanaraman, B.[1] and Gonzalez, C.[3]

[1]*Free Rad. Res. Ctr., Med. Coll. of Wis., Milwaukee, WI*
[2]*Department of Biomed., Iowa State University, Ames, IA, USA*
[3]*Autonomous University, SLP, Mexico*
[4]*NCTR, Jefferson, AR, USA*

Mitochondrial dependent oxidative stress plays a central role in normal aging and neurodegeneration. In this study, we report that ex vivo low temperature X-band EPR spectroscopy by monitoring the paramagnetic proteins in the brains of aged and MPTP/METH-treated animals. C57B6 mice of 8–10 weeks old and 48–52 weeks old were treated with saline, MPTP (4 x 20 mg/kg, ip) or METH (4 x 10 mg/kg, ip); sacrificed 1, 3 and 7 days post-treatment. Brains were removed and freezed over dry ice and store at–80°C until EPR analysis. The X-band EPR of striatum, frontal cortex, cerebellum, and brain stem tissues of mice were recorded. The EPR spectra of brain tissues of adult and aged mice measured at 10 K revealed that they exhibit signals due to the high (g = 6.0) and low (g = 3.0) spin hemes and a trinuclear 3Fe-4S (g = 2.015), 4Fe4S (1.98) and 2Fe2S clusters (g = 1.94). An expanded EPR of the Fe-S cluster of the aged brain comparison with mitochondrial and cytosolic aconitases clearly revealed the oxidative damage of m-aconitase. A consistent age dependent decrease in the 3Fe-4S signal was observed in the striatum suggests MPTP/METH-induced oxidative damage. Collectively, the present ex vivo EPR data reported for the first time that MPTP/METH treatment induces a selective oxidative damage of m-aconitase, [3Fe4S]+ cluster in the aged brain, suggesting that both aging and METH/MPTP-induced dopaminergic neurotoxicity have a common mechanism that generates ROS/RNS and caused oxidative damage to iron-sulfur clusters and thereby increased iron overload to further exacerbate the neurotoxicity. [NIH grants NS 038644 and 039958].

MO05-02

REDUCTION OF PPAR-γ IN METHAMPHETAMINE-INDUCED NEUROTOXICITY AND PROTECTIVE EFFECTS OF INTERFERON-γ

Asanuma, M.[1], Miyazaki, I.[1], Kikkawa, Y.[1], Takeshima, M.[1], Miyoshi, K.[1] and Kita, T.[2]

[1]*Department of Brain Science, Okayama University. Graduate School of Medicine, Dental and Pharmaceutical Science, Okayama*
[2]*Laboratory of Pharmacology, Kyushu Nutrition Welfare University, Graduate School of Food and Nutrition, Kitakyushu, Japan*

An inhibitory transcription factor, peroxisome proliferator-activated receptor-γ (PPAR-γ), inhibits AP-1, NF-κB, and NF-AT which activated by methamphetamine (METH) injection. Our previous studies showed that treatment of several nonsteroidal anti-inflammatory drugs that act as PPAR-γ ligands ameliorated METH neurotoxicity. Moreover, we demonstrated that the systemic or central injections of interferon-γ (IFN-γ) adjunct to METH injections significantly prevented METH-induced dopaminergic neurotoxicity through intracerebral molecular pathways (Hozumi et al., 2008). IFN-γ is known to activate PPAR-γ. In this study, we examined effects of IFN-γ or PPAR-γ agonist on METH-induced changes in PPAR-γ, its related molecules and glial cell activation. Repeated METH injections (4 mg/kg X 4, i.p. with 2 h-interval) markedly reduced nuclear PPAR-γ expression. Systemic IFN-γ injections prior to METH injections attenuated the reduction of nuclear PPAR-γ expression and increasing tendency of NF-κB expression in the striatum. IFN-γ injections also enhanced METH-induced activation of striatal astrocytes. Although treatment of an intrinsic PPAR-γ ligand 15d-PG J2 attenuated striatal METH toxicity, 15d-PGJ2 inhibited METH-induced astrocyte activation. These results suggest that IFN-γ indirectly induces PPAR-γ activation and NF-κB inhibition in the striatum provably via astrocyte activation to exert its protective effect, in contrast to direct PPAR-γ activation by 15d-PG J2.

MO05-03

ABSTRACT TITLE: CONSUMPTION OF AFLATOXIN-CONTAMINATED FOODS: A RISK FACTOR TO HEPATIC ENCEPHALOPATHY

Bbosa, G. S.[1], Kitya, D.[2] and Lubega, A.[1]

[1]*Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences, Kampala*
[2]*Department of Surgery, Mbarara University of Science and Technology. Mbarara, Uganda*

Mycotoxins are metabolic products of fungi produced by ubiquitous fungal genera of Aspergillus and Penicillium species. The most

common toxins being aflatoxins B1, B2, G1, G2 and ochratoxin A. They are commonly encountered in grains and foods which are not properly kept or poorly processed. Prolonged consumption of these contaminated-foods cause damage to the liver and the mitochondrial DNA causing abnormal metabolism of ammonia. This increases glutamine and glutamate synthesis that causes neurotoxicity and hepatic encephalopathy risk. A cross sectional study was conducted to determine aflatoxin levels in the common foods in Kampala, Uganda. A total of 50 food samples were randomly collected and tested for aflatoxin levels by aflatest equipment using standard procedures. The food types were millet (37%), sorghum (24%), cassava (20%), eshabwe (sauce derived from cow ghee) (16%) and groundnuts (3%). SPSS version 10 was used for data analysis. Aflatoxin levels in food samples ranged from 0–55 ppb (mean level of 15.7 ppb ± 14.9). Eshabwe had the highest aflatoxin levels of 18.6 ppb which was significant (P-value < 0.01). Some common foods consumed in Uganda are contaminated with aflatoxin that is higher than acceptable levels (EU acceptable of 4.0 ppbs). This may be a contributing factor to increased hepatocellular carcinoma cases and associated hepatic encephalopathy and so prevention of mycotoxins in these foods requires community sensitisation as well as regular monitoring for their occurances.

MO05-04

THE ROLE OF APOPTOSIS INDUCING FACTOR AND POLY(ADP-RIBOSE)POLYMERASE IN THE MECHANISM OF NEURONAL DEATH EVOKED BY GENOTOXIC STRESS

Cieslik, M., Jacewicz, M. and Strosznajder, J. B.

Departament of Cellural Signaling, Medical Research Center Polish Academy of Sciences, Warsaw, Poland

Poly (ADP-ribose) polymerase (PARP-1) is a nuclear enzyme involved in DNA repair but its overactivation may play a key role in apoptosis and necrosis in various pathological conditions. PARP catalyzes the conversion of NAD^+ to poly(ADP-ribose) polymer (PAR), which is a new important signaling molecule regulatig several transcription factors. PAR released from nucleus induces cell death through stimulation of apoptosis inducing factor (AIF) release from mitochondria. However, the role of PARP/PAR/AIF death signaling seems to depend on the cell line and the type and duration of stress.In this study the role and relationship between AIF and PARP/PAR in death signaling evoked by genotoxic stress in cultured mouse hippocampal neurons (HT22) was investigated. HT22 cells were treated with 50–500 uM DNA alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).MNNG-treated cells demonstrated concentration- and time-dependent enhancement of PAR level and alteration of mitochondrial function evaluated by MTT test. Mitochondrial AIF level significantly decreased after MNNG treatment. After 24 h in the presence of 500 uM MNNG only 15% of HT22 cells survived. At this concentration 40 uM caspase-3 inhibitor and 20 uM Pifitrin have no protective effect on cells survival. However, PARP-1 inhibitors: 5 mM 3-aminobezamide (3AB) and 20 uM PJ34 protect most cells against MNNG-induced death signaling. Concomitantly our study indicated that PARP inhibitors enhanced the endogenous cytoprotective pathway regulated by PI3-K/AKT. Summarizing, our data indicated that PARP/PAR/AIF signaling pathway is responsible for HT22 cell death and that PARP-1 inhibitors protect mitochondrial integrity and function.

MO05-05

EFFECTS OF D-CYCLOSERINE ON MPTP-INDUCED NEUROINFLAMMATION AND DEFICITS IN EPISODIC-LIKE MEMORY IN WISTAR RATS

Ho, Y. J.[2], Wang, A. L.[1], Huang, W. N.[2], Ho, S. C.[2] and Liou, Y. M.[1]

[1]*Department of Life Sciences, National Chung Hsing University*

[2]*School of Psychology, Chung Shan Medical Universit, TaiChung, Taiwan, ROC*

It has been suggested that glutamatergic hyperactivation is involved in memory dysfunction in Parkinson's disease (PD). This study was aimed at clarifying the role of D-cycloserine (DCS), a partial agonist of N-methyl-D-aspartate (NMDA) receptor, in episodic-like memory (ELM) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD animal model. Male Wistar rats were stereotaxically administered with MPTP (1 μmol in 2 μl of saline) or vehicle into the substantia nigra pars compacta (SNc). One day after the surgery, DCS (5 or 10 mg/kg/day; i.p.) was administered daily for 14 days. Motor function and ELM were measured on 7th and 13th days, respectively, after the MPTP treatment. One day after ELM task, rats were sacrificed and the brains were taken for detecting histological changes, including dopaminergic tyrosine hydroxylase (TH), microglial activation, and hippocampal cell loss. The results showed that total distance and center time in the open field test were not different between the groups. The performance of ELM was partially impaired in MPTP-lesioned animals. Low dose of DCS did not influence the performance of ELM in MPTP-treated rats. However, DCS at the dosage of 10 mg/kg recovered the ELM performance in MPTP-treated rats. DCS treatment also suppressed MPTP-induced TH depletion in the SNc and striatum, microglial activation in the SNc, amygdala, and hippocampus, as well as cell loss in the hippocampal CA1 area. These results suggest that NMDA receptor may participate in MPTP-induced neuroinflammation and deficits in ELM.

MO05-07

THE EFFECT OF LIDOCAINE∂HCL ON THE PHYSICAL PROPERTIES OF NEURONAL MEMBRANES

Jang, H. O., Park, J. S., Kwon, S. G., Kim, M. S., Kim, H. I., Bae, Y. J., Seo, S. J., Jeon, Y. C., Chung, I. K., Bae, M. K. and Yun, I.

College of Dentistry and Research Institute for Oral Biotechnology, Pusan National University, Yangsan, Republic of Korea

Fluorescent probe techniques were used to evaluate the effect of lidocaine•HCl on the physical properties (transbilayer asymmetric lateral and rotational mobility, annular lipid fluidity and protein distribution) of synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. An experimental procedure was used based on selective quenching of 1,3-di(1-pyrenyl)propane (Py-3-Py) and 1,6-diphenyl-1,3,5-hexatriene (DPH) by trinitrophenyl groups, and radiationless energy transfer from the tryptophans of membrane proteins to Py-3-Py. Lidocaine•HCl increased the bulk lateral and rotational mobility, and annular lipid fluidity in SPMV lipid bilayers, and had a greater fluidizing effect on the inner monolayer than the outer monolayer. The magnitude of increasing effect on annular lipid fluidity in SPMV lipid bilayer induced by lidocaine•HCl was significantly far greater than magnitude of increasing effect of the drug on the lateral and rotational mobility of bulk SPMV lipid bilayer. It also caused membrane proteins to cluster. These effects of lidocaine•HCl on neuronal membranes may be responsible for some, though not all, of the local anesthetic actions of lidocaine•HCl.

MO05-08

RESPONSE OF NEUROTRANSMITTERS IN HYPERGLYCEMIC STROKE RAT MODEL

Kang, S. W.[1,3,4], Choi, S.[1,2], Lee, G. J.[1,2], Choi, S. K.[5], Chae, S. J.[1,2], B., H. K.[1,2,6], Park, H. K. Oh, H. K.[1,2,6] and Chung, J. H.[3,4]

[1]*Department of Biomedical Engineering, School of Medicine*
[2]*Healthcare Industry Research Institute*
[3]*Department of Pharmacology, School of Medicine*
[4]*Kowhang Medical Research Institute*
[5]*Department of Neurosurgery*
[6]*Program of Medical Engineering, Kyung Hee University, Seoul, Korea*

An increase in the excitotoxic amino acid glutamate (GLU) associated with increased neuronal damage might be thought of the cause for the increased damage seen in hyperglycemia in stroke. So we investigated the effects of functional responses of neurotransmitter GLU and cerebral blood flow (CBF) in hyperglycemic stroke through the real time *in vivo* monitoring. Five Sprague-Dawley rats were used to hyperglycemia, induced with streptozocin, and five normoglycemic rats were used to controls. A development of global ischemia was elicited by an eleven vessel occlusion (11VO) model. Experimental protocols consisting of ten minutes pre-ischemic, ten minutes ischemic and forty minutes reperfusion periods were applied to both groups. CBF levels were significantly higher during reperfusion in hyperglycemia group compared with control group ($P < 0.05$). GLU releases in extracellular space were significantly increased during ischemia ($P < 0.0001$) and reperfusion ($P < 0.001$) in hyperglycemia group compared to control group. Adverse effects of hyperglycemic stroke and endothelial cell swelling with reductions in vascular luminal diameters might lead to a reduction of CBF response during reperfusion. Exacerbated outcome of ischemic stroke caused by increased damage in hyperglycemic stroke might lead to an increase of extracellular GLU concentration.

MO05-09

REPIN INHIBITS UBIQUITIN-PROTEASOME AND INDUCES PROTEIN AGGREGATION AND PROTEASOME DYSFUNCTION IN PARKINSON'S DISEASE MODEL

Kanthasamy, A. G.[1], Ali, S.[2], Huang, D.[1], Anantharam, V.[1], Tukov,F.[3] and Kanthasamy, A.[1]

[1]*Department of Biomedical Sciences, Iowa Center for Advanced Neurotoxicology Iowa State University, Ames, IA*
[2]*NCTR/FDA, Neurochemical Laboratory, Divsion of Neurotoxicology, Jefferson, AR*
[3]*Department of Medicinal Chemistry, Mississippi University, University, MS, USA*

Consumption of Russian knapweed (Centaurea repens) is known to cause equine nigropallidal encephalomalacia, a Parkinsonian-like movement disorder in horses. Repin is one of the principal sesquiterpene lactones of Russian knapweed and has two highly reactive electrophiles (β-lactone and epoxyketone), similar to proteasome inhibitors. Therefore, we tested a novel hypothesis that repin may cause dopaminergic neurotoxicity by inhibiting the ubiquitin proteasome system (UPS). Repin (0-10 μM) produced a dose- and time-dependent inhibition of all three proteasome enzyme activities including peptidylglutamylpeptide hydrolyzing (PGPH), trypsin, and chemotrypsin-like (CTL) in a dopaminergic neuronal N27 cell model. Repin inhibited PGPH to a greater extent than the other two proteasome enzymes and caused an accumulation of ubiquitinated proteins. However, repin did not inhibit proteasome activity in a cell-free system. Repin also induced a time-dependent increase in cytochrome C release, caspase-2, -3 and -9 activities, DNA fragmentation and cytotoxicity. Interestingly, repin dose-dependently inhibited caspase-8 activity. Furthermore, repin caused formation of protein aggregates comparable to those resulting from known proteasome inhibitors in human α-synuclein expressing dopaminergic cells. Together, these results demonstrate repin indirectly inhibits proteasome activities and induces dopaminergic neurotoxicity via a caspase-dependent mechanism. (NIH grants ES010586, NS038644 and NS065167).

MO05-10

ASSOCIATION BETWEEN POLYMORPHISMS OF MATRIX METALLOPROTEINASE 11 (MMP11) AND KAWASAKI DISEASE IN THE KOREAN POPULATION

Kim, S. K.[1], Ban, J. Y.[2], Yoon, K. L.[3], Kang, S. W.[1] and Chung, J. H.[1]

[1]*Kohwang Medical Research Institute, School of Medicine, Kyung Hee University, Seoul*
[2]*Department of Dental Pharmacology, College of Dentistry, Dankook University, Chungnam*
[3]*Department of Pediatrics, East-West Neo Medical Center, Kyung Hee University, Seoul, Korea*

Kawasaki disease (KD) is an acute febrile vasculitis occurring predominantly in infants and young children. The matrix metalloproteinases (MMPs) are a family of secreted zinc metalloproteinases that degrade the collagens of the extracellular matrix important in normal tissue remodeling and repair processes. We investigated the associations between single nucleotide polymorphisms (SNPs) of MMP7 (rs10502001, Arg77His), MMP11 (rs738792, Ala38Val), MMP12 (rs652438, Ile357Val), and MMP26 (rs2499953, Lys43-Glu) and the risk of kawasaki disease (KD) in 101 Korean KD patients and 300 healthy controls. The results were analyzed using logistic regression models, adjusting for gender as covariates. The four SNPs were in Hardy-Weinberg disequilibrium. Only MMP-11 polymorphism (rs738792) was associated with KD. The SNP rs738792 showed a statistically significant association with KD in the codominant (OR = 1.61, 95% CI = 1.11–2.34, $P = 0.0110$) and dominant (OR = 1.92, 95% CI = 1.21–3.06, $P = 0.0057$) models, respectively. However, there was no association between MMPs polymorphisms and the development of coronary artery lesions (CALs). In conclusion, MMP-11 may be associated with KD in Korean children.

MO05-11

ACUPUNCTURE REDUCED THE KAINIC ACID-INDUCED INFLAMMATORY RESPONSES IN THE MICE HIPPOCAMPUS

Kim, S. N.[1,2], Doo, A. R.[1], Kim, S. T.[3], Lee, H. J.[1] and Park, H. J.[1]

[1]*Acupuncture and Meridian Science Research Center*
[2]*BK21 Oriental Medical Science Center, Kyung-Hee University, Seoul*
[3]*Division of Meridian and Structural Medicine, School of Korean Medicine, Pusan National University, Gyeongsamnam-do, Republic of Korea*

Epilepsy is one of the most common serious brain diseases that affect people of all ages. Acupuncture has been used for an epilepsy

treatment, but the mechanism is unknown. We examined the effects of acupuncture on kainic acid (KA)-induced cell death and pro-inflammatory cytokines in the mice hippocampus. EEG recordings were performed after KA injection for 1 h. 8-week-old male C57BL/6 mice were intraperitoneally injected 30 mg/kg of KA, and acupuncture treatment was subsequently administered bilaterally to acupoint HT8 with two pretreatment sessions before injection (total 3 times). For the EEG recording, bipolar electrodes were implanted. After kainic acid injection, brain waves were recorded during 1 h. Twenty four hours later, the destruction of neuronal cells in the hippocampus and changes of proinflammatory cytokines, Il1b and Tnfa were measured. Acupuncture reduced the neuronal cell death by KA in the mice hippocampi. The expressions of Il1b and Tnfa were decreased by acupuncture treatment. Furthermore acupuncture attenuated the electrographic seizures in EEG. Acupuncture might inhibit the KA-induced cell death by suppressing proinflammatory cytokines such as Ilab and Tnfa in the hippocampus. Considering currently available anti-epileptic drugs are unable to modify the disease process and have adverse side effects, our results may suggest that acupuncture be an alternative therapy for epilepsy. This work was supported by KOSEF(R11-2005-014) and the Brain Korea 21 project in 2009.

MO05-12

NEUROPROTECTION BY ATP AGAINST NMDA TOXICITY

Kodama, A., Kato, S., Kambe, Y., Nakamichi, N., Takarada, T. and Yoneda,Y.

Laboratory of molecular pharmacology, kanazawa university graduate school of natural science and technology, kanazawa, japan

Extracellular ATP is believed to play a role as a signal mediator through activation of ionotropic P2X receptors and metabotropic P2Y receptors in the brain. In this study, we have attempted to demonstrate the possible modulation by ATP signals of the neurotoxicity of N-methyl-D-aspartate (NMDA). For this purpose, neurons were prepared from the hippocampus of embryonic 18-day-old rats. An RT-PCR analysis revealed the expression of mRNA for P2X2, P2X3, P2X4, P2X6, P2X7, P2Y1, P2Y2, P2Y4, P2Y6, P2Y12 and P2Y14 isoforms, in addition to A1, A2a and A2b isoforms of P1 receptors for adenosine, in neurons cultured for 8 days. A significant decrease was seen in MTT reduction in neurons briefly exposed to glutamate (Glu) at 10 to 500 μM for 1 h when determined 24 h and 48 h after the exposure, whereas prior and sustained exposure to 1 mM ATP significantly prevented the decrease by Glu. Similarly significant protection was induced by UTP and ADP against the decrease in MTT reduction in neurons exposed to NMDA at 50 to 100 μM for 1 h. Hippocampal neurons were loaded with the Ca^{2+}-sensitive dye fluo-3 AM, followed by exposure to 10 μM Glu or 100 μM NMDA in either the presence or absence of 1 mM ATP for 10 min and subsequent addition of the calcium ionophore A23187 for quantification of the fluorescence intensity using a confocal microscope. A marked increase was seen in the fluorescence in neuronal cells exposed to Glu or NMDA, while ATP at 1 mM significantly attenuated the increase by Glu and NMDA. These results suggest that ATP may preferentially protect neurons from the cytotoxicity of NMDA through a mechanism relevant to intracellular free Ca^{2+} levels in cultured hippocampal neurons.

MO05-13

EFFECTS OF METHYLMERCURY ON PROLIFERATION OF MOUSE AND MONKEY ES CELL-DERIVED NEURAL STEM CELLS

Kume, N.[1], Otsu, M.[12], Kobayashi, H.[1], Inada, D.[1], Yoshie, T.[1], Nakayama,T.[3], Suzuki, Y.[4], Kondo, Y.[4] and Inoue, N.[1]

[1]*Grad. Sch. Human Health Sci., Laboratory Regener. Neurosci., Tokyo Metropolitan University*

[2]*Department of Chemistry, Sch. of Med., Kyorin University, Tokyo*

[3]*Department Biochem., Sch. of Med., Yokohama City University, Yokohama*

[4]*Mitsubishi Tanabe Pharma Corp., Regenerative Medicine Group, Osaka, Japan*

Methylmercury (MeHg), which caused Minamata disease, has been reported to act as a neurotoxic agent during brain development and produce microcephaly as well as various syndromes characterized by mental retardation and motor dysfunction. In this study, we investigated the effects of MeHg on the neural stem cells, prepared from mouse and monkey embryonic stem (ES) cells by the Neural Stem Sphere (NSS) method. Cultured with mitogen fibroblast growth factor-2 (FGF-2), the neural stem cells proliferated exponentially. However, when the neural stem cells were exposed to MeHg (from 10 nM to 3 μM) and cultured for 4 days, the proliferation of the cells was dose-dependently inhibited by MeHg and almost completely inhibited at low concentration. TUNEL analysis demonstrated that MeHg induced apoptosis of the cells. The monkey neural stem cell was slightly more resistant to MeHg than the mouse neural stem cell. These results suggest that MeHg inhibits proliferation of the neural stem cells via apoptosis and consequently causes neurotoxic effects in the development of central nervous system including microcephaly. This work was partly supported by Grants-in-Aid for Scientific Research from JSPS, a grant of LRI by JCIA and Selective Research Fund of Tokyo Metropolitan University.

MO05-14

A SEROTONIN-1A AGONIST UP-REGULATES THE EXPRESSION OF ENDOGENOUS ANTIOXIDANT ENZYME GENES

Lee-Armandt, J. P., Gillespie, R. A. and Druse-Manteuffel, M. J.

Loyola University Medical Center, Maywood, USA

Prenatal exposure to ethanol can damage the development of serotonergic neurons in raphe nuclei. *In vitro* studies suggest that ethanol damages developing neurons by increasing the generation of reactive oxygen species which then augments apoptosis. However, a 5-HT1A agonist ipsapirone augmented the expression and the activity of catalase while antioxidants attenuated ethanol-associated apoptosis in fetal rhombencephalic neurons. In this study, an acute treatment (2 h) of ipsapirone induced an up-regulation of SOD1 and SOD2 expression. At this time point there was also increased SOD2 expression in neurons treated with ethanol and in those treated with ethanol and ipsapirone. A long exposure (24 h) to ethanol and ethanol with ipsapirone increased expression of both genes in these neurons. Interestingly, a 48-hour co-treatment with ethanol and ipsapirone decreased SOD1 but increased SOD2 expression. With the exception of the 24 h time point, the changes in the combined enzyme activities of SOD1 and SOD2 paralleled the changes in SOD1 expression. This is not surprising, because the activity of SOD2 was below levels of detectability when the

activities of the two enzymes were analyzed separately. Inhibitors of two pro-survival pathways, PI-3K and MAPK, were able to prevent up-regulation of both genes at 2 h. In summary, these studies suggest that endogenous antioxidant enzymes play an important role in neuroprotection against ethanol-induced oxidative stress. They also suggest that ipsapirone mediates regulation of these enzymes to stabilize redox status of the cell. Timely regulation of both gene expression and enzyme activity might contribute to the neuroprotective effects of ipsapirone on ethanol-mediated apoptosis.

MO05-15

CHRONIC ETHANOL EXPOSURE INDUCES INFLAMMATORY BRAIN DAMAGE AND ITS ROLE IN VASCULOGENESIS

Mukherjee, S.[1], Das, S. K.[2], Vasudevan, D. M.[1] and Chattopadhyay, S.[3]

[1]*Department of Biochemistry, Amrita School of Medicine, Kochi*
[2]*Department of Biochemistry, Agartala Govt Medical College, Tripura*
[3]*BioOrganic Division, Bhabha Atomic Researrch Centre, Mumbai, India*

Ethanol has a negative impact on human health. Moderate to heavy doses of alcohol have deleterious effects and especially on brain and its different compartments. In this study, we investigated the role of natural antioxidants like grape polyphenols and Vit E in combating the ROS balance in alcoholism for a dose of 4 g /Kg b.w/day of ethanol for a period of 3 months in male Wistar rats. Ethanol intoxication upregulated COX-2 expression, iNOS expression, p38 MAPK pathways and also cell death in different compartments of brain (cerebrum, thalamic area, mid brain hippocampal region and cerebellum), which was evidenced by Caspase-3 and Caspase-8 assays. These findings indicated that exposure to higher concentration of ethanol induced brain damage triggering the inflammatory processes and leads to neuronal apoptosis by TUNEL assay.Natural antioxidants showed protective effect against ethanol induced brain damage by regulating the inflammatory processes with the alteration of the VEGF receptors (flk1 and flt1) and VWF-VIII facilitating vasculogenesis.

MO05-16

PROTECTIVE EFFECTS OF ROSEMARY PHENOLICS DITERPENE AGAINST APOPTOTIC EVENTS AND HO-1 MODULATION IN GLIAL CELLS BY SNP

Park, E. and Chun, H. S.

Department of Biotechnology (BK21 Program) and Research Center for Proteineous Materials, Chosun University, Gwangju, Republic of Korea

Accumulating evidences from epidemiological and dietary intervention studies have implicated that naturally occurring polyphenols may play a useful role in preventing neurodegeneration. Carnosol and carnosic acid are major polyphenolic diterpenes found in Lamiaceae herbs including rosemary. Carnosic acid is easily converted into carnosol by oxidation. Although neuronal cell survival by carnosol has been reported, the effect of carnosol against the glial cell death has not been studied. Therefore, we examined the protective effects of carnosol against sodium nitroprusside (SNP)-induced C6 glial cell damage. In this study, we found for the first time that carnosol-mediated protection in C6 glial cells was involved in the attenuation of apoptotic cell death and modulation of antioxidative molecules. SNP (100 μM)-induced apoptotic characteristics including DNA fragmentation, chromatin condensation, caspase-3 activation, and c-jun N-terminal protein kinase (JNK) phosphorylation, were significantly suppressed by carnosol (10 μM). In addition, carnosol pretreatment restored the level of reduced glutathione (GSH) which was diminished by SNP treatment. We also demonstrated that the heme oxygenase-1 (HO-1) induction in C6 glial cells was associated with suppression of SNP-mediated cell death. [This work was supported by the SRC/ERC program of MOST/KOSEF(#R-11-2000-083-00000-0)].

MO05-17

B23/NPM INTERACTS WITH NUCLEAR GAPDH, INHIBITING GAPDH-MEDIATED CELL DEATH CASCADE

Park, J. H.[1], Kim, C. K.[2], Ahn, J. Y.[2] and Lee, K. H.[1]

[1]*Department of Anatomy*
[2]*Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, South Korea*

Glyceraldhyde-3-phosphate dehydrogenase (GAPDH) has been implicated in apoptotic cell death wherein a variety of cellular stress, translocating the nucleus during apoptosis. Here we report that GAPDH forms complex with B23/NPM, nucleolar antiapoptotic protein, both *in vivo* and *in vitro*. The C-terminus of B23/NPM (240–294 fragment) specifically interacts with GAPDH and N-terminal domain of GAPDH is dispensable for this binding. Forced expression of GAPDH in the nucleus with NLS-tagging augments its binding to B23/NPM, whereas site-directed mutation of C152, S-nitrosylation site, or K227, the binding site of Siah1, E3 ligase, in GAPDH alters its binding to B23, implying that B23/NPM associates with nuclear translocated GAPDH under apoptotic stimuli. Moreover, we show that B23/NPM reduces the cytotoxicity triggered by GAPDH-Siah1 in PC12 cells. Therefore, our findings suggest that B23/NPM antagonizes apoptosis through interacting with nuclear GAPDH and inhibiting GAPDH death cascade.

MO05-18

ANTI-ISCHEMIC HERB SHOWS NEUROPROTECTIVE EFFECTS AGAINST NEURONAL DAMAGES INDUCED BY OXYGEN/GLUCOSE DEPRIVATION

Qian, H., Xiaojing, L. and Pengtao, L.

Beijing University of Chinese Medcine, Beijing, China

Cerebral ischemia followed by oxygen reperfusion could induce apoptosis in hippocampal neurons in the stroke. Extension of axons and dendrites in neurons may compensate for and repair damaged neuronal networks in the hypoxia brain. In this study, we investigated whether an anti-ischemic herb (AIH), an extract containing ginsenoside Rg1 and geniposide and being clinically used for ischemia stroke treatment in the brain, can protect against ischemic neuronal damage in brain vasculature. We found that AIH had neuroprotective role to cultured primary rat hippocampal neurons under both normal and oxygen/glucose-deprivation (OGD) conditions. AIH also protected cerebral microvascular endothelial cells (CMECs) from cell death induced by OGD. By using CMECs-conditioned media for treating rat hippocampal neurons, we observed that AIH stimulated CMECs to secrete some neuroprotective/neurotrophic factors that increased viability of neurons both under normal condition and during re-oxygenation phase after OGD insult. Conditioned media

from AIH-treated CMECs also promoted neurite outgrowth of hippocampal neurons. These findings suggest that AIH has a marked neuroprotective and neurotrophic effects and provide insight into the mechanism of clinical efficacy of this drug against stroke.

MO05-19

NEUROTOXIC EFFECTS OF AMPROLIUM-EVOKED THIAMINE DEFICIT ON CHOLINERGIC SN56 NEUROBLASTOMA CELLS

Szutowicz, A., Zygmanska-Bizon, D., Bielarczyk, H., Jankowska-Kulawy, A. and Romianowski, P.

Department of Laboratory Medicine, Medical University of Gdansk, Gdansk, PL

Several cholinergic encephalopathies are accompanied by energy deficits caused by the inhibition of pyruvate and ketoglutarate dehydrogenase (PDH, KDH) activities in affected brains. Thiamine deficit is one of factors in pathomechanisms of these pathologies. Therefore, we investigated whether amprolium-evoked deficits of thiamine might through impairment of acetyl-CoA metabolism affect function and viability of cholinergic neurons. Two day exposure of differentiated (DC) or nondifferentiated (NC) SN56 cholinergic cells cultured in Minimal Eagles Medium to 1-5 mM amprolium caused concentration-dependent inhibition of their MTT reducing capacity by 30 and 15%, respectively. In same conditions, respective fractions of trypan blue positive cells reached values 43 and 10%. On the contrary, after cell homogenization, neither PDH nor KDH were found to be inhibited by preceding cell culture with amprolium. Also cell number was not altered by these conditions. On the other hand, amprolium decreased acetyl-CoA levels in whole DC and NC by 39 and 46%, respectively. The inhibitor altered choline acetyltransferase activity neither in NC nor in DC. However, acetylcholine content in DC was suppressed by amprolium for over 40% while in NC it was without effect. These findings indicate that increased ACh synthesis in DC made them more vulnerable to acetyl-CoA shortages caused by amprolium-evoked inhibition of thiamine uptake. Low rate of ACh synthesis in NCs, enabled them to save more acetyl-CoA for energy metabolism and thereby facilitating their survival under pathologic conditions. Supported by MNiSW projects PO5A 11030, NN401 2333 33 and MUG fund W-20.

MO05-20

INTERACTIONS OF ZINC AND CALCIUM IN FUNCTIONAL AND STRUCTURAL IMPAIRMENT OF CHOLINERGIC CELLS

Szutowicz, A., Dys, A., Ronowska, A., Jankowska-Kulawy, A., Romianowski, P. and Bielarczyk, H.

Department of Laboratory Medicine, Medical University of Gdansk, Gdansk, PL

Release of glutamate from glutamatergic terminals is accompanied by co-release of zinc that is taken up from the synaptic cleft into postsynaptic cells by Zn-transporters and Ca-channels. Exitotoxicity-evoked overload of postsynaptic cholinergic neurons with Zn could impair their neurotransmission and structural integrity through alterations in subcellular comapartmentation of Ca. Short-term, 30 min exposure of SN56 cholinergic cells to Zn yielded concave up non saturable accumulation plots up to 60 nmol/mg protein at 0.15 mM extracellular Zn. It points out coexistence of low density, high-affinity and high density-low affinity Zn-transporting entities in cell plasma membranes. In such conditions Zn caused 30% decrease of mitochondrial and 100% increase of cytoplasmic Ca. On the other hand, 24 h culture with 0.15 mM Zn increased its intracellular content from 1.4 to about 6 nmol/mg protein. Simultaneously 40% decrease of whole cell Ca was observed. The rate of cell death significantly correlated with Zn-evoked changes in intracellular distribution of Ca ($r = 0.98$). Zn evoked no significant changes in the content of ZnT3 and ZnT4 proteins. Inhibition of mitochondrial Na-Ca exchanger by accumulated Zn might deplete Ca in mitochondria yielding inhibition of acetyl-CoA transfer to cytoplasm and subsequent suppression of acetylcholine synthesis and release. Inhibition of aconitase by acutely accumulated Zn may aggravate its detrimental influences on cell integrity. Time-dependent decrease of Zn accumulation in SN56 cells might result from adaptative changes in density of nonspecific sites but not ZnT transporters. Supported by MNSW grants NN401 2333 33, P05A 110 30 and GUM projects St57, W-20.

MO05-21

PRECONDITIONING WITH CRUCIFEROUS ALLYL NITRILE PROTECTS AGAINST NEUROTOXICITY OF ITS OWN

Tanii, H., Higashi, T. and Saijoh, K.

Department of Hygiene, Kanazawa University, Kanazawa, Japan

Allyl nitrile is generated from cruciferous vegetables. A large dose of allyl nitrile induces in rodents persistent behavioral abnormalities similar to the excitement, choreoathetosis and circling syndrome (ECC syndrome), while a repeated exposure to this chemical at subneurotoxic levels shows induction of phase 2 detoxification enzymes in tissues including stomach, kidneys and lungs although no information is available on the brain. In this study we examined if the repeated low level exposure to allyl nitrile had inductive ability of phase 2 detoxification enzymes in the brain, resulting in protection against neurotoxicity of its own. The repeated exposure to allyl nitrile (0.1–0.4 mmol/kg, po, 5days) showed 1) inductive ability in the striatum, medulla oblongata plus pons and cortex as seen in increases in activities of glutathione S-transferase and quinine reductase and in level of glutathione, 2) protection against behavioral abnormalities induced by a large dose with this chemical (1.2 mmol/kg, po), and 3) protection against reduction in the density of GABA cells observed in substantia nigra and interpeduncular nucleus 2 days after dosing with this chemical (1.2 mmol/kg, po). The results suggest that a repeated low level exposure to allyl nitrile is beneficial to your health.

MO06 Prion diseases

MO06-01

PEPTIDYLARGININE DEIMINASE-MEDIATED PROTEIN CITRULLINATION IN THE PATHOGENESIS OF PRION DISEASES

Choi, E. K.[1], Jang, B.[1], Jin, J. K.[1], Jeon, Y. C.[1], Cho, H. J.[1], Ishigami, A.[2,3], Choi, K. C.[1], Carp, R. I.[4], Maruyama, N.[3] and Kim, Y. S.[1]

[1]*Hallym University, Chuncheon, South Korea*
[2]*Toho University, Chiba*
[3]*Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*
[4]*New York State Institute for Basic Research in Developmental Disabilities, New York, USA*

Protein citrullination by peptidylarginine deiminase (PAD), which results in the conversion of peptidylarginine to peptidylcitrulline, is markedly increased in abnormal conditions. To elucidate the involvement of protein citrullination by brain-specific PAD2 in the pathogenesis of prion diseases, we examined the profiles of citrullinated proteins using the brains of sporadic Creutzfeldt-Jakob disease (sCJD) and scrapie-infected mice. Increased expression and activity of PAD2 were found in the reactive astrocytes that contained increased levels of citrullinated proteins. The study of electron microscopy using immunogold labeling revealed that PAD2 and citrullinated proteins were widely distributed in various cell compartments including endoplasmic reticulum, mitochondria, and nucleus of hippocampal neurons and astrocytes in infected brains. Using 2-DE and MALDI-TOF mass spectrometry, we identified various citrullinated proteins that were structural and energy metabolism-associated proteins; vimentin, GFAP, myelin basic protein, enolase, and aldolase. This study suggests that accumulated citrullinated proteins and abnormal activation of PAD2 may function in the pathogenesis of prion diseases and serve as potential therapeutic targets. [This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-331-E00287) and by a grant (Code #20080401034016) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.]

MO06-02

HYPOXIA INHIBITS THE NEURONAL CELL DEATH INDUCED BY PRP106–126

Park, S. Y., Seo, J. S., Seol, J. W., Moon, M. H., Jeong, J. K. and Lee, Y. J.

College of Veterinary Medicine, Chonbuk National University, Jeonju, South Korea

In this work, we were interested in the effect of hypoxia on the neurotoxicity induced by prion peptide. After PrP106-126 peptide treatment in human neuroblastoma cells under low oxygen environment, we tested the cell viability and intracellular signals using crystal violet, MTT, western blot and immuno fluorescence assays. The results showed that PrP106-126 peptide induced apoptosis in human neuroblastoma cells and the apoptosis inhibited by hypoxic conditions. We investigated the intracellular signaling responsible for the PrP106-126-dependent cell death of SH-SY5Y, a cell line derived from a human neuroblastoma. As a result, hypoxia inhibited the reduction of mitochondrial membrane potential, caspase-3 activation and NF-kB activation induced by PrP106-126 peptide. And hypoxia increased the anti-apoptotic Bcl-2, IAP-2 proteins and activation of Akt phosphorylation. These results demonstrated that hypoxia inhibit the PrP106-126 peptide-induced neuroblastoma cell death with mechanism by Akt activation and NF-kB inhibition, and suggested that hypoxic conditions or HIF-1α may serve as a potentially exciting therapeutic method for various prion diseases.

MO07 Axonal transport

MO07-01

CHARACTERIZATION OF THE MOVEMENT OF NELL2 IN THE RAT HIPPOCAMPAL PROGENITOR CELL LINE, HIB5

Hwang, E.[1], Ha, C.[1,2], Shayusupova, M.[1], Yoo, J.[1], Kim, D.[1], Kim, E.[1], Park, N.[1], Kang, D.[1], Han, J.[1], Hong, S.[1], Chang, S.[3], Lee, B.[4] and Park, J.[1]

[1]*Department of Physiology, Gyeongsang National University, Junju*
[2]*Department of Pharmacology and BK21, Yonsei University College of Medicine*
[3]*Department of Biomedical Sciences, Seoul National University Graduate School, Seoul*
[4]*Department of Biological Sciences, University of Ulsan, Ulsan, Korea*

NELL2 is a secreted glycoprotein known to be almost exclusively expressed in the nervous system, but little is known about the secretion mechanism of this protein. By monitoring the localization and movements of EGFP-labeled NELL2 in HiB5 cells, we determined the subcellular localization of NELL2 and its mechanism of secretion. NELL2-GFP revealed an expression pattern typical of other secreted proteins, especially with respect to its localization in endoplasmic reticulum, Golgi apparatus, and punctates. Vesicles containing NELL2 exhibited bidirectional movements in HiB5 cells and cultured hippocampal neurons, The majority of the vesicles moved in an anterograde direction along the neuronal axon, with an average velocity of 0.528 μm/s, while some vesicles (~28.5%) showed retrograde movement with an average velocity of 0.295 μm/s. The movement patterns of NELL2 vesicles were dependent upon the presence of microtubules. The 29 amino acids at the N-terminus of NELL2, including the putative signal peptide was important for its secretion since NELL2 mutants in which this region was deleted were not detected in the medium. These results strongly suggest that NELL2 is secreted by neuronal transport at a high velocity, and may affect the cellular activity of neighboring cells and itself in a paracrine or autocrine manner.

MO07-02

A COMPREHENSIVE MUTATIONAL ANALYSIS SYSTEM REVEALED MUTATIONAL SPECTRUM OF HEREDITARY SPASTIC PARAPLEGIA

Ishiura, H., Takahashi, Y., Goto, J. and Tsuji, S.

Department of Neurology, The Univerisity of Tokyo, Tokyo, Japan

Objective: HSP is a genetically heterogeneous neurodegenerative disorder characterized by progressive lower limb spasticity and pyramidal weakness. More than 40 (SPGs 1–45) genetic loci and 19 causative genes have been identified. Because little is known about genotype-phenotype correlations, comprehensive mutational analysis system is demanded.

Method: We established a high-throughput DNA microarray resequencing system (Affymetrix) for analysis of 13 causative genes (*L1CAM*, *PLP1*, *atlastin*, *spastin*, *NIPA1*, *paraplegin*, *KIAA0196*, *KIF5A*, *HSP60*, *BSCL2*, *spartin*, *maspardin*, and *REEP1*). Sanger method was also employed in *spastin* and *REEP1* to detect small insertion/deletion mutations. For patients with thin corpus callosum and cognitive dysfunction, *SPG11* was analyzed with Sanger method. In addition, to detect large rearrangements, we designed a custom-made high density oligonucleotide CGH array (Agilent) in which sequences of 16 causative genes (*CYP7B1*, *SPG11*, and *ZFYVE27* in addition to 13 genes above) were tiled. The study enrolled 128 HSP patients.

Results: We detected 32 *spastin*, 2 *atlastin*, 2 *KIAA0196*, 2 *REEP1*, 4 *SPG11* mutations. Collectively, 33% (42/128) were given diagnosis. Twenty-three out of 32 mutations (71.8%) in *spastin* were nonsense, splice site, insertion/deletion, or large rearrangement, and 8 out of the rest of 9 *spastin* mutations were located in functional AAA cassette, suggested haploinsufficiency is the disease mechanism.

Conclusion: The combination of three methods found various types of mutations effectively. Mutations detected by the system helps to understand disease mechanisms and protein functions.

MO08 Molecular mechanisms involved in Alzheimer disease

MO08-01

THE ASSOCIATION OF BDNF GENE POLYMORPHISMS AND PLASMA LEVELS WITH ALZHEIMERS'S DISEASE

Ahn, K., Song, J. H. and Jo, S. A.

National Institute of Health, Seoul, Korea

Brain-derived neurotrophic factor (BDNF) is an activity-dependent secreted protein that is critical to organization of neuronal networks and synaptic plasticity. From the studies of a variety of animal models and of humans, reduced BDNF secretion is one mechanism of age-related cognitive decline. Multiple transcription initiation sites and the presence of anti-gene makes BDNF gene expression complex. We analyzed 17 SNPs in the 120 kb genomic region spanning BDNF gene and nearby genes on chromosome 11p13 of DNA samples of 271 Alzheimer's disease (AD) patients, 347 subjects with mild cognitive impairment (MCI) and 489 healthy controls (average age of total samples, 70.8_6.4; male/female, 470/637). Association with AD was observed for 4 SNPs and with MCI for 3 SNPs ($P < 0.05$). One haplotype was associated with both AD and MCI.BDNF concentrations were measured by sandwich ELISA method (Chemicon Int.) in plasma samples of 120 AD patients, 60 MCI, 50 depression and 70 healthy controls. Compared to the controls, the BDNF levels of AD patients and depression were significantly lower ($P < 0.001$). The association between plasma BDNF concentrations and the genotype of Val66M was observed ($P = 0.01$). Our results suggests that the polymorphisms in BDNF gene may cause to increase the risk for AD and plasma BDNF level could be a biomarker of cognitive impairment (AD/MCI) or depression.

MO08-02

SINGLE NUCLEOTIDE POLYMORPHISMS OF GLYCOGEN SYNTHASE KINASE 3 BETA AND CYCLIN-DEPENDENT KINASE 5 GENES IN ALZHEIMER'S DISEASE

Czapski, G. A.[1], Styczynska, M.[2], Barcikowska, M.[2] and Strosznajder, J. B.[1]

[1]*Department of Cellular Signalling, Mossakowski Medical Research Centre PAS, Warsaw, Poland*

[2]*Department. of Neurodegenerative Disorders, Mossakowski Medical Research Centre PAS, Warsaw, Poland*

Overactivation of Glycogen Synthase Kinase 3 beta (GSK-3B) and Cyclin-Dependent Kinase 5 (CDK5) has been implicated in Alzheimer's disease (AD), the main cause of dementia in the elderly. GSK-3B and CDK5 are responsible for aberrant phosphorylation of tau, the major component of the neurofibrillary tangles, which besides deposits of amyloid β are pathological hallmarks of AD. In this study we assessed the association between single nucleotide polymorphism (SNP) in these kinases genes and the risk of early (EOAD) and late onset (LOAD) Alzheimer's disease. The following SNPs rs334558 (A/G), rs9278 (A/G), rs2069454 (C/G) and rs2069442 (C/G) were analysed by TaqMan SNP genotyping assay or PCR-RFLP assay. In this study 198 Polish LOAD cases, 71 EOAD cases and 104 controls were analysed. Among tested SNPs only the distribution of genotypes in rs334558 SNP in GSK3B gene was significantly different in patients with late onset AD comparing to healthy control group. The analysis of environmental factors indicated enhancement of serum level of total cholesterol/HDL and decreased level of LDL in EOAD and LOAD groups comparing to control group. Moreover lower concentration of vitamin B12 and higher homocysteine were observed in LOAD group compared to controls. Further analysis is carried out by us to clarify the role of polymorphisms in the genes encoding CDK5 and GSK-3B kinases in pathogenesis of AD.

This study was supported by MSHE Grant 0146/B/P01/2008/35 and by Scientific Network 28/E-32/BWSN-0053/2008.

MO08-03

ISCHEMIA-REPERFUSION INJURY AND PROCESSING OF AMYLOID PRECURSOR PROTEIN

Dobrota, D.[1], Babusikova, E.[1], Turner, A. J.[2] and Nalivaeva, N. N.[2]

[1]*Department of Medical Biochemistry, Comenius University in Bratislava, Jessenius Faculty of Medicine, Martin, Slovakia*

[2]*Institute of Molecular and Cellular Biology, University of Leeds, Leeds, UK*

Alzheimer'disease (AD) is linked to certain common brain pathologies (e.g. ischemia, stroke, trauma), which facilitate its development and progression. The molecular mechanisms of this phenomenon are still unknown. Amyloid precursor protein (APP) plays one of the key roles in the pathogenesis AD via accumulation of toxic amyloid peptide (Abeta). Accumulation of Abeta depends on the activity of various enzymes participating in its production (beta-secretase and gama-secretase) and degradation (neprilysin, NEP; endothelin-converting enzyme, ECE). In this study we determined the effect of the global ischemia-reperfusion injury in adult male rats on the APP metabolism. We have found that levels of APP increased in hippocampus and cortex after ischemia, as well as levels of sAPPbeta increased after ischemia. After 2 h reperfusion the levels of APP in the brain reduced to the control values. The amount of sAPPbeta after reperfusion was increased compared to the control but decreased to the ischemia. Levels of Abeta degradating enzyme decreased significantly after *in vivo* ischemic insult. Reperfusion has beneficial effects on NEP, ECE, as well as amount of beta-secretase decreased after reperfusion. Ischemia shifts APP processing towards amyloidogenic beta-secretase pathway in the brain and in long term, might lead to accumulation of neurotoxic amyloid beta peptide.

Supported by: AV 1105/2004, MZ 2007/57-UK-17.

MO08-04

OLIGOMERS OF β-AMYLOID (1-42) PEPTIDE INDUCE CO-LOCALIZATION OF AB AND TAU PROTEINS ASSOCIATED WITH CALPAIN ACTIVITY

Gonzalo-Ruiz, A., Gonzalo, P. and Perez, J. L.

University of Valladolid (Campus of Soria), Soria, Spain

The aim of this study was investigated whether injection of Aβ1-42 oligomers in the rat brain induces histopathological alterations resembling those observed in human AD brain. In rats, anaesthetised with equitesina(0.33 ml/ 100 gr), injections of Aβ oligomers were placed into the posterior cingulate cortex. Control animals were injected with Aβ(42-1) sequence. After 24 h and 72 h, animals were deeply anaesthetised and perfused with fixative. Injections of Aβ oligomers induce Aβ-IR structures-like diffuse amyloid forms in

retrosplenial cortex, and extending to hippocampus and to several cortical regions. Aβ-positive material was also observed in both leptomeningeal and cortical blood vessels, at an early time point (24 h) of Aβ toxicity. In Aβ oligomers-treated animal calpain and tau-IR were observed around the injection site, in cortical and leptomeningeal blood vessel,and along the subventricular zone of the lateral ventricles, at an early (24 h) time point. Double-immunohistochemical studies showed that Aβ co-localized with calpain and tau proteins at the injection site and around blood vessels, at an early (24 h) time point. The injection of scrambled peptide showed light background staining surrounding the injection site, and no structures-like diffuse amyloid forms were observed in such control animals. Since alterations in calcium homeostasis in AD pathogenesis associated with calpain activation has been proposed to play an important role in the development of cytoskeletal pathology and neurodegeneration, the present findings suggest that calpain is involved in Ca^{2+} mediated apoptosis in rat brain as *in vivo* model of AD pathology. Supported by Junta de Castilla y León (SAN673/VA14/08; VA030A07).

MO08-05

INHIBITION OF ABETA ACCUMULATION BY BACE INHIBITORS AND VACCINATION

Ishiura, S.

The University of Tokyo, Tokyo, Japan

There is compelling evidence that aggregation and accumulation of amyloid beta peptide (Abeta) play a pivotal role in the development of Alzheimer's disease. Abeta is generated from amyloid precursor protein (APP) by beta- and gamma-secretase-mediated cleavages. Since BACE1 (beta-site APP cleaving enzyme 1)-knockout mice produce much less Abeta and grow healthily without apparent side effects, BACE1 inhibitor is thought to be one of the most attractive targets for the development of therapeutic interventions for AD. Here, we report *in vivo* inhibitory effects of a novel BACE1 inhibitor, KMI-429, which is a transition-state mimic, effectively inhibits beta-secretase activity in cultured cells in a dose-dependent manner. We injected KMI-429 into the hippocampus of APP transgenic Tg2576 and wild-type mice and measured the release of sAPPbeta (soluble extracellular fragment of APP generated by beta-secretase), the levels of C-terminal fragment of APP and Abeta by quantitative Western blotting and sandwitch ELISA. KMI-429 significantly reduced Abeta production in soluble fraction compared with vehicle *in vivo*, but changes in Abeta levels in insoluble fraction were not so affected. In contrast, the intrahippocampal injection of KMI-429 in wild-type mice remarkably reduced Abeta production in both soluble and insoluble fractions. Preclinical studies in Tg2576 mice demonstrate the therapeutic potential of altering Abeta deposition by inducing humoral immune response to Abeta. We expressed Abeta in green pepper and the leaf is administered as oral vaccine. Abeta levels in the brain of Tg2576 mice are significantly reduced after vaccination. These results indicate that the BACE1 inhibitor KMI-429 and vaccination would be promising candidates for a treatment for AD.

MO08-06

NEUROPROTECTIVE EFFECT OF PORIA COCOS AGAINST SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT AND ITS UNDERLYING MOLECULAR MECHANISMS

Jegal, K. H., Park, S. J., Kim, C. Y., Lee, C. and Jang, J .H.

College of Oriental Medicine, Daegu Haany University, Daegu, South Korea

Cholinergic deficits have been associated with memory loss and correlated with the severity of Alzheimer's disease (AD). In line with this notion, scopolamine, a muscarinic cholinergic receptor antagonist has widely been used to impair learning acquisition and short-term memory and utilized as a model for screening anti-amnesic drugs. Pharmacological intervention to increase cholinergic transmission is a potential candidate for the development of preventive and/or therapeutic agents against cognitive impairment in AD. In this study, we have investigate the neuroprotective effect of Poria cocos Wolf (Hoelen cum radix), a fungus in the Polyporaceae family which has been widely used in the traditional herbal medicine, on the scopolamine-induced learning and memory impairments in Sprague-Dawley rats. Poria cocos significantly improved mean escape latency in Morris water-maze test and spontaneous alteration in Y-maze task. Moreover, Poria cocos increased mRNA levels of muscarinic acetylcholine receptor (mAchR), particularly M2 type in rat hippocampus compared with scopolamine injected alone group. The protein expression of choline acetyltransferase (ChAT), a key enzyme in the synthesis of acetylcholine and brain-derived neurotrophic factor (BDNF), a representative endogenous neuroprotective molecule was also elevated by Poria cocos, which seems to be mediated by activation of cAMP response element-binding protein (CREB). These results suggest that Poria cocos may have memory and cognitive enhancing potentials for the prevention and/or treatment of amnesia.

MO08-07

THE ROLE OF ATBF1 (AT-MOTIF BINDING FACTOR 1) IN ALZHEIMER'S DISEASE

Jung, C. G.[1], Kawaguchi, M.[2], Miura, Y.[3], Akatsu, H.[4], Hosono, T.[1], Yamaguchi,T.[1], Horike, H.[1], Uhm, K. O.[1] and Michikawa, M.[1]

[1]*Department. of Alzheimer's disease, National Institue for Longevity Sciences, Obu*

[2]*Department. of Pathology, Niigata Rosai Hospital, Niigata*

[3]*Department. Mol. Neurobiol., Med., Nagoya City Univ., Nagoya*

[4]*Choju Medical Institute, Fukushimura Hospital, Toyohashi, Japan*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by selective neuronal loss and formation of plaques containing amyloid-β peptide (Aβ). It has been reported that Aβ induces DNA damage, resulting in neuronal cell death in AD. It is established that Aβ-induced neurotoxicity occurs through the induction of apoptotic pathways including pro-apoptotic factors, Ataxia telangiectasia mutated (ATM) and p53. The specific signaling pathway, however, mediating this Aβ-induced neuronal cell death has not been completely elucidated. We have previously reported that ATBF1 is highly expressed in postmitotic neurons and it induces cell cycle arrest associated with neuronal differentiation in the developing brain. Recently, we found that ATBF1 is highly expressed in the AD brain compared with age-matched control. The aim of this study is to investigate whether ATBF1 plays any role in the pathogenesis of AD brain. We hypothesize that up-regulated

expression of ATBF1 in AD brain is a response to DNA damage induced by Aβ. We found that ATBF1 expression was up-regulated in the cultured rat cortical neurons treated with different genotoxic compounds (etoposide, homocysteine) and Aβ (1-42). Apoptosis induced by Aβ and etoposide in cultured neurons was attenuated by inhibition of ATBF1 with siRNA. Our data suggest that ATBF1 expression is enhanced by Aβ, and enhanced level of ATBF1, in turn, activates ATM signaling pathways responsible for the neuronal cell death.

MO08-08

AUTOPHAGY INDUCTION ENHANCES AB GENERATION VIA SECRETASE ACTIVATION

Jung, E. S., Hwang, J. Y., Jin, S. M. and Mook-Jung, I.

Department of Biochemistry and Cancer Research, Seoul National University College of Medicine, Seoul, Korea

Alzheimer's disease (AD) is known to be a neurodegenerative disease caused by amyloid β peptide (Aβ), which is generated from amyloid precursor protein (APP). Although the neuronal death is the direct cause of AD, the alteration of APP processing during cell death has not been thoroughly investigated and there are only few reports presenting the existence of machinery for Aβ generation. Autophagy plays an important role in maintaining cellular homeostasis by degrading and recycling components for biosynthesis and energy production. There are several reports that autophagic vacuoles, intermediate of the process, are abnormally accumulated in AD patient brains and in a model of AD pathology. Besides, generation of Aβ is increased when autophagy is induced. It was shown that the autophagosome contains all the components of Aβ producing machineries, β-& γ -secretases and APP and Aβ secretion facilitated in autophagy-inducing condition. However, the mechanism of enhancement of Aβ secretion has not been fully elucidated. Here, we hypothesized that the secretase activities are modulated during autophagy process. The activities of both Aβ and γ -secretase were significantly increased by inducing autophagy using amino acid depletion or mTOR inhibition. The association of secretase activity and autophagy was confirmed by autolysosomal inhibitor, bafilomycin A1. Treatment of bafilomycin A1 inhibited autolysosomal formation and decreased secretase activity. Finally, it was shown that the activation of secretase was not due to the increase of proteins comprising secretases. Based on these results, we suggest that the autophagosome can be a new drug target to develop a treatement for Alzheimer's disease.

MO08-09

D1 RECEPTOR STIMULATION PREVENTS LOSS OF AMPA AND NMDA RECEPTORS INDUCED BY AEPTOR STIMULATION PREVENT LOSS OF AMPA AND NMDA RECEPTORS INDUCED BY Aβ OLIGOMERS

Jurgensen, S., Mussi, G. E., Brito-Moreira, J. and Ferreira, S. T.

Federal University of Rio de Janeiro, Institute of Medical Biochemistry, Rio de Janeiro, Brazil

Soluble oligomers of the amyloid-β (Aβ) peptide accumulate in the brains of Alzheimer's disease (AD) patients and are increasingly recognized as the major neurotoxins in AD. Aβ oligomers impair synaptic plasticity by inhibiting long term potentiation (LTP) and inducing loss of surface AMPA and NMDA receptors, key molecules for neurotransmission and neuroplasticity. Recent studies have shown that activation of dopamine D1 receptors reinforces glutamatergic transmission via phosphorylation of AMPA and NMDA receptor subunits (GluR1 and NR1, respectively) at specific sites by protein kinase A (PKA). Phosphorylation of GluR1 and NR1 affects their trafficking, rendering these subunits more resistant to endocytosis and ready to be inserted into synaptic sites. We hypothesized that stimulation of D1 receptors might prevent the endocytosis of GluR1 and NR1 induced by Aβ oligomers. Immunocitochemical analysis showed that treatment of mature hippocampal neurons in culture with Aβ oligomers (400 nM for 4h) significantly decreased the levels of surface AMPA and NMDA receptors. Interestingly, pre-treatment of the cultures with SKF81297, a selective D1 receptor agonist, prevented loss of both types of receptors. SCH23390, a selective D1 receptor antagonist, blocked the protective effect of SKF81297, further substantiating the involvement of D1 receptors. Results suggest that stimulation of D1 receptors may provide a novel pharmacological approach to prevent Aβ oligomer-induced loss of plasticity-related receptors and memory impairment in AD.

MO08-10

PROTECTIVE EFFECT OF EPIGALLOCATECHIN-3-GALLATE AGAINST BETA-AMYLOID-INDUCED OXIDATIVE AND/OR NITROSATIVE CELL DEATH

Kim, C. Y., Lee, C. and Jang, J. H.

Department of Pathlogy, College of Oriental Medicine, Molecular Neurotoxicology Laboratory, Daegu Haany University, Daegu, South Korea

β-Amyloid (Aβ), a major component of senile plaques has been regarded to play a crucial role in the development and neuropathogenesis of Alzheimer's disease (AD). Increasing data indicate that Aβ-induced damages in neurons and glia are mediated via oxidative as well as nitrosative stress. Epigallocatechin-3-gallate (EGCG), one of these candidates is a major polyphenolic compound present in green tea and has been reported to exhibit potent antioxidant and anti-inflammatory properties. In this study, we have investigated the effect of EGCG against Aβ-induced oxidative and/or nitrosative cell death in BV2 microglia. Aβ treatment led to apoptosis in BV2 cells as revealed by DNA fragmentation, perturbation of mitochondrial transmembrane potential, and alterations in the expression of apoptosis-regulator Bcl2 family proteins. EGCG pretreatment effectively ameliorated Aβ-induced cytotoxicity and manifestation of proapoptotic signals. Furthermore, BV2 cells exposed to Aβ underwent nitrositive stress as shown by the increased expression of inducible nitric oxide (iNOS) synthase and subsequent production of nitric oxide (NO) and peroxynitrite, which were effectively suppressed by EGCG pretreatment. To elucidate a molecular mechanism underlying the neuroprotective effect of EGCG, we have examined the cellular metabolism of reduced glutathione (GSH) with antioxidant properties. EGCG treatment fortified cellular GSH pool through elevated mRNA expression of γ-glutamylcysteine ligase (GCL), the rate limiting enzyme in the glutathione biosynthesis. These results suggest that EGCG may have preventive and/or therapeutic potential in AD patients by augmenting cellular anti-oxidant defense capacity.

MO08-11

FRET -BASED ASSAY DEVELOPMENT FOR MODULATOR SCREENING BETWEEN GAMMA SECRETASE AND APP INTERACTION IN LIVE CELL

Kim, J. E. and MooK-Jung, I.

Department of Biochemistry and Biomedical Science , Seoul National University College of Medicine, Seoul, Korea

Amyloid plaque is one of major characteristic features of Alzheimer's disease (AD). Major component of the plaques is Aβ peptide which is generated by sequential cleavages of the amyloid precursor protein (APP) with β-secretase and γ-secretase. Since Aβ peptide is known as a crucial factor for AD pathogenesis, understanding of Aβ generation mechanism is important. γ-secretase is an essential protease to produce Aβ peptide from β-secretase cleavage product of APP (APP-C99) and it is composed of 4 different molecules such as presenilin, nicastrin, Aph-1 and Pen-2, indicating that APP-C99 and γ-secretase are directly interacting to generate Aβ peptide. To investigate the dynamic interaction between APP-C99 and γ-secretase in live cells, fluorescence resonance energy transfer (FRET) assay is developed. FRET assay provides real time interaction of γ-secretase to APP-C99 and we can monitor various drug responses whether it can promote or inhibit Aβ generation in live cells by dynamic interaction between APP-C99 and γ-secretase in live cells. We found rapamycin, an autophagy activator, and camptotehcin, topoisomerase inhibitor, accelerated the interaction between APP-C99 and γ-secretase using dynamic FRET system in live cells. In contrast, AY9944, an inhibitor of cholesterol biosynthesis, attenuated the interaction between APP-C99 and γ-secretase. Biochemical measurement of Aβ production using ELISA system for Aβ peptide under same drug treatment showed consistent outcome of those drug responses. Taken together, we developed efficient FRET assay system to monitor γ-secretase activity in live cells and it can be used for modulator screening of γ-secretase activity in live cells.

MO08-12

AMYLOID - PEPTIDE ACCELERATES ALTERATIONS OF EXPRESSION AND LOCALIZATION OF TIGHT JUNCTION PROTEINS VIA RAGE

Kook, S. Y., Son, S. M., Hong, H. and Mook-Jung, I.

Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea

The blood-brain barrier (BBB) is the specialized barrier formed by the endothelial cells and an important role in regulating transport of various molecules and maintaining integrity and brain homeostasis. Deposition of amyloid β-peptide (Aβ) in the brain is the prominent feature of Alzheimer's disease (AD). Accumulation of Aβ leads to increase activated microglia and astrocytes, resulting in alteration of brain microenvironment and BBB breakdown. The brain endothelium forming the BBB is connected by tight junction proteins including ZO-1 and occludin, restricting paracellular permeability in selective. The receptor for advanced glycation end products (RAGE) binds soluble Aβ and is thought to be a major transporter of Aβ across BBB. Influx of Aβ by RAGE from blood to brain elevates deposition of Aβ in the brain including around BBB. From the point of view the overexpression of RAGE in AD brain vasculature, as well as RAGE-dependent binding and transport of Aβ, we hypothesized that enhanced RAGE-mediated Aβ may accelerate BBB disruption by inducing alteration of tight junction proteins and paracellular permeability. We attempt to explore tight junction proteins may be altered by Aβ following up-regulation of RAGE in the immortalized mouse brain capillary endothelial cell line, bEnd3 cells, as an *in vitro* BBB model. We observed that Aβ altered the expression of ZO-1 and occludin mRNA and protein as well as the localization of those proteins *in vitro*. It indicates that Aβ causes the alteration of mRNA, protein and localization of tight junction proteins and it is accelerated in RAGE-overexpressing bEnd3 cells, suggesting that RAGE-Aβ interaction might be important for change of the BBB integrity in AD patients.

MO08-13

HUMAN TRUNCATED TAU INDUCES FUNCTIONAL CHANGES OF THE BLOOD–BRAIN BARRIER

Kovac, A.[1], Zilka, N.[1] and Novak, M.[1,2]

[1]*Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*

[2]*Axon Neuroscience GmbH , Vienna, Austria*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that destroys the higher structures of the brain. It is generally accepted that there are two major driving forces behind neurodegeneration in AD, intrinsically disordered proteins β-amyloid and misfolded tau. Both proteins gain toxic function during transition from their physiological to pathological forms. Several independent studies showed that amyloid β was toxic to human brain endothelial cells and increased microvascular permeability. In contrast, little is known about the relationship between functional impairment of blood-brain barrier and misfolded tau. In this study, we tested whether human non-mutated truncated tau protein is able to induce changes of rat *in-vitro* blood-brain barrier model. We found that stimulation of complete *in-vitro* BBB model revealed very strong polarity-dependent effect. Abluminal but not luminal treatment of BBB model with human truncated tau protein induces significant decrease of transendothelial electrical resistance and increase of paracelular marker mannitol permeability. Further, we found that truncated tau treatment does induce increase of adenylate kinase in astroglia-microglia culture but had no effect on endothelial cells. These data strongly suggest that human truncated tau protein is able to change functional properties of BBB and thus contribute to cerebral microvascular changes in Alzheimer's disease.

MO08-14

NEUROPROTECTIVE ROLE OF NRF2-MEDIATED HEME OXYGENASE-1 UP-REGULATION AGAINST BETA-AMYLOID-INDUCED OXIDATIVE CELL DEATH

Lee, C. and Jang, J. H.

Department of Pathlogy, Molecular Neurotoxicology Laboratory, College of Oriental Medicine, Daegu Haany University, Daegu, South Korea

β-Amyloid (Aβ) is the major component of senile plaques and considered to have a causal role in development and progress of Alzheimer's disease. Increasing evidence supports that Aβ-induced cell death is mediated by oxidative stress. Induction of heme oxygenase-1 (HO-1), the rate-limiting enzyme in heme degration process, has been associated with adaptive survival response aginst oxidative insults, but its underlying molecular mechanisms remain largely unresolved. Treatment of SH-SY5Y cells with Aβ time-dependently increased the mRNA and protein expression of HO-1. Furthermore pretreatment of these cells with ZnPP, an inhibitor of

HO-1 activity, aggravated Aβ-induced apoptotic cell death. Aβ treatment resulted in a transient activation of upstream redox-sensitive transcription factor, NF-E2-related factor 2 (Nrf2). Conversely, knockdown of Nrf2 gene expression *in vitro* as well as *in vivo* experimental system abolished Aβ-induced HO-1 expression. N-acetylcysteine, a representative antioxidant, suppressed Aβ-caused HO-1 induction suggesting the possible involvement of reactive oxygen species in this process. In another experiment, several neuroprotective phytochemicals attenuated Aβ-induced apoptosis via up-regulation of Nrf-2-mediated HO-1 expression. Taken together, these findings suggest that HO-1 may act as a survival and protective mediator against Aβ-caused oxidative cell death.

MO08-15

HUMAN UCB-MSCS IMPROVE NEUROPATHOLOGY AND COGNITIVE IMPAIRMENT IN ALZHEIMER'S DISEASE MOUSE MODEL

Lee, H. J.[1], Lee, J. K.[2], Lee, H.[2], Jin, H. K.[2] and Bae, J. S.[1]

[1]*Department of Physiology, School of Medicine and Brain Korea 21, WCU Project*

[2]*Stem Cell Neuroplasticity Research Group, Kyungpook National University, Daegu, Korea*

Neuroinflammation of cytotoxic microglia by deposition of amyloid-β peptide (Aβ) in Alzheimer's disease (AD) has been associated with the production of proinflammatory cytokines and the process contributes to the pathogenesis of AD. Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) may have a potential therapeutic role in the treatment of neurological disorders by opposing proinflammatory Th1 and stimulating anti-inflammatory Th2 responses but, as yet, their actual therapeutic impact and mechanism of action has not been ascertained in AD. We demonstrate that whether hUCB-MSCs is capable of driving improvement of AD neuropathology and memory deficits in amyloid precursor protein (APP) and presenilin1 (PS1) double-transgenic mice. Here, we report that hUCB-MSCs transplantation into APP/PS1 mice significantly improved spatial learning and memory decline. Furthermore Aβ deposition, β-secretase 1 and tau hyperphosphorylation dramatically reduced in hUCB-MSCs transplanted APP/PS1 mice. Interestingly, these effects were associated with reversal of disease-associated defective microglial neuroinflammation, as evidenced by decreased microglia-induced proinflammatory cytokines, elevated alternatively activated microglial markers. In conclusion, we propose that hUCB-MSCs produced their sustained neuroprotective effect by inducing a feed-forward loop involving alternative activation of microglial neuroinflammation, thereby ameliorating disease pathophysiology and reversing the cognitive decline associated with Aβ deposition in AD mice.

MO08-16

BM-MSCS REDUCES AMYLOID-BETA DEPOSITION AND RESCUES MEMORY DEFICITS IN ALZHEIMER'S DISEASE MICE BY MODULATION OF IMMUNE RESPONSES

Lee, J. K.[1], Lee, H.[1], Lee, H. J.[2], Jin, H. K.[1] and Bae, J. S.[2]

[1]*Stem Cell Neuroplasticity Research Group*

[2]*Department of Physiology, School of Medicine and Brain Korea 21, WCU Project, Kyungpook National University, Daegu, Korea*

Alzheimer's disease (AD) is characterized by the deposition of amyloid-β peptide (Aβ) and the formation of neurofibrillary tangles. Transplantation of bone marrow-derived mesenchymal stem cells (BM-MSCs) has been suggested as a potential therapeutic approach to prevent various neurodegenerative disorders, including AD. However, the actual therapeutic impact of BM-MSCs and their mechanism of action in AD have not yet been ascertained. The aim of the present study was therefore to evaluate the therapeutic effect of BM-MSC transplantation on the neuropathology and memory deficits in amyloid precursor protein (APP) and presenilin 1 (PS1) double-transgenic mice. Here, we show that intra-cerebral transplantation of BM-MSCs into APP/PS1 mice significantly reduced Aβ deposition. Interestingly, these effects were associated with restoration of defective microglial function, as evidenced by increased Aβ-degrading factors, decreased inflammatory responses, and elevation of alternatively activated microglial markers. Furthermore, APP/PS1 mice treated with BM-MSCs had decreased tau hyperphosphorylation and improved cognitive function. In conclusion, BM-MSCs can modulate immune/inflammatory responses in AD mice, ameliorate their pathophysiology, and improve the cognitive decline associated with Aβ deposits. These results demonstrate that BM-MSCs are a potential new therapeutic agent for AD.

MO08-17

CURCUMIN REDUCES Aβ GENERATION BY PPARγ ACTIVATION AND BACE1 INHIBITION *IN VITRO*

Li, Y., Zhang, X. and Si, L.

Institute of Neuroscience, Chongqing, China

Alzheimer disease (AD) brain is characterized by β-amyloid (Aβ) deposits in association with glial-mediated inflammation. Aβ is generated through cleavage of the Alzheimer precursor protein APP by the β- and γ-secretase enzymes. Curcumin reduces the risk of AD and reduces microglial reactivity in AD brains, however, the molecular mechanisms by which curcumin exerts these effects are not known. Curcumin is known to bind to and activate the nuclear peroxisome proliferator-activated receptor-γ (PPARγ) receptor. This receptor inhibits the expression of proinflammatory genes and could therefore mediate the observed anti-inflammatory effects of curcumin. Recent *in vitro* data have suggested that curcumin may negatively regulate microglial activation and APP processing via PPARγ activation. We report that curcumin treatment of human SHSY5Y neuroblastoma cells increased the expression of PPARγ mRNA and protein and decreased the expression levels of the APP processing enzyme BACE1, and all the benefits were neutralized by the blocking agents of PPARγ-GW9662. Importantly, Curcumin significantly reduced the production of Aβ40/42. These findings strongly support a major role of PPARγ in the modulation of Aβ generation and suggest that the neuro-protection of curcumin in AD involves PPARγ activation and BACE1 inhibition. Curcumin is therefore of potential therapeutic interest in AD.

MO08-18

GLYCATION OF TAU PROTEIN ENHANCE OXIDANT STRESS IN DIABETIC RATS

Liu, Y., Li, F. and He, R.

State Key Laboratory of Brain and Cognitive Sciences, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Alzheimer's disease (AD) is characterized pathologically by the presence of intracellular (neurofibrillary tangles mainly consisting of

tau protein) and extracellular (senile plaques including α-synuclein) filamentous protein aggregates. Nonenzymatic glycation, a process that occurs over long times, has been proposed as a primary factor involved in this pathogenesis, leading to increased insolubility and resistance against proteolytic degradation of these proteins. This study tested the hypothesis that protein glycation results in enhanced reactive oxygen species (ROS) which promotes neuronal dysfunction. Male Sprague Dawley rats were randomized to diabeted, starved and sham-operated controls. Blood glucose, plasma malondialdehyde (MDA), NO synthase (NOS) activity, superoxide dismutase (SOD) activity, lactate dehydrogenase (LDH) activity, superoxide anion (O2-) and blood nitric oxide (NO) production were determined. High level of glucose in blood resulted in marked elevations of plasma MDA, NOS activity, LDH activity and NO production abundance, but did not change the plasma SOD activity. Moreover, the decreased level of superoxide anion radical was observed in a time dependent manner in diabetic rats rather than that of starved and sham-operated SD rats. Tau proteins were highly expressed in the brain of diabetic rats. In contrast, the level of monomeric α-synuclein expression was lower in diabetic rats as compared with control. These findings imply that oxidation/glycation is involved in the onset and/or progression of neurodegenerative diseases. Analyses of the glycosylated modifications of these proteins may offer promising therapeutic opportunities to prevent and treat neurofibullary degeneration of AD and other neurodegenerative diseases.

MO08-19

APOLIPOPROTEIN E (1-272) FRAGMENT IS ASSOCIATED WITH MITOCHONDRIAL PROTEINS AND AFFECTS MITOCHONDRIAL FUNCTION IN NEURONAL CELLS

Michikawa, M.[1], Nakamura, T.[1], Watanabe, A.[1], Hosono, T.[1] and Fujino,T.[2]

[1]*National Center for Geriatrics and Gerontology, Obu, Japan*
[2]*Ehime University, Matsuyama, Japan*

Apolipoprotein E (ApoE4) allele ε4 is a strong risk factor for developing Alzheimer's disease (AD). Secreted ApoE has a critical function in redistributing lipids among central nervous system cells maintain normal lipid homeostasis. In addition, previous reports have shown that ApoE4 is cleaved by a protease in neurons to generate ApoE4(1-272) fragment, which is associated with neurofibrillary tangle-like structures and mitochondria, causing mitochondrial dysfunction. However, it remains still unclear how the ApoE fragment associates with mitochondria and induce mitochondrial dysfunction. In order to clarify the molecular mechanism, we have carried out experiments to identify intracellular ApoE-binding molecules and their functions in modulating mitochondria function. Here we found that ApoE4 binds to ubiquinol cytochrome c reductase core protein 2 (UQCRC2) and cytochrome C1, both of which are components of mitochondrial respiratory complex III, and cytochrome c oxidase subunit 4 isoform 1 (COX IV 1), which is a component of complex IV, in Neuro-2a cells. Interestingly, these proteins associated with ApoE4(1-272) more strongly than intact ApoE4 (1-299). Further analysis showed that in Neuro-2a cells expressing ApoE4(1-272), enzymatic activities of mitochondrial respiratory complex III and IV were significantly decreased compared with Neuro-2a cells expressing ApoE4(1-299). These results suggest that C-terminal-truncated fragment of ApoE4(1-272) bind to mitochondrial complexes and affect their activities.

MO08-20

AMYLOID BETA; A PUTATIVE INTRA-SPINAL MICROTUBULE-DEPOLYMERIZER TO INDUCE SYNAPSE-LOSS OR DENTRITIC SPINE SHORTENING IN AD

Mitsuyama, F.

Fujita-Health University, Toyoake, Japan

A loss or shortening of dendritic spines has been described in patients with AD, and in mouse models for these disorders. Such alteration is thought to be responsible for cognitive deficits long before or even in the absence of neuronal loss. Recently, we have reported that dendritic shaft's microtubules ramified into spines and reached only to the stimulated postsynaptic membranes, resulting in the enlarged protrusion of dendritic spines by LTP-producing stimulation in CA1 neurons under the microtubule-preserving fixation. This means that the nucleus is connected only with simulated postsynaptic membranes by microtubules. This newly produced microtubule track only to the stimulated postsynaptic membrane might be the route of the bi-directional transportation of signals during LTP formation. This lead us the hypothesis of the 'endless memory amplifying circuit' that means gene expression-promoting molecules are translocated from postsynaptic membrane to the cell body and enter into nucleus and activate transcription factors, and gene products, which will probably promote plasticity, may be re-translocated only to the stimulated postsynaptic membrane along microtubules. From our experimental results, to maintain the newly produced microtubules in dendritic spines may be essential to form memory, and the dendritic spines may be enlarged by the pushing force of microtubules to the postsynaptic membranes as a tent pole, which are polymerized by the postsynaptic stimulation. So, our experimental results strongly suggest that amyloid beta may induce a loss or shortening of dendritic spines via depolymerization of microtubules in the dendritic spines, resulting in the disruption of the endless memory amplifying circuit, and finally cognitive deficits.

MO08-21

IRON-RELATED GENES EXPRESSED IN BRAIN

Nakamura, Y., Nakamichi, N., Takarada, T. and Yoneda, Y.

Laboratory. Mol. Pharmacol., Kanazawa Univ. Grad. Sch. Nat. Sci. Tech., Kanazawa, Japan

Iron is believed to play a dual role as an active center of the electron transfer system in mitochondria and as a cytotoxin through generation of reactive oxygen radicals in different eukaryotic cells. In recent studies, iron has been shown to be drastically accumulated in particular brain regions in patients suffering from a variety of neurodegenerative diseases such as Parkinson's disease. In this study, we have evaluated expression profiles of different genes related to iron mobilization across plasma membranes in neurons. RT-PCR analysis clearly revealed marked expression of mRNA for ferritin, transferrin (Tf) and transferrin receptor-1 (TfR1), but not for hepcidin, ferroportin or TfR2, in mouse neocortical neurons cultured for 8 days. In the neuronal cell line Neuro2A cells cultured in the presence of retinoic acid (RA), neurite outgrowth was clearly seen together with a significant increase in mRNA expression of both Tf and TfR1. On immunocytochemistry a number of cells were immunoreactive for TfR1 in Neuro2A cells treated with RA. In mouse embryonal carcinoma P19 cells endowed to proliferate for self-replication and differentiate into neurons and astroglia, exposure to RA led to neurite outgrowth toward neuronal differentiation

along with a significant increase in TfR1 mRNA expression within 8 days in culture. However, TfR1 mRNA expression was significantly decreased in response to subsequent differentiation into an astroglial lineage in P19 cells previously cultured in the presence of RA within 16 days. These results suggest that TfR1 mRNA would be highly expressed in neurons rather than astroglia in the brain.

MO08-22

REGULATION OF GENE EXPRESSION OF NEPRILYSIN, AN AMYLOID-DEGRADING ENZYME: IMPLICATION FOR ALZHEIMER'S DISEASE

Nalivaeva, N. N.[1,2], Belyaev, N. D.[1], Makova, N.[1] and Turner, A. J.[1]

[1]*Institute of Molecular & Cellular Biology, University of Leeds, Leeds, UK*

[2]*I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, RAS, St.Petersburg, Russia*

Amyloid β-peptide (Aβ) accumulation as toxic oligomers is believed to be one of the key events in the brain leading to neurodegeneration and Alzheimer's disease (AD). However, formation of Aβ is a physiologically relevant process and in the brain there are enzymic mechanisms for its removal. One of the main Aβ-degrading enzymes is a metalloprotease neprilysin (NEP), whose expression in the brain cortex significantly reduces with age and its upregulation is considered as a potential therapeutic strategy in AD. Currently considerable controversy surrounds whether the intracellular domain of the amyloid precursor protein (AICD) regulates expression of NEP. By comparing two neuroblastoma cell lines differing substantially in NEP expression and employing chromatin immunoprecipitation (ChIP), we have demonstrated that AICD is directly bound to the NEP promoter in the human neuroblastoma cells expressing high levels of NEP (NB7) but not in the cells expressing low levels of this enzyme (SH-SY5Y). The methylation status of the NEP promoter did not affect expression in both cell lines whereas the histone deacetylase inhibitors trichostatin A and valproate partially restored NEP expression and activity in SH-SY5Y. ChIP analysis also revealed AICD binding to the NEP promoter in rat primary neurons but not in HUVEC cells. A decrease of NEP expression in rat brain cortex with age seems also to be related to AICD binding to the NEP promoter. We suggest that chromatin remodelling of key AD-related genes could provide a novel therapeutic strategy. Supported by UK MRC and by RAS programme 'Fundamental Sciences to Medicine'.

MO08-23

EARLY BIOCHEMICAL CHANGES IN 5 × FAD (FAMILIAL ALZHEIMER'S DISEASE) TRANSGENIC MICE AT PRE-SYMPTOMATIC STAGE

Noh, H., Kim, W., Jeong, Y., Cha, Y. and Seo, H.

Division of Molecular & Life Science, Hanyang University, Ansan, South Korea

The 5 × FAD transgenic mice overexpress mutant human APP(695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) Familial Alzheimer's Disease (FAD) mutations and human PS1 harboring two FAD mutations, M146L and L286V. Expressions of these transgenes are regulated by neural-specific elements of the mouse Thy1 promoter to drive the specific overexpression in the brain. To determine the biochemical changes in this AD transgenic model at pre-symptomatic stages, we collected the blood from 5 × FAD mice at 4 weeks of age from orbital sinus and prepared RNA samples using Blood RNA Isolation Kit (Ambion). We performed RT-PCR using blood RNA samples with matching primers for each cytokine gene. We determined that the expression of several cytokines including NF-κB increased in mRNA levels in blood. We also detected the expression levels of amyloid precursor protein (APP) processing related genes including full-size APP, secreted APP (APPs), amyloid beta (Aβ), from blood samples. These data suggest that 5 × FAD mice express the increased levels of mRNA of several cytokines and APP processing related products in blood. This study will provide the opportunity of the future application for early diagnosis of AD at pre-symptomatic stage.

MO08-24

INCREASED EXPRESSION OF SPLA2-IIA IN HIPPOCAMPUS OF APP TRANSGENIC RATS

Sheng, W., Agca, C., Agca, Y., He, Y., Cui, J., Sun, A. Y., Simonyi, A. and Sun, G. Y.

University of Missouri, Columbia, MO, USA

Increased production of amyloid beta peptide from aberrant amyloid precursor protein (APP) has been regarded as an important basis in the pathogenesis of Familial Alzheimer's disease (FAD). Although a number of transgenic mouse models overexpressing human mutant APP have been made available for AD research, development and characterization of rat models are still lacking. Our laboratory is interested in type IIA secretory phospholipases A2 (sPLA2-IIA), an important inflammatory protein in humans, but its expression in many mouse strains is lacking. Upregulation of sPLA2-IIA mRNA has been reported in AD brain as compared with age-matched non-dementia controls (Moses et al., J. Neuroinflammation, 2006). Recently, Fisher 344 rat lines overexpressing human APPsw/Ind driven by the ubiquitin-C promoter were generated via lentiviral vector (Agca et al., BMC Neuroscience, 2008). The APP21 rat line expressed 7.5 times more APP mRNA as compared to wild type (WT) rats. Furthermore, human APP was expressed exclusively in neurons but not in astrocytes. In this study, we examined sPLA2-IIA protein in cerebral cortex, hippocampus and cerebellum in adult APP Tg rats and age-matched WT controls. In agreement with previous reports, WT rats showed constitutive expression of sPLA2-IIA protein in the cerebellum which was higher than those in cortex and hippocampus. In the Tg rats, levels of sPLA2-IIA protein expression in the cortex and cerebellum were similar to the WT controls, but levels of sPLA2-IIA in the hippocampus were higher than those in the WT controls. Results show for the first time a link between APP overexpression in neurons with the inflammatory protein sPLA2-IIA, and suggest possible use of this Tg rat model to unravel the role of APP and inflammatory responses.

MO08-25

CALCIUM-INDUCED PROTEOLYTIC CLEAVAGE OF RAGE BY MATRIX METALLOPROTEINASE

Son, S.[1], Lee, S.[2] and Mook-Jung, I.[1]

[1]*Department of Biochemistry and Biomedical Sciences, Seoul National University*

[2]*Department of Biochemistry, College of Medicine, Yeonsei University, Seoul, Korea*

The receptor for advanced glycation end products (RAGE), a member of the immunoglobulin superfamily of cell surface

molecules, is expressed ubiquitously in the brain. As multiligand receptor, RAGE interacts with several known ligands including amyloid β peptide (Aβ). Because RAGE expression is elevated in the brains of Alzheimer's disease (AD) patients and RAGE is transporter of Aβ from the periphery to the brain, RAGE is believed to play an important role in AD pathogenesis. Three isoforms of RAGE such as full length (fRAGE), truncated form (dnRAGE) and secreted form of RAGE (sRAGE) are generated by alternative splicing in the body. Since sRAGE shares the ligand binding site with fRAGE, it works as an endogenous competitor for fRAGE, suggesting the positive effect of sRAGE by blockade of fRAGE activation. In this study, we found sRAGE is generated by proteolytic cleavage under dysregulation of intracellular calcium homeostasis. MMP-9, not MMP-2, is activated by calcium ionophore, followed by more sRAGE is generated. When BAPTA-AM or EGTA, both calcium chelators, is applied to the cells, sRAGE generation is decreased. Also, MMP-9 directly cleaves fRAGE to generate sRAGE *in vitro*. siRNA of MMP-9 treatment markedly reduces sRAGE production in cells. Taken together, we demonstrate that MMP-9 acts as a RAGE sheddase to generate sRAGE, suggesting that sRAGE generation can be regulated.

MO08-26

LOSS OF PRESENILIN IS ASSOCIATED WITH PML EXPRESSION AND INVULNERABLE TO DNA DAMAGE INDUCED CELL DEATH

Song, H., Boo, J. and Mook-Jung, I.

Department of Biochemistry and Biomedical Sciences, College of Medicine, Seoul National University, Seoul, Korea

Presenilin (PS) is a main component of γ-secretase complex to generate Aβ peptide that is known as a causal factor for pathogenesis of Alzheimer's disease. Even though PS function is well studied in the view of γ-secretase component, PS has non-proteolytic functions associated with apoptosis, calcium homeostasis and wnt signaling. Promyelocytic leukaemia (PML) has macromolecular multiprotein complexes called PML nuclear-bodies (PML-NBs). PML NBs in response to DNA damage, senescence and apoptosis recruit numerous proteins including CBP, p53 and HIPK2. In this study, we investigated the effect of PS on PML and cell death during campthothecin (CPT)-induced apoptosis. Under CPT-induced DNA damage condition, PS knock-out MEFs showed aberrant decrease of PML, PML-NB formation and Cell death. Reconstruction of PS1 in PS knockout MEFs rescued CPT-induced cell death and PML expression. In addition, these processes were regulated in PML transcription level in a p53 dependent manner. These results indicate that PS may regulate PML expression under DNA damage condition.

MO08-27

GENETIC ASSOCIATION OF UBE2I WITH ALZHEIMER'S DISEASES

Song, J. H., Ahn, K., Jo, S. A. and Koh, Y. H.

Biomedical Sciences, Division of Brain Diseases, National Institute of Health, Seoul, Korea

Alzheimer's disease (AD) is the most common type of dementia characterized by a progressive loss of cognitive function. Genetic variability may affect AD progression. Ubiquitin-conjugating enzyme E2I (UBE2I) encodes a protein that conjugates small ubiquitin-related modifier (SUMO) to target proteins resulting in a change of their localization, activity or stability. The previous studies exploring the importance of UBE2I in AD were based on the sumoylation of APP or tau proteins. To investigate the association between genetic variations of UBE2I and AD, we genotyped 5 common SNPs in UBE2I gene located on chromosome 16p13.3. DNA samples of 312 Korean AD patients, 489 controls and 347 mild cognitive impairment (MCI) subjects were analyzed. Average age of participants was 70.9 _ 6.4 years and 58.5 % was female. The cognitive functions and memory impairment of subjects were examined using CERAD neuropsychological battery. All analyses were adjusted for age, sex, the presence of APOE-ε4 allele and duration of education. SAS 9.1 program was used for the statistical analysis. For all subjects, the AG + AA genotype frequency of an SNP (G > A) in intron 6 was higher in AD patients than in the controls (OR = 1.448, 95% CI: 1.007-2.082, p=0.046). For men, A allele of an SNP in intron 3 (C > A) showed protective effect against AD (OR = 0.415, 95% CI: 0.179–0.961, p=0.040). For women, GA genotype of an Exon 7 SNP (G>A) was associated with MCI (OR = 2.757, 95% CI: 1.117–6.804, P = 0.028). There was no significant difference between AD and MCI groups. Stratification by the presence/absence of ApoE-ε4 allele gave no significant difference among groups. These SNPs may not create a functional change by themselves, but they might affect the risk for AD in combination with other unknown UBE2I or SUMO polymorphisms.

MO08-28

ALZHEIMER'S Aβ1-42 PEPTIDE INTERACTION WITH α1-ANTICHYMOTRYPSIN *IN VITRO*

Sun, Y. X.[1], Janciauskiene, S.[2] and Jia, J.[1]

[1]*Department of Neurology, Xuan Wu Hospital of Capital Medical University, Beijing, China*

[2]*Department of Medicine, University Hospital of Malmo, Lund University, Malmo, Sweden*

Objective: To characterize the kinetic equilibrium reaction between Aβ1-42 and α1-antichymotrypsin (ACT) *in vitro*, and its role in the pathogenesis of Alzheimer's disease (AD).

Methods: Aβ1-42 and ACT were dissolved in Tris buffer solution at a molar ratio of 10:1 and incubated at 37_C for 7 hours to make Aβ1-42/ACT complex. The kinetics of formation and breakdown of this complex were determined from SDS-PAGE, ACT inhibition assays and fluorescence measurements using fluorescein-labelled Aβ1-42 (fAβ1-42).

Results: ACT and Aβ1-42 formed an SDS-labile complex after 7 h incubation, which was distinct from the stable complex after long-time incubation (72 hours). After interaction with Aβ1-42, the protease inhibitor activity of ACT was diminished about ten times compared with ACT alone (SI=1.1 vs SI=10.5), and to some extent, ACT activity decreased with increasing concentrations of Aβ1-42. From fluorescence measurements of fAβ1-42/ACT, a dissociation constant KD was calculated (8.75μM), and chymotrypsin addition resulted in nearly 100 times acceleration of Aβ1-42 dissociation.

Conclusion: The complex formation between Aβ1-42 and ACT is a long-term, kinetic interaction model. ACT is an acute phase protein abundant in amyloid deposits in Alzheimer's brains. Upon complex formation with Aβ1-42, ACT loses its effect on inhibiting proteases and Aβ1-42 deposition was affected, this might be a mechanism for AD pathogenesis.

MO08-29

RESVERATROL AND NITRIC OXIDE CHOLINERGIC NEUROTOXICITY

Szutowicz, A., Gul-Hinc, S., Bielarczyk, H. and Jankowska-Kulawy, A.

Department of Laboratory Medicine, Medical University of Gdansk, Gdansk, PL

Resveratrol (RV) a red grape-derived antioxidant is claimed to be efficient in preventing free-radical evoked brain lesions in Alzheimer's dementia (AD) and other cholinergic encephalopathies, through the protection of mitochondrial energy metabolism in the neurons. NO excess is one of recognized pathogens in AD brains. Therefore, we investigated whether RV might overcome cytotoxic effects of NO excess on septal murine cholinergic neuroblastoma SN56 cells. One day exposure of nondifferentiated SN56 cells (NC) to 0.2 mM sodium nitroprusside (SNP) caused 30 and 27% suppression pyruvate dehydrogenase (PDH) activity and acetyl-CoA level as well as the increase of nonviable cells fraction to 30%. In cAMP/retinoic acid-differentiated cells (DC), similar 30% inhibition of PDH by SNP caused 50% suppression of acetyl-CoA content and 47% loss of cell viability. RV (0.005 mM) itself affected none of parameters, neither in NC nor in DC. It reversed neither SNP-evoked inhibition of PDH nor suppression of acetyl-CoA content in NC and DC. Despite of that RV partially prevented SNP-induced increase of DC and NC mortality. Greater cytotoxicity of SNP in DC than in NC correlated with deeper acetyl-CoA deficits in the former. These shortages of acetyl-CoA might be linked with higher rates of acetylcholine metabolism in DC. These data indicate that protective effects of RV in NO-challenged cholinergic cells were not connected with alterations of their acetyl-CoA metabolism. Supported by MNiSW projects NN401 2333 33, P05A 11030 and GUM projects St57 and W-20.

MO08-30

EFFECTS OF AMYLOID-β PEPTIDE ON GLUTAMINE TRANSPORTER MRNA EXPRESSION AND CELL VIABILITY IN CULTURED RAT CORTICAL CELLS

Thangnipon, W.[1], Buntup, D.[1], Chayasadom, A.[2], Surarit, R.[3] and Jutapakdeegul,N.[1]

[1]*Neuro-Behavioural Biology Center, Institute of Science and Technology for Research and Development, Mahidol University, Nakorn Pathom*

[2]*Department of Periodontology, Faculty of Dentistry, Khon Kaen University, Khon Kaen*

[3]*Department of Physiology and Biochemistry, Faculty of Dentistry, Mahidol University, Bangkok, Tailand*

Alzheimer's disease (AD) is a major neurodegenerative disorder in which there is an overproduction and accumulation of amyloid-β (Aβ) peptides. In the initial stages of AD, glutamate receptors are dysregulated by Aβ accumulation resulting in the disruption of glutamatergic synaptic transmission. We examined in rat cortical cell cultures the effects of Aβ(25–35)-induced neurotoxicity on glutamine transporters involved in the glutamate cycle. In primary mixed cell cultures prepared from the cerebral cortex, incubation with 10 μM Aβ(25–35) for 12 h markedly suppressed system A transporter 1 (SAT1) mRNA expression but had no effect at 24 h. On the other hand, Aβ(25–35) had no effect on SAT1 mRNA level in neuronal cell cultures. Treatment of both types of cell cultures with Aβ (25–35) resulted in a significant decrease in cell survival in a concentration- and time-dependent manner as determined by MTT assay. These results indicated that Aβ may impair neuronal function and transmitter synthesis, and perhaps reduce excitotoxicity, through a reduction in neuronal glutamine uptake.

MO08-31

NEUREGULIN-1 SHOWS NEUROPROTECTIVE EFFECTS IN *IN VITRO* ALZHEIMER'S DISEASE MODELS

Woo, R. S., Lee, J. H., Yu, H. N., Oh, K. M., Song, D. Y., Baek, C. H. and Baik, T. K.

Department of Anatomy & Neurosciences, Eulji University College of Medicine, Daejeon, South Korea

Neuregulin-1 (NRG1) plays important roles in the development and plasticity of the brain, and it has been shown to potent neuroprotective properties. Although there are various data, little is known about its role in Alzheimer's disease. In this study, we investigated the therapeutic potential for Alzheimer's disease of NRG1. We found that NRG1 attenuates the neurotoxicities induced by amyloid β peptide (Aβ)1-42 or Aβ1-40 treatment and the expression of a Swedish double mutation (KM595/596NL) of amyloid precursor protein (Swe-APP) or the C-terminal fragments of APP(APP-CTs) in neuronal cells. We also show that NRG1 decrease in production of reactive oxygen species (ROS) and and attenuates mitochondrial membrane potential loss induced by Aβ1-42, Swe-APP, or APP-CTs in the neuronal cells. In addition, NRG1 down-regulates expression of the pro-apoptotic protein Bax. This effect was blocked by inhibition of ErbB4, suggesting the involvement of ErbB4, a key NRG1 receptor. Together, these results demonstrate the efficacy of NRG1 in *in vitro* Alzheimer's disease model. Our findings further indicate that NRG1 could be used as a therapeutic agent in Alzheimer's disease.

MO08-32

NEUROPROTECTIVE EFFECT OF AN ANTI-ISCHEMIC HERB ON BETA-AMYLOID PROTEIN(1-42)-INDUCED NEUROTOXICITY IN HIPPOCAMPAL NEURONS

Xiaojing, L. I.[1,2], Qian, H.[1] and Pengtao, L.[1]

[1]*Beijing University of Chinese Traditional Medicine*

[2]*State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Science, Beijing, China*

Neuropathologic studies suggest an increasingly association between cerebral blood flow (CBF) and Alzheimer disease (AD) in recent years. After acute therapy, neuronal loss and amyloid plaques increases the risk of neurodegeneration, but the increased risk does not negative the overall benefit of this therapy. To investigate the effects of an Anti-Ischemic Herb (AIH), an extract containing ginsenoside Rg1 and geniposide and being clinically used for ischemia stroke treatment in the brain, on neuronal damage induced by Abeta and potential mechanisms of the effects, Prediction of Activity Spectra for Substances (PASS) and biochemical analysis combining primary cultured hippocampal neurons were adopted. Neurons that were treated with Abeta1-42 (10 microM) were shrunken perikaryon with loss of neurite processes; the survival rate of neurons decreased almost to 50% ($P < 0.01$). Lactate dehydrogenase (LDH) release increaded, neurite outgrowth and MAP2 (Microtubuln Associate Protein-2) activity level all decreased obviously ($P < 0.01$ or $P < 0.05$). However, neurons pre-treated with AIH (1 and 10 microM) had a survival rate increase compared

with neurons treated with Abeta alone; LDH release decreased distinctly, and the increase in neurite outgrowth and MAP2 activity in Abeta-treated neurons was attenuated evidently ($P < 0.01$ or $P < 0.05$). Thus, we conclude that AIH exerted neuroprotection obviously. AIH protected neurons against the toxicity of Abeta, most likely through an neurotrophic pathway. AIH could provide insight into the mechanism of clinical efficacy of neuroprotective agents of AD.

MO08-33

A BIOMARKER CANDIDATE FOR ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT

Yun, S. M.[1], Jang, B. G.[1], Ahn, K.[1], Chae, S.[1], Kim, D. K.[2], Park, M. H.[3], Jo, S. A.[1] and Koh, Y. H.[1]

[1]*Department of Biomedical Sciences, National Institute of Health*
[2]*Sungkyunkwan University School of Medicine, Seoul*
[3]*Department of Neurology, Korea University Medical College, Ansan-si, Korea*

An early and accurate diagnosis of Alzheimer's disease (AD) is important in order to initiate treatment. Combining memory testing with biomarker measurements will help diagnosing AD earlier and with greater accuracy. In an effort to find biological indicators for AD, we screened the blood plasma samples with various antibodies. Previous studies reported that carbonic anhydrase II (CA-2) were found to be increased in AD brain hippocampus. The present study was focused on the determination of CA-2 protein change in AD blood plasma. We compared 115 AD patients (av. age 74.9 years), 83 persons with amnestic mild cognitive impairment (MCI) (av. age 73.5 years), and 185 cognitively healthy controls (av. Age 69.3 years), using immunoblots, ELISA and dot blot methods. We measured the protein amount of CA-2 by ELISA using an anti-CA-2 antibody and found that the plasma level of CA-2 was significantly higher in AD patients than in control or amnestic MCI groups. The CA-2 level was higher in males than females but the difference was not statistically significant. There was an age-dependent increase of CA-2. These results show that the elevated plasma CA-2 concentration might be a risk factor for AD. Possible usefulness as an AD biomarker and the molecular connection between biological function of CA-2 and pathogenesis of AD is being investigated.