Poster Session 3A
Neuroprotection

PS3A-01

The neuroprotective effect of insulin involves activation of membrane-bound Ras and downstream antiapoptotic pathway through the association of GRF-SOS adaptor protein

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Insulin has been known to play key roles in a variety of important biological functions, however the functions of insulin in the CNS have largely remained unclear. Interruption of insulin production and/or insulin receptor's (IR) activity causes deficits in learning and memory formation, which seems to be one of the etiological factor of diabetic encephalopathy and Alzheimer's dementia. It has been shown, that insulin causes activation of a series of signal transduction pathways, following hormone binding to IR of neural cells. We have studied the mechanism of insulin's neuroprotective effect on primary neuronal cells and streptozotocin (STZ)-induced diabetic brains. It has been found, that insulin increase the content of membrane-bound Ras-protein ($p21^{Ras}$} and respectively amplify the activation of Akt-kinase antiapoptotic signal pathway, that results in enhancing electron transport in mitochondria and changes in transcription activity of the cell. Furthermore, the activating effect of insulin mediated by the association of GRF-SOS adaptor protein to the synaptic membrane, since the amount of this protein is increased by addition insulin to the primary neural cells and is minimal in the synaptic membranes of STZ-induced diabetic rat brains. It is concluded that insulin-induced association of GRF-SOS adaptor protein to the synaptic membrane cause activation of membrane-bound Ras and switch antiapoptotic processes through Akt pathways.

Acknowledgment: This work supported by INTAS 2001-0666 grant.

PS3A-02

Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives

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Gallic acid and its derivatives are a group of naturally occurring polyphenol antioxidants which have recently been shown to have potential healthy effects. In order to understand the relationship between the structures of gallic acid derivatives and their antioxidant activities and neuroprotective effects, we examined their free radical scavenging effects in liposome and anti-apoptotic activities in human SH-SY5Y cell induced by 6-hydroxydopamine autooxidation. It was found that these polyphenol antioxidants exhibited different hydrophobicity and could cross the liposome membrane to react with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical in a time and dose dependent manner. At the same time, the structure–antioxidant activity relationship of gallic acid derivatives on scavenging DPPH free radical in the liposome was also analyzed based on theoretical investigations. Analysis of cell apoptosis, intracellular GSH levels, production of ROS and the influx of Ca^{2+} indicated that the protective effects of gallic acid derivatives in cell systems under oxidative stress depend on both their antioxidant capacities and hydrophobicity. However, the neuroprotective effects of gallic acid derivatives seem to depend more on their molecular polarities rather than antioxidant activities in the human SH-SY5Y cell line. In conclusion, these results reveal that compounds with high antioxidant activity and appropriate hydrophobicity are generally more effective in preventing injury of oxidative stress in neurodegenerative diseases.

PS3A-03

Possible neuroprotective mechanism of resveratrol on 3- nitropropionic acid-induced neurotoxicity, an animal model of Huntington's disease

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Huntington's disease is neurodegenerative disease occurring due to destruction of neurons in the basal ganglia. 3-nitropropionic acid (3-NP), a complex II inhibitor of the electron transport chain, causes Huntington's disease like symptoms. Recently it has been reported that oxidative stress are involved in the pathogenesis of HD. The present study was designed to investigate effects of resveratrol, in 3-NP-induced cognitive impairment and oxidative stress in rats. Intraperitoneal administration of 3-NP (20 mg/kg for 4 days) showed loss in body weight, declined motor function (locomotor activity, movement pattern and vacuous chewing movements) and poor retention of memory. Resveratrol is well known antioxidant with COX-I inhibiting property. Chronic treatment with resveratrol (5 and 10 mg/kg, p.o.) once daily for a period of 8 days beginning 4 days prior to 3-NP administration significantly improved the 3-NP-induced motor and cognitive impairment. Biochemical analysis revealed that systemic 3-NP administration significantly increase lipid peroxidation, nitrite levels, and depleted reduced glutathione levels and reduced succinate dehydrogenase activity in the rat brain. The results of the present study clearly indicate that resveratrol (5 and 10 mg/kg, p.o.) by its antioxidant activity showed neuroprotection against 3-NP-induced motor and cognitive impairment and associated oxidative stress.

PS3A-04

Protective effect of naproxen (non-selective COX-inhibitor) or rofecoxib (selective COX-2 inhibitor) on immobilization stress-induced behavioral and biochemical alterations in mice

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Chronic stress precipitates many neuropsychiatric disorders and alters the various oxidative stress parameters in brain. Cyclooxygenase (COX) is reported to play an important role in pathogenesis of various neurodegenerative disorders including stroke and seizures. In the present study, we examined the effect of naproxen (non selective COX-inhibitor having much potency towards COX-I isoform) or rofecoxib (a selective COX-2 inhibitor) in subchronic Immobilization stress. Mice were subjected to immobilized stress for 6 h daily for a period of 7 days. Naproxen (7 mg/kg, i.p.) or rofecoxib (2 mg/kg, i.p.) was administered daily for 7 days before challenging them to immobilization stress. Behavioral analysis revealed the hyperlocomotor activity and increased anxiety response. Subchronic stress decreased percent retention of memory and also caused hyperalgesia in mice. Biochemical analysis revealed that chronic Immobilization stress significantly increased lipid peroxidation and nitrite levels and decreased the reduced glutathione and adrenal ascorbic acid levels. Chronic treatment with naproxen or rofecoxib significantly attenuated the immobilization stress-induced behavioral and biochemical alterations. These results suggested that the use of COX-inhibitors (naproxen or rofecoxib) could be a useful neuroprotective strategy in the treatment of stress.

PS3A-05

Dietary restriction alters the expression of glial fibrillary acidic protein (GFAP) in kainic acid induced model of temporal lobe epilepsy in adult wistar rats

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Responses to neurodegeneration are complex, involving activation of microglia, astrocytes and synaptic remodeling. Reactive astrocytosis occurs prominently in response to all forms of CNS injury or disease. The kainic acid induced temporal lobe epilepsy is a well-characterized and established model to study the neurodegeneration. Recent studies have shown that dietary restriction (DR) can have profound effect on brain function and vulnerability to injury and disease and can also enhance synaptic plasticity, which may increase the ability of brain to resist aging and restore function following injury. We examined the effect of 3 months of DR (alternate day feeding regimen) on the expression of glial fibrillary acidic protein (GFAP) by immunoblotting and immunohistochemistry after kainate-induced temporal lobe epilepsy in adult wistar rats. Kainic acid administration significantly increased the expression of GFAP in ad libitum (AL) fed rats but DR significantly decreased the expression of GFAP in kainic acid treated rats, particularly in the cerebral hemisphere and diencephalon regions of the brain as revealed by immunoblotting experiments. These results were further confirmed by immunohistochemistry of GFAP in the rat brain. Kainic acid administration significantly enhanced the expression of GFAP as revealed by reactive astrogliosis in the brain areas particularly dentate gyrus of the hippocampus region, amygdaloid hippocampal region and hypothalamus, whereas DR was found to modulate the effect of kainic acid significantly. Dietary restriction was also observed to exert neuroprotection by markedly reducing neuronal cell death in the CA3 region of hippocampus after kainate administration as conformed by hematoxylin-eosin and Hoechst staining.

PS3A-06

Effect of $APP_{319–335}$ on the expression of β-amyloid associated protein in APP V717I transgenic mice

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Background: Alzheimer's disease is a kind of primary neurodegenerative disease that attacks on age. More and more researches indicate that the developing, metabolizing and the toxic of β-amyloid (Aβ) play an important role in the development of AD, although the mechanism of AD is not unknown. Amyloid protein precursor 319–335 ($APP_{319–335}$), the 17-mer peptide, has several pharmacology effects on the different mechanisms of AD. It activates the channel of signal transduction by insulin receptor substrate 1 (IRS-1), distinctly ameliorated learning and memory function and changed the neuron ultrastructure of the hippocampus in several kinds of dementia models.

Objective: To investigate the effect of $APP_{319–335}$ on the expression of Aβ associated protein in neurons of hippocampus in APP V717I transgenic mice.

Methods: The transgenic mice were randomly divided into model group and $APP_{319–335}$ treatment group. In the last group, mice were injected $APP_{319–335}$ 0.16 mg/kg three times a week subcutaneously. C57BL/6J mice of the same age and background were used as controls. 7 month later, the water maze test was conducted; then cryostat section of the brain were studied by immunohistochemistry for $Aβ_{1–42}$, $Aβ_{1–16}$, APP-N fragments, presenilin-1(PS-1) C fragments and β-secretase (BACE).

Results: (i). Full-course swimming time was obviously longer in model group than those in treatment group and control group. In the use of $APP_{319–335}$, the results were similar to Group C. (ii). In model group, the expression of $Aβ_{1–42}$, $Aβ_{1–16}$, APP-N fragments, PS-1 C fragments and BACE were increased. Treatment group can normalize expression of proteins as above.

Conclusion: There were high Aβ levels in the brain of APP transgenic mice. $APP_{319–335}$ can reduce the levels of Aβ by restrain the expression of BACE and PS-1; therefore, it may improve learning ability and memory.

PS3A-07

Histo-pathological effect of alcohol and stress on hippocampal region of female albino rat

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Hippocampus is the region involved in integrative brain function and certain aspects of learning and memory. Study was done to investigate effects of alcohol, stress and their synergistic action on hippocampal region of female albino rat in control, stress and alcoholic conditions using behavioral, and histological parameters.

The neuroprotectiveness of Celastrus paniculatus was also studied under the above defined conditions. Histological changes in hippocampal region of female albino rat were observed, when an animal is subjected to different conditions and treatment under synergistic action of stress and alcohol. Neural degeneration of hippocampal regions CA1, CA2, CA3, CA4, and dentate gyrus (DG) were analyzed by histological studies under a light microscope. Immobilization stress given to female albino rat over a period of 30 days caused neural degeneration in CA3, CA2, CA1 and CA4 regions respectively in decreasing order. Chronic alcohol administration (3 g/Kg of body weight) caused maximum neural degeneration in CA1 hippocampal subregion expressed as dark cell with irregular shaped perikarya showing apoptotic bodies, while synergistic action affected CA3 and CA4 subregion besides CA1, CA2 and DG. All above described changes were revealed by histological slides with normal thionin staining. Each treatment leading to neural degeneration was correlated with behavioral changes with histopathology and hippocampal status of animal. Neuroprotectiveness of Celastrus paniculatus was observed in CA3 region of female albino rat which were subjected to synergistic action of stress and alcohol.

PS3A-08

Neuroprotection by L-deprenyl against oxidative stress-mediated dopaminergic neurodegeneration induced by rotenone in rats

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Parkinson's disease (PD) is a late-onset, progressive neurological disorder characterized by selective nigrostriatal dopaminergic degeneration in A_9 substantia nigra pars compacta (SNpc) neurons. The present study investigated oxidative damage and neuroprotective effect of the antiparkinsonian drug, L-deprenyl in neuronal death produced by intranigral infusion of a potent mitochondrial complex-I inhibitor, rotenone in rats. Unilateral stereotaxic intranigral infusion of rotenone caused significant decrease of striatal dopamine levels as measured employing HPLC-electrochemistry, and loss of tyrosine hydroxylase immunoreactivity in the perikarya of ipsi-lateral substantia nigra (SN) neurons and their terminals in the striatum. Rotenone-induced increases in the salicylate hydroxylation products, 2,3- and 2,5-dihydroxybenzoic acid, indicators of hydroxyl radials in mitochondrial P_2 fraction were dose-dependently attenuated by L-deprenyl. L-Deprenyl (0.1–10 mg/kg; i.p.) treatment dose-dependently attenuated rotenone-induced reductions in complex-I activity and glutathione (GSH) levels in the SN, tyrosine hydroxylase immunoreactivity in the striatum or SN as well as striatal dopamine. Amphetamine-induced stereotypic rotations in these rats were also significantly inhibited in deprenyl-administered animals. The rotenone-induced elevated activities of cytosolic antioxidant enzymes superoxide dismutase and catalase showed further significant increase following L-deprenyl. Our findings suggest that unilateral intranigral infusion of rotenone reproduces neurochemical, neuropathological and behavioural features of PD in rats and L-deprenyl can rescue the dopaminergic neurons from rotenone-mediated neurodegeneration in them. These results not only establish oxidative stress as one of the major causative factors underlying dopaminergic neurodegeneration as observed in Parkinson's disease, but also support the view that deprenyl is a potent free radical scavenger and an antioxidant.

PS3A-09

Post-treatment with 17β-estradiol following traumatic brain injury reduces neuronal degeneration in rat

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The neuroprotective effects of17β-estradiol, post-treatment after traumatic brain injury (TBI), on neuronal degeneration in cerebral cortex (ctx) and hippocampus (CA2-3) in female rats were examined. Eighteen female Sprague-Dawley rats were injured by lateral fluid percussion. Immediately after injury, animals were administered a single intraperitoneal injection of 1 mg/kg 17β-estradiol (E2-1 mg/kg), 4 mg/kg 17β-estradiol (E2-4 mg/kg) or equal volume distill water vehicle (dH_2O) (n = 6/group). At 24 h after injury, animals were euthanized and brains were stained by Fluoro-Jade (FJ) and TUNEL to label degenerating neurons and apoptotic cells, respectively. Numbers of positive cells were observed and stereologically quantified. E2-1 mg/kg and E2-4 mg/kg groups showed lower numbers of FJ- and TUNEL-positives than dH_2O group in ctx ($P < 0.05$), but this effect was not seen in CA2-3 of hippocampus. These results demonstrated that post-treatment with exogenous 17β-estradiol after TBI can provide neuroprotection against degeneration and apoptosis in core injury area (ctx). Both exogenous and cycling endogenous 17β-estradiol showed similar protective potential against delay degeneration in hippocampus (CA2-3).

PS3A-10

The inhibitory effect of melatonin on endothelial nitric oxide synthase in bovine cerebral arteries

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Melatonin plays role in a variety of physiological responses include free radical scavenger and also broad-spectrum antioxidant. Recently melatonin has been shown to constrict and potentiate contractile response to adrenergic stimulation of cerebral arteries via melatonin receptors. However, the distribution of specific melatonin receptors throughout the cerebral vasculature has not been demonstrated. In the present study, we have attempted to identify melatonin receptor mRNAs of bovine cerebral arteries by using RT-PCR, semi-quantitative RT-PCR and radioligand binding assays, respectively. In addition, the existence of melatonin receptors and the function of melatonin on endothelial nitric oxide synthase (eNOS) expression has also been demonstrated. The results indicated that mt_1A melatonin receptor mRNA was expressed in bovine cerebral arteries. The data showed the highest level of expression occurred in the middle and the lowest level occurred in the vertebral cerebral artery ($P < 0.001$). The melatonin receptor

subtypes in different regions of cerebral arteries were identified and characterized by using selective 2-[125I] iodomelatonin binding. The arterial 2-[125I] iodomelatonin binding site was saturable. Saturation studies revealed that the binding represented a single site of high affinity binding for the melatonin receptor and show relative order of binding capacities (Bmax) in the area of the middle > posterior > anterior > vertebral cerebral artery (20.932 ± 0.435, 18.448 ± 0.383, 17.715 ± 0.409, and 14.02 ± 0.46) fmol/mg protein, and the dissociation constant values (Kd) were (36.02 ± 0.064, 33.15 ± 0.022, 31.71 ± 0.085, and 26.39 ± 0.086) pM, respectively. In addition, hydrogen peroxide induced induction in eNOS protein level has been demonstrated in the bovine isolated cerebral arteries and the said effect was abolished by melatonin. This is the first evidence showing the expression of mt_1A melatonin receptor and its function in the bovine cerebral arteries. However, further studies are necessary to understand the significant role of melatonin and its receptors in regulating physiological function.

Acknowledgement: Thailand Research Fund.

PS3A-11

Gas6: a novel survival factor for human and murine oligodendrocytes

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Oligodendrocytes, express the Axl, Mer and Rse family of receptor tyrosine kinases that are activated by the ligand, growth arrest-specific protein 6 (gas6). We examined whether rhgas6 (5.6 nM or 400 ng/ml) protects oligodendrocytes when challenged with insulin withdrawal or tumor necrosis factor-αTNFα cytotoxicity. TNFα is one of the major secreted cytokines associated with white matter injury in multiple sclerosis and periventricular leukomalacia. Oligodendrocyte viability was assessed by O4 and TUNEL staining. The presence of rhgas6 in the medium protected against insulin withdrawal with greater than 60–75% oligodendrocyte survival observed in the cultures with lower insulin concentrations (25–100 ng/ml), demonstrating a survival effect of rhgas6 independent of insulin/IGFR. In cultures treated with TNFα (100 ng/ml) the oligodendrocyte survival rate was 18%, compared with 65% and 63% in cultures treated with the caspase inhibitors IETD-fmk and zVAD-fmk respectively. Oligodendrocyte cultures treated with TNFα and rhgas6 had a survival rate of 64% and reduced active caspase-3 immunoreactivity relative to TNFα-only treated cultures. To determine whether rhgas6 signaling protects against TNFα-induced toxicity via the PI3 kinase/Akt pathway we examined whether the addition of TNFα alone, or rhgas6 and TNFα would activate the prosurvival kinase Akt. A significant increase in phosphoAkt (Ser473) immunoreactivity was detected 15 min after gas6 administration to TNFα-treated oligodendrocytes, but not in gas6-untreated, TNF(-treated cultures. The gas6 protective effect was abrogated by the Axl decoy receptor Axl-Fc, and by the PI3 kinase/Akt inhibitor LY294002. Oligodendrocyte cultures established from wildtype and Rse-/- mice, but not from Axl-/- mice, were protected from TNFα-induced cell death when maintained in rhgas6 suggesting that rhgas6 enhances oligodendrocyte survival only in the presence of the Axl receptor. We conclude that rhgas6 potently inhibits TNFα-induced oligodendrocyte apoptosis and identify the gas6/Axl/PI3 kinase/Akt signaling pathway as an important mediator of oligodendrocyte survival following cellular stress and cytokine challenge.

PS3A-12

NMDA receptor-mediated neuroprotection against cyclosporinA toxicity: regulation of polysialyltransferases expression

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Immunophilins are members of a highly conserved family of proteins all of which have cis-trans peptidyl-prolylisomerase (PPI) enzymatic activity that is involved in protein folding processes. CyclosporinA is used to treat patients with immune-mediated diseases, chronic diseases requiring organ transplantation, or malignancies. These conditions often require higher cyclosporinA doses. NMDA receptors exhibit a dichotomy of signaling with excessive stimulation leading to neuronal damage that occurs during neurodegenerative disorders, whereas the normal burst of activity results in plastic responses with the expression of molecular substrates of long-term plasticity, growth and survival. Treatment with a subtoxic concentration of NMDA protects all of the vulnerable neurons. We studied the effect of subtoxic dose of NMDA (100 mg/kg bw) *in vivo* in mediating neuroprotection against toxic dose of CyclosporinA (50 mg/kg bw) by studying the expression of antioxidant enzymes in different regions of brain (cereberal hemispheres, cerebellum, diencephelon and brain stem). Further to characterize the neuroplastic changes in response to subtoxic dose of NMDA we studied the polysialyltransferse (PST) mRNA expression. The various enzyme activities were significantly higher in the diencephelon region in NMDA pretreated rats exposed to toxic doses of cyclosporinA. Similar increased expression of PST in this region points to the ability of diencephelon region to maintain neuroplastic conditions. Thus understanding the regulation of antioxidant enzymes and PST mRNA expression by NMDA receptor-mediated activity may help in delineating the mechanisms of survival pathways that exist in neurons will provide important insight into how neurons utilize intracellular proteins as neuroprotectants against the causes of acute neurodegeneration.

PS3A-13

Dual modality neuroprotection using non-selective poly (ADP-ribose) polymerase (PARP) and pancaspase inhibitors following experimental stroke in rats; is there additional benefit?

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Energy requiring apoptosis and presumably unregulated necrosis are considered conceptually and morphologically distinct forms of cell death which have been initially identified as two exclusive pathways. However, several characteristics of apoptosis have been observed in the necrotic core lesion in ischemia that led to the controversial theory that cell death advances via a number of hybrid pathways among a continuum between the two processes. ATP availability has been shown to influence the decision between

apoptosis and necrosis. The aims of our study are (i) to determine if dual modality administration of non-selective Poly (ADP-ribose) polymerase (PARP) inhibitor 3-aminobenzamide (3-AB) and pancaspase inhibitor Carbobenzoxy-Val-Ala-Asp-fluoromethylketone (z-VAD-fmk) can further reduce infarct volume compared to single modality of either inhibitor following ischemic insult, (ii) to ascertain the 'critical hour' for pharmacological intervention, and (iii) to correlate intracellular ATP level with infarct volume. Untreated control group infarct volume was registered at 45.97 ± 1.86%. 3-AB treated group, at 10 mg/kg, 30 mg/kg and 50 mg/kg, infarct volume were determined at 39.99 ± 1.15%, 26.98 ± 2.22% and 38.14 ± 3.70% respectively. z-VAD-fmk treated group, at 1 mg/kg, 3 mg/kg and 5 mg/kg were recorded at 35.78 ± 1.95%, 24.13 ± 3.89% and 39.80 ± 2.69% respectively. 3-AB and z-VAD-fmk were optimised at 30 mg/kg and 3 mg/kg respectively. Infarct volume reduction, with concomitant administration of the inhibitors at optimized dosage were determined at 7.228 ± 1.988%, 21.02 ± 1.063%, 24.40 ± 2.121% for 30 min, 6 h, 24 h post injury respectively. In conclusion, the dual modality administration of both 3-AB and z-VAD-fmk show further increased in infarct volume reduction with the ischemic model, up to the 24th 'critical hour' post injury. However, only the 3-AB treatment group showed an inverse relation between the infarct volume and the intracellular ATP level.

PS3A-14

Effect of honokiol and magnolol on neuron toxicity in cerebellar granule cells

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The present study was to investigate the neuroprotective effects of honokiol and magnolol, two major bioactive constituents of the bark of *Magnolia officinalis*, against neuron toxicity induced by glucose deprivation, excitatory amino acids and H_2O_2 in cultured rat cerebellar granule cells. Cell membrane damage was measured with a lactate dehydrogenase (LDH) release assay and MTT assay. We found that honokiol and magnolol alone did not affect cell growth but significantly reversed mitochondrial dysfunction and cell damage induced by glucose deprivation. Furthermore, the glutamate receptor blocker MK-801 and antioxidant vitamin E provided protection against this damage, in which honokiol was more potent than magnolol in protecting against glutamate-, NMDA- and hydrogen peroxide (H_2O_2)-induced neuronal toxicity. These results demonstrated that the neuroprotective effects of honokiol and magnolol may be related to their anti-oxidative actions and antagonism of excitotoxicity induced by excitatory amino acids, suggesting that both honokiol and magnolol may be potential therapeutic agents for neurodegenerative diseases.

Poster Session 3B
Neurotransmission and signal transduction

PS3B-01

Differential effect of alpha-syntrophin knockout on aquaporin-4 and kir4.1 expression in retinal macroglial cells in mice

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Aquaporin-4 water channels and the inwardly rectifying potassium channels Kir4.1 are coexpressed in a highly polarized manner at the perivascular and subvitreal endfeet of retinal Muller cells and astrocytes. The present study was aimed at resolving the anchoring mechanisms responsible for the coexpression of these molecules. Both aquaporin-4 and Kir4.1 contain PDZ-domain binding motifs at their C-termini and it was recently shown that mice with targeted disruption of the dystrophin gene display altered distribution of aquaporin-4 and Kir4.1 in the retina. To test our hypothesis that alpha-syntrophin (a PDZ-domain containing protein of the dystrophin associated protein complex) is involved in aquaporin-4 and Kir4.1 anchoring in retinal cells, we studied the expression pattern of these molecules in alpha-syntrophin null mice. Judged by quantitative immunogold cytochemistry, deletion of the alpha-syntrophin gene causes a partial loss (by 70%) of aquaporin-4 labeling at astrocyte and Muller cell endfeet but no decrease in Kir4.1 labeling at these sites. These findings suggest that alpha-syntrophin is not involved in the anchoring of Kir4.1 and only partly responsible for the anchoring of aquaporin-4 in retinal endfeet membranes. Furthermore we show that wild type and alpha-syntrophin null mice exhibit strong beta1 syntrophin labeling at perivascular and subvitreal Muller cell endfeet, raising the possibility that beta1 syntrophin might be involved in the anchoring of Kir4.1 and the alpha-syntrophin independent pool of aquaporin-4.

PS3B-02

Farnesylation and nitrosylation change the intrinsic GTPase activity of $p21^{Ras}$

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Ras proteins perform functional roles in a large number of biological processes leading to changes in cell morphology, gene expression, survival and apoptosis. Ras is regulated by a series of post-translational modifications, including farnesylation, and nitrosylation, but the role of these modifications on the activity of Ras is not fully understood. Using several chromatographic steps, soluble and membrane-bound Ras protein from bovine brain were purified. To investigate the effects of nitrosylation and farnesylation on Ras-protein, the purified preparations were incubated with either S-nitroso-cysteine or farnesyltransferase and farnesyl diphosphate. We have found that in the presence of S-nitroso-cysteine GTPase the activity of soluble Ras was increased, while the farnesylation cause a significant inhibition of GTPase activity. In contrast, inhibition of GTPase activity was observed after incubation of S-nitroso-cysteine with membrane-derived Ras. These results suggest that different cysteine residues were S-nitrosylated in the membrane-derived and soluble Ras-proteins and these modifications can regulate GTPase activities.

Acknowledgment: This work was supported by the INTAS grant 2001-0666.

PS3B-03

Rapid decreases in intrinsic excitability triggered by excitation involving Kv1.4 membrane insertion

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The neuronal input can affect membrane excitability through modulating ion channels. However, the channels involved remain unclear. Here, we report that in response to glutamate voltage-dependent potassium channel (Kv) 1.4 was regulated to stabilize neuronal intrinsic excitability. Glutamate treatment led to reduction in the instantaneous firing frequency, the increment of firing threshold values and the shortening of accumulative spike duration. Glutamate increased I_A from 67.5 ± 9.0 pA/pF to 135.2 ± 13.2 pA/pF at the potential value of +70 mV, but did not affect half-maximal activation of I_A. Further, activation of NMDA-type of glutamate receptor mediated the increase of Kv1.4 in membrane. Over-expressing Kv1.4 suppressed the neuronal excitability, whereas down-regulating Kv1.4 with siRNA enhanced the excitability. Taken together, rapid membrane insertion of Kv1.4 by NMDA receptor activation suppressed neuronal excitability triggered by excitation.

PS3B-04

Store-operated channels in a microglia-derived cell line examined with a gene-encoded calcium indicator

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We investigated the regulatory mechanisms of ATP-induced Ca^{2+} influx in microglia by expressing a Ca^{2+} indicator protein ratiometric pericam (rmPericam) in murine microglia cell line MG5 cells. In MG5 cells, lower levels of ATP (<1 mM) induced only rapid and transient increase of $[Ca^{2+}]_i$ whereas higher levels of ATP (>1 mM) induced an additional phase of sustained $[Ca^{2+}]_i$ increase. This sustained $[Ca^{2+}]_i$ elevation was abolished when extracellular Ca^{2+} was reduced to 10 μM. ATP-induced sustained $[Ca^{2+}]_i$ increase was blocked by an inhibitor for store-operated Ca^{2+} influx (SOC), SKF96365, but not by antagonists for P2XR (e.g. PPADS and suramin). Therefore, the sustained $[Ca^{2+}]_i$ increase was likely due to Ca^{2+} entry through SOC. In response to ADP-β-S, both the initial transient $[Ca^{2+}]_i$ increase and the prolonged SOC were observed in MG5 cells even when the extracellular Ca^{2+} was depleted, indicating that the initial rapid and transient component could be induced by P2YR activation and the following prolonged SOC required the initial ATP response. In separate experiments, we unexpectedly found that higher levels of GTP or GDP (>1 mM) and polyphosphoric acid also evoked both the rapid and transient $[Ca^{2+}]_i$ increase and the subsequent sustained $[Ca^{2+}]_i$ increase in MG5 cells. Taken together, our results suggested that the sustained SOC in microglia might be induced by unknown components of store-operated Ca^{2+}-channels which are sensitive to extracellular polyphosphate group.

PS3B-05

Sustained phosphorylation of tyrosine hydroxylase at serine 40: a novel mechanism for maintenance of catecholamine synthesis

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine synthesis. Its activity is controlled acutely by phosphorylation (mins) and chronically by protein synthesis (days). Using bovine adrenal chromaffin cells we found that nicotine, acting via nicotinic receptors, induced the phosphorylation of TH at Ser40 for up to 48 hours. At 10 min Ser40 phosphorylation was not mediated by PKC and was not inhibited by the antidepressant imipramine. At 24 h Ser40 phosphorylation was mediated predominantly by PKC and was inhibited by the antidepressant imipramine; it was not inhibited by the ERK inhibitor UO126 or the protein synthesis inhibitor cycloheximide. At 48 h Ser40 phosphorylation was mediated by PKC. The mechanism(s) for the acute phosphorylation of TH at Ser40 therefore differ from that for sustained phosphorylation and there is a switch in the protein kinase(s) responsible over time. Sustained phosphorylation of TH at Ser40 was also observed with histamine and angiotensin II, but not with bradykinin and muscarine. Sustained phosphorylation of TH at Ser40 correlated with sustained TH activation and catecholamine synthesis for the first 24 hours. As TH protein synthesis increased between 24–48 h it became an additional mechanism of TH activation and catecholamine synthesis. This study reports the existence of a previously undiscovered sustained phase of TH activation that occurs via a mechanism different to the acute phase of TH activation that is specifically inhibited by an antidepressant drug and that continues in parallel with TH protein synthesis during the early stages of the chronic phase.

PS3B-06

Changes in expression of gamma-glutamylcysteine synthetase in the cochlea following acoustic overstimulation

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Sensorineural hearing loss can be caused by a variety of insults, including acoustic trauma, exposure to ototoxic drugs, infections or aging. There is compelling evidence that reactive oxygen species (ROS) are formed in the cochlea during acoustic overstimulation. Glutathione (GSH) protects against the hearing loss through scavenging ROS generated by noise and ototoxic drugs. In this study, we investigated the changes in expression of gamma-glutamylcysteine synthetase (GCS) gene, which is the rate-limiting enzyme in de novo GSH synthesis, in the cochlea by intense noise exposure. Nuclear extracts were prepared from the cochlear at various time points after intense noise exposure (4 kHz octave band, 125 dB, 5 h), and then subjected to electrophoretic mobility shift assay to determine DNA binding of transcription factors, such as activator protein-1 (AP-1), nuclear factor kappa B (NF-κB), antioxidant-response element (ARE) binding protein, and cyclic AMP- response element binding protein (CREB). AP-1 DNA binding was increased 2 to 12 h after the exposure, with a peak at 2 h afterward. In addition to AP-1, NF-κB DNA binding was increased immediately after exposure. However, ARE and CREB binding were not significantly affected by the exposure. Supershift analysis revealed that Jun-D and Fos-B of Fos/Jun family proteins participate to potentiation of AP-1 DNA binding at 2 h after the exposure. At 12 h after the exposure, c-Jun antibody also led to a shift of AP-1/probe complex. Furthermore, the supershift analysis indicated that p65 was involved in NF-κB DNA binding at 2 h after the exposure. RT-PCR revealed that noise exposure was effective in elevating expression of GCS mRNA in the cochlea 2 h later, with the elevation being sustained at least until 24 h later. Taken together, both AP-1 and NF-κB may participate in the expression of GCS gene in the cochlea after intense noise exposure.

PS3B-07

The existence of dopamine transporter immunoreactive terminals in bovine pineal gland

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Dopaminergic innervation has been proposed to be one of the systems controlling mammalian pineal gland function. The dopamine receptors have been characterized in the pineal and the biphasic effects of dopamine on melatonin production have been demonstrated. Recently, the dopamine transporter (DAT), a plasma membrane transport protein of dopaminergic neuron, has also been characterized in the bovine pineal. DAT provides an excellent marker for most dopaminergic neurons and their projections; and DAT expression is more restricted than the expression of genes encoding dopamine biosynthetic enzymes. Therefore, the aim of the present study is to identify the dopaminergic innervation (neurons/nerve fibers) in the bovine pineal gland. The localization and expression of DAT have been performed by using an immunofluorescent and a reverse transcriptase polymerase chain reaction technique. DAT-immunoreactivity was found in nerve terminals throughout the gland, but not in pinealocytes or neuronal-like cells. Amplification of RNAs with the primers of DAT resulted in a product of the predicted size, 405 base pairs. However, DAT mRNA was found in the bovine substantia nigra, but not in pineal. The colocalization of DAT with tyrosine hydroxylase (TH) or dopamine beta hydroxylase (DBH) immunoreactivities was observed in nerve terminals. The rest of DBH immunoreactivity was detected in nerve fibers, not colocalized with DAT. The present results reveal the dopaminergic innervation in the bovine pineal with their perikarya origin located possibly outside the gland. These dopaminergic nerve fibers distinguish from the noradrenergic nerve fibers.

Acknowledgment: This work was supported by the National Institute of Health/National Institute of Neurological Disorder and Stroke (No.1RO1-NS38764-04) and Srinakharinwirot University Research Assistantship to PB.

PS3B-08

Activation of TRPC5 Ca^{2+} channels by crosslinking of GM1 ganglioside in a neuronal cell line

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The TRPC5 channel, a member of non-voltage dependent cation channel family of the transient receptor potential canonical (TRPC) genes, is abundant in CNS and plays a role in axon outgrowth. Previous studies showed that crosslinking of GM1 on the surface of NG108-15 and other neuronal cells with cholera toxin B subunit (Ctx B) induced Ca^{2+} influx together with formation of axon-like neurites. This Ca^{2+} influx was voltage-independent and insensitive to blockers of voltage-operated Ca^{2+} channels. However, it was blocked by SK and F96365, an inhibitor of the TRPC channel. RT-PCR assay revealed expression of TRPC1, 5 and 6 in undifferentiated NG108-15 cells. In differentiated NG108-15 cells that did not respond to Ctx B, TRPC6 was eliminated and TRPC5 was significantly reduced. RT-PCR applied to Ctx B-unresponsive Neuro2a cells showed expression of TRPC3 and 6 but not TRPC1 and TRPC5. Taken together, these findings suggest a correlation between Ctx B-trigged Ca^{2+} influx and TRPC5 channels. To test this hypothesis, TRPC5 protein in NG108-15 cells was knocked down by siRNA transfection, utilizing four different oligonucleotides. This resulted in attenuation of Ctx B-induced Ca^{2+} influx, inward-current (determined with voltage-clamp analysis) as well as neurite outgrowth; each nucleotide had the same effect. Our results provide evidence that Ca^{2+} influx and axonogenesis induced by Ctx B are mediated by TRPC5, with GM1 functioning as intrinsic factor associated directly or indirectly with such channels. The natural ligand reacting with plasma membrane GM1 to trigger this process remains to be elucidated.

Acknowledgment: Supported by NIH grant 2RO1 NS033912.

PS3B-09

CDK5 phosphorylates and stabilizes p27kip1, contributing to actin organization and cortical neuronal migration

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Cyclin-dependent kinases (CDKs) are key regulators for cell cycle progression in proliferating cells, including neural precursors in the ventricular zone of embryonic cerebral cortices, and their activities are suppressed by $p27^{kip1}$, a CDK inhibitor (CKI), in G0 or early G1 phases of cell cycle. Unlike conventional CDKs, it has been known that Cdk5, a unique neuronal CDK, is not involved in cell cycle regulation, but in cortical neuronal migration through phosphorylating microtubule-regulatory proteins. Here we show that Cdk5 phosphorylates and stabilizes p27 as an upstream regulator, maintaining the amount of p27 in post-mitotic neurons. *In vivo* RNAi experiments showed that reduced amount of p27, which mimicked an inhibition of upstream signal from Cdk5, caused suppression of cortical neuronal migration, but little affected the expression of proliferative markers, PCNA and Ki67, in migrating neurons. p27-Knockdown neurons in the lower part of the intermediate zone displayed relatively round morphology with poor processes which contained decreased F-actin, while non-transfected control neurons normally exhibited multipolar morphology with abundant F-actin. We further revealed that the Cdk5-p27 pathway activated an actin-binding protein, cofilin, which was also involved in cortical neuronal migration *in vivo*. Our findings shed light on new relationship between CDK and CKI in G0-arrested cells to regulate cytoskeletal reorganization and neuronal migration during corticogenesis.

PS3B-10

Phosphorylation and regulation of $GABA_B$ receptor subunits by 5′-AMP activated protein kinase

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$GABA_B$ receptors mediate slow and prolonged synaptic inhibition in the brain, and are members of the G protein-coupled receptor (GPCR) superfamily. Unlike other members of the GPCR superfamily, functional $GABA_B$ receptors are heterodimers formed between $GABA_B$R1 and $GABA_B$R2. It is emerging that $GABA_B$ receptors utilize unique modes of cellular regulation compared to other monomeric GPCRs. Here we have investigated the role of 5′AMP-activated protein kinase (AMPK), as an endogenous regulator of $GABA_B$ receptor function. Using a combination of molecular approaches we have established that AMPK associates with $GABA_B$ receptors in the brain via a direct interaction with the cytoplasmic tail of $GABA_B$R1. Using mass spectrometry coupled with site-specific mutagenesis, we have identified multiple phosphorylation sites for AMPK within the cytoplasmic tails of both $GABA_B$R1 and R2. Moreover, the activation of AMPK seems regulating the cell surface stability of $GABA_B$ receptors, through their functionally coupling K^+ channels. Together our results highlight a novel role for AMPK in regulating the functional properties of $GABA_B$ receptors, by direct phosphorylation. Given the role of AMPK as a sensor of cellular stress this potential mechanism may be relevant in regulating the efficacy of synaptic inhibition under anoxic conditions and during periods of high synaptic activity.

PS3B-11

Changes in the expression of DJ-1 in the hippocampal neural cells damaged by trimethyltin

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DJ-1 has been identified as a novel oncogene that transforms mouse NIH3T3 cells in cooperation with activated ras. Deletion and point mutation of DJ-1 are responsible for the onset of familial Parkinson's disease, PARK7, and lead to neural death. In addition, expression of DJ-1 is enhanced by oxidative stresses. Although exact functional significance of DJ-1 has still unknown, it is thus proposed that DJ-1 is protective against neural damage under oxidative stresses. In this study, we tested expression of DJ-1 in the hippocampus damaged by endocrine disrupting chemical, trimethyltin (TMT). TMT was systemically injected into mice to cause neural damage in the dentate gyrus selectively. Immunohistochemical analysis indicated that DJ-1 was markedly increased in the molecular layer of the dentate gyrus on days 4 and 14 post TMT injection. On day 14 post TMT injections, enhanced expression of DJ-1 was observed in the stratum lucidum of the CA3. In glutathione-depleted mice, TMT was more effective in enhancing expression of DJ-1, compared with that in untreated mice. Furthermore, double staining of DJ-1 and glial fibrillary acidic protein (GFAP) demonstrated that most of cells highly immunoreactive to DJ-1 were co-localized with GFAP in the dentate gyrus of TMT-treated animals, but not of untreated animals. These results suggest that DJ-1 is enhanced in the dentate astrocytes activated by TMT treatment.

PS3B-12

Differential internalization and recycling of the human serotonin 2A ($5\text{-}HT_{2A}$) receptor by serotonin

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The $5\text{-}HT_{2A}$ receptor has been implicated in various psychiatric disorders and is a major target of many antipsychotic and antidepressant drugs. We tagged the human $5\text{-}HT_{2A}$ receptor at its C-terminus to EGFP and studied its internalization and recycling kinetics using its natural ligand, serotonin in HEK 293 cells – a human cell line. When compared to the rat isoform [Bhattacharyya, S. *et al.* (2002) *PNAS,* 99, 22, 14470–14475] we find that, although the human and the rat $5\text{-}HT_{2A}$ receptors differ by only a few amino acids, the human receptor is more sensitive to serotonin. In addition, human $5\text{-}HT_{2A}$ receptors take longer time to recycle to the cell surface after internalization and there is an altered dependence on the kinases involved in internalization. Though human $5\text{-}HT_{2A}$ receptors undergo clathrin-mediated endocytosis similar to its rat counterpart, there is an additional involvement of beta-2 arrestin in facilitating this process. In all we find that, the human $5\text{-}HT_{2A}$ receptor differs from its rat isoform sufficiently enough in its internalization and recycling processes that previous findings with the rat $5\text{-}HT_{2A}$ receptor cannot be extrapolated onto human $5\text{-}HT_{2A}$ receptors. This has significant implications both in our understanding of how human $5\text{-}HT_{2A}$ receptors function and for future clinical approaches.

Acknowledgment: We thank Dr. S. Mandal and Mr. P. S. Beed for making the human-$5\text{-}HT_{2A}$–EGFP construct and initial experiments.

PS3B-13

Involvement of calcium mobilization in platelet-activating factor-induced pro-inflammatory cytokine expression in human microglia

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Platelet-activating factor (PAF) is a potent phospholipid compound produced by a variety of cells in the nervous system. It has been thought that an *in vivo* accumulation of PAF, synthesized and released by activated microglia, is a main initiator of neuronal dysfunction and death in HIV-associated dementia (HAD), and a secondary mediator of neuronal loss in ischemia. In the present study, the effects of PAF on human fetal microglia cultures were investigated. Our results showed that PAF (100 nM) induced the expression of pro-inflammatory cytokines TNF-α and IL-6, as detected by RT-PCR, and caused a biphasic increase in intracellular calcium (Ca^{2+}) level as monitored by calcium imaging using fura-2.

The expression of TNF-α and IL-6 was inhibited by BN52021 (PAF receptor antagonist) and SKF96365 (inhibitor of store-operating calcium channel: SOC), suggesting that the actions of PAF is mediated via surface PAF receptors and that Ca^{2+} influx from extracellular space is one of the signal transductions involved in the induction of cytokine expression in microglia. These results suggested that PAF may be an important mediator inducing microglia cell activation in some neurodegenerative diseases that are associated with neuronal cell death. Finally, PAF may be a potential target in a therapeutic approach for patients suffering from HAD and stroke.

PS3B-14

Activation of NMDA receptors and calcium influx mediated cell death during cerebral malaria in mice brain

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Human cerebral malaria (CM) is a major life threatening complication of *Plasmodium falciparum* infection and is characterized by various neurological symptoms including behavioral changes, paralysis, hemiplegia and convulsions, often proceeding to coma and death. Over stimulation of N-methyl-D-aspartate (NMDA) receptors is believed to play a major role in neuronal death in several acute and chronic neurodegenerative diseases, which results in intracellular Ca^{2+} overload and subsequent sustained activation of Ca^{2+} dependant cysteine protease cascade. Calcineurin is a Ca^{2+} and calmodulin dependent protein phosphatase. Calcineurin is known to participate in a number of cellular processes and Ca^{2+}-dependent signal transduction pathways by dephosphorylation. One of the known substrates of calcineurin is the NMDA receptor. Further, over expression of calcineurin is implicated in apoptosis. Endoplasmic reticulum (ER) participates in the initiation of apoptosis via calcium mediated signalling. Prolonged ER stress stimulates the activation of pro-caspase-12 followed by caspase 3 activation. This enzyme is localized in the ER membrane. Our earlier studies indicated activation of m-Calpain. Caspases are a family of intracellular cysteine aspartases involved in the initiation and execution of apoptosis. In the CNS, participation of caspases has been well documented in experimental apoptosis models and its activation has been implicated as a step in the signaling pathway leading to apoptosis. Depending on the severity of insult, NMDA receptor activation induces either apoptosis or necrosis. Hence, a better understanding of the biochemical pathways involved in the status of intracellular calcium influx, calcineurin and NMDA receptor expression, caspases might represent important implications during the pathology of cerebral malaria. In the present study an experimental model has been used to investigate the morphological and biochemical changes associated with CNS dysfunction during cerebral malaria. Mice infected with *Plasmodium berghei* ANKA will develop cerebral symptoms at around 12th day and the animals were sacrificed and brains were excised for biochemical studies. Intracellular calcium levels were studied using Fura-2AM method in the tissue sections. This indicated the calcium levels were elevated in the infected brains compared with the control. Since, intracellular calcium levels were enhanced, calcineurin and NMDA (NR1, NR2A and NR2B) receptor status was studied using western blot analysis. NR1 levels were increased in cerebellum and NR2A and NR2B levels were elevated in both cerebral cortex and cerebellum. Further, pro-caspase levels were decreased indicating the cleavage of caspase 12 to active from. Subsequently caspase 3 was activated and apoptosis was observed in the infected mice brain.

PS3B-15

The Ca^{2+}-dependent binding of syntaxin-1A and myosin-V regulates exocytosis

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Myosin-V is an actin-based processive motor that conveys intracellular cargoes. We show that myosin-V on cortical synaptic vesicles interacts with syntaxin-1A, a *t*-SNARE involved in exocytosis, at or above 0.3 μM Ca^{2+}. Interference with formation of the syntaxin-1A-myosin-V complex reduces the exocytotic frequency in adrenal chromaffin cells. The syntaxin-1A-binding site was in the neck of myosin-V, a region that contains six calmodulin-binding IQ-motifs. We also found that syntaxin-1A binding by myosin-V in the presence of Ca^{2+} depends on the release of calmodulin from the neck, allowing syntaxin-1A to occupy the vacant IQ-motif. Using an anti-myosin-V neck antibody, we demonstrated that the step most important for the antibody's inhibitory activity is the late phase, which is involved in supplying readily releasable vesicles. Our results demonstrate that the interaction between myosin-V and syntaxin-1A is involved in exocytosis and suggest that the myosin-V neck contributes to the regulation of exocytosis by Ca^{2+}.

PS3B-16

Desensitization of NMDA receptor channels by repeated stimulation in cultured rat cortical neurons

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Activation of N-methyl-D-aspartate (NMDA) receptors is believed to induce rapid opening of an ion channel permeable to Ca^{2+} ions, often followed by signaling cascade to the nucleus for long-term consolidation in the brain. Rat cortical neurons were cultured in DMEM/F12 in the absence of FBS for 3 to 15 days *in vitro*, followed by incubation with fluo-3 AM for determination of intracellular free Ca^{2+} ions on fluorescence image analysis. Brief exposure to NMDA for 5 min rapidly increased the fluorescence intensity in a reversible manner, with a persistently constant increase throughout the sustained exposure for 60 min. Irrespective of the cellular maturity, the second brief exposure resulted in a less efficient increase in the fluorescence than that found after the first brief exposure in a manner dependent on intervals between the two repetitive exposures. *In vitro* maturation significantly shortened the interval required for the reduced responsiveness to the second brief exposure, while in immature neurons prolonged intervals were required for the reduced responsiveness to the second brief exposure to NMDA. Brief exposure to NMDA led to a significant

decrease in biotinylated membrane proteins immunoreactive to NR1, NR2A and NR2B subunits in proportion to the time after washing. A marked decrease was seen in immunoreactivity to extracellular loop of NR1 subunit after brief exposure to NMDA when determined in cultured neurons not permeabilized on immunocytochemical detection. These results suggest a novel concept that desensitization would undergo with NMDA receptor channels through the decrease in the number of NMDA receptors expressed at cell surfaces in a manner triggered by dissociation, but not by association, of the agonist NMDA in cultured rat cortical neurons.

PS3B-17

Changes of non-adrenergic, non-cholinergic innervation in the neural pathways of the guinea pig urinary bladder following chronic bladder outlet obstruction

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This study was aimed to examine the effect of chronic bladder outlet obstruction (BOO) on expressions of non-adrenergic, non-cholinergic (NANC) neuro-transmitters or -modulators, including neuronal nitric oxide synthase (nNOS), calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal polypeptide (VIP) and neuropetide Y (NPY) in the neural pathways of the guinea pig bladder. Partial urethral ligation was done in young male guinea pigs (body weight: about 300 g). Animals were sacrificed at 4 and 12 weeks after partial BOO and immunohistochemistry for nNOS, CGRP, SP, VIP and NPY was carried out in the urinary bladder, lumbosacral spinal cord (L6-S2) and their corresponding dorsal root ganglia (DRG). Results were then compared to controls (normal and sham-operated). At 4 weeks after urethral obstruction, decreased immunhistochemical expressions of nNOS, CGRP, SP and VIP were detected in the intramural ganglion cells and nerve fibers of the urinary bladder. However, there were partial restorations at 12 weeks in all the above mentioned proteins. NPY immunoreactivity did not show obvious changes following BOO. The number of CGRP and SP immunoreactive neurons in the DRG showed increases at 4 weeks post-BOO. At 12 weeks post-operation, the increase in CGRP immunoreactive neurons was maintained, but was partially decreased for SP immunoreactive cells. No apparent change was observed in the number of nNOS, VIP and NPY immunoreactivities in the DRG. Some immunonegative DRG neurons appeared to be encircled by satellite cells immunoactive to all the antibodies tested. In the spinal cord, nNOS immunoreactive neurons were found mainly in laminae 1 and 2. The number of immunopositive cells showed significant increase 4 weeks after BOO, but decreased marginally at 12 weeks. Immunoreactivities for CGRP, SP and VIP were mainly observed in nerve fibers located between laminae 1 to 3. At 4 weeks post-BOO, immuno-expressions for CGRP, SP and VIP showed significant increase. However, SP and VIP immunoreactivities decreased at 12 weeks, but CGRP immuno-expression continued to show an increase. There was no significant change in NPY immunoreactivity in the dorsal horn (DH). Chronic BOO resulted in the changes of expressions of nNOS, CGRP, SP, VIP in the urinary bladder, DRG and DH of spinal cord, indicating their possible involvements in neural control changes, and this may be linked to the development of overactive bladder.

PS3B-18

Calcium regulation by hydrogen sulphide in microglia cells

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Hydrogen sulphide (H_2S), which is produced endogenously from L-cysteine in mammalian tissues, has been suggested to function as a neuromodulator in the brain. However, the role of H_2S in microglial cells is unclear. In this study, the effect of exogenous and endogenous H_2S on intracellular calcium homeostasis was investigated using BV-2 microglial cells, followed by conformational studies with primary cultures of microglia. Sodium hydrosulphide (NaHS), a H_2S donor, caused a concentration-dependent (0.1–0.5 mM) increase in intracellular calcium concentration ($[Ca^{2+}]i$). This effect was significantly attenuated in the presence of a calcium-free extracellular solution, Gd^{3+} (100 μM) and La^{3+} (100 μM), two non-selective Ca^{2+} channel blockers, or thapsigargin (2 μM), an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase. These observations suggest that the increase in $[Ca^{2+}]i$ in response to H_2S involves both calcium influx across the plasma membrane and calcium release from intracellular stores. H-89, a selective PKA inhibitor, failed to affect $[Ca^{2+}]i$ response to NaHS, suggesting that the action of H_2S does not involve cAMP/PKA pathway. Using RT-PCR, two H_2S-producing enzymes, cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), were found to be expressed in BV-2 microglial cells whereas only CSE was detected in primary cultures of microglia. Inhibition of either enzyme with amino-oxyacetate (a CBS inhibitor), β-cyano-L-alanine, D,L-propargylglycine (two CSE inhibitors) significantly decreased $[Ca^{2+}]i$, suggesting that endogenous H_2S may have a positive tonic influence on $[Ca^{2+}]i$ homeostasis. These findings support the possibility that H_2S may serve as a neuromodulator to facilitate signaling between neurons and microglial cells.

PS3B-19

Activation of the human FPRL-1 receptor promotes Ca^{2+} entry via store operated channel in U87 astrocytoma cells

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The human formyl-peptide receptor like 1 (FPRL-1) is a variant of the G_i-coupled formyl-peptide receptor. Functional FPRL-1 is endogenously expressed in the U87 astrocytoma cell line [1] and there is accumulating evidence to suggest that FPRL-1 may be involved in neuroinflammation associated with the pathogenesis of Alzheimer's disease. In this study, we examined the ability of FPRL-1 to mobilize intracellular Ca^{2+} and regulate mitogen-activated protein kinases in U87 astrocytoma cells, as well as Chinese hamster ovary (CHO) cells stably expressing FPRL-1. Trp-Lys-Tyr-Met-Val-Met (WKYMVM), a specific agonist for FPRL-1, can stimulate Ca^{2+} mobilization in both U87 and FPRL-1/CHO cells with EC_{50} values around 16.3 μM and 70.9 nM, respectively. These effects can be inhibited by the FPRL-1 selective antagonist, WRW4. To delineate the mechanism by which the FPRL-1 receptor stimulated Ca^{2+} mobilization, several pharmacological inhibitors were used. Pertussis toxin completely inhibited FPRL-1 mediated Ca^{2+} increase, indicating the involvement of G_i proteins. Pretreat-

ment with inositol 1,4,5-trisphosphate receptor inhibitor 2-APB and the selective store-operated channel inhibitor SKF96365 were found to abolish the WKYMVM-induced Ca^{2+} increase. Ca^{2+} mobilization were also prevented when the assays were performed in a Ca^{2+} free buffer. Intracellular Ca^{2+} mobilizations in both cell lines were unaffected by the phospholipase Cβ inhibitor U73122 or selective ryanodine receptor inhibitors (ruthenium red and dantrolene). Immunoblotting analysis revealed that WKYMVM can activate the mitogen-activated protein kinase (MAPK) pathway. Whereas addition of WKYMVM peptide led to JNK, ERK and P38 phosphorylations in FPRL-1/CHO cells, the agonist only induced phosphorylation of JNK and ERK in U87 cells. Phosphorylations of JNK and ERK in both cell types were not affected by the treatment with BAPTA, a calcium chelator. In conclusion, stimulation of G_i-coupled FPRL-1 can lead to calcium mobilization and the differentiation activation of MAPK pathways in transfected CHO cells and U87 cells.

Acknowledgment: This work was supported in part by grants from the Research Grants Council of Hong Kong (HKUST 3/03C), the University Grants Committee (AoE/B-15/01), and the Hong Kong Jockey Club.

PS3B-20

Phosphorylation of CAMKII at Thr253 occurs *in vivo* and enhances binding to isolated post synaptic densities

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Autophosphorylation of Ca^{2+}-calmodulin stimulated protein kinase II (CaMKII) at two sites (Thr286 and Thr305/306) is known to regulate the subcellular location and activity of this enzyme *in vivo*. CaMKII is also known to be autophosphorylated at Thr253 *in vitro* but the functional effect of phosphorylation at this site and whether it occurs *in vivo*, is not known. Using antibodies that specifically recognize CaMKII phosphorylated at Thr253 together with FLAG-tagged wild type and phospho- and dephospho-mimic mutants of alpha-CaMKII, we have shown that Thr253 phosphorylation has no effect on either the Ca^{2+}-calmodulin dependent or autonomous kinase activity of recombinant alpha-CaMKII *in vitro*. However, the Thr253Asp phosphomimic mutation increased alpha-CaMKII binding to subcellular fractions enriched in postsynaptic densities (PSDs). The increase in binding was similar in extent, and additive, to that produced by phosphorylation of Thr286. Thr253 phosphorylation was dynamically regulated in intact hippocampal slices. KCl induced depolarisation increased Thr253 phosphorylation and the phospho-Thr253-CaMKII was specifically recovered in the subcellular fraction enriched in PSDs. These results identify Thr253 as an additional site at which CaMKII is phosphorylated *in vivo* and suggest that this dynamic phosphorylation may regulate CaMKII function by altering its distribution within the cell.

Poster Session 3C
Myelin and lipids

PS3C-01

Ca^{2+}-dependent synaptic vesicle fusion occurs at PA-rich domain of presynaptic membrane

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Phosphatidic acid (PA) has been implicated in Ca^{2+}-dependent exocytosis of secretary granules at an exocytotic site of presynaptic membrane called 'active zone'. However, a specific role of PA in membrane fusion of synaptic vesicles or a local production of PA at the presynapses has remained unclear. Using both an intracellularly expressed GFP probe for PA and anti-PA monoclonal antibody, we first demonstrated that there was PA-rich domain at the presynapse of mouse hippocampal pyramidal neurons, and synaptic fusion occurred at the PA-rich domain of the presynaptic membrane. When PA production by phospholipase D (PLD) was selectively inhibited by n-butanol, the number of recycling vesicles was significantly decreased, suggesting a role for PA-rich domain to facilitate synaptic vesicle fusion. Inhibition of PA production also attenuated potassium-induced Ca^{2+}-influx, therefore PA might regulate voltage-sensitive calcium channels in the active zone. Extracellular administration of PA liposome induced a rapid Ca^{2+}- influx, indicating that PA by itself could cause a local membrane phase separation. These results suggest that PA is locally produced at the site of the synaptic vesicle fusion and plays a role in phase separation of presynaptic membrane upon the intracellular Ca^{2+}-rise. In this study, we provide a new insight into PA as a mediator for Ca^{2+}-dependent membrane phase separation. PA may directly bind to Ca^{2+}-bound synaptic vesicle and facilitate synaptic vesicle fusion followed by transmitter release.

PS3C-02

Effects of tetrahydroxystilbene glucoside on rat model of β-amyloid increase induced by hypercholesterolemia

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Aim: To investigate the effects of 2,3,5,4'-tetrahydroxystilbene -2-O-β-D-glucoside (TSG) on learning and memory ability, brain β-amyloid (Aβ) content, blood fat and hemorheology in rat model induced by hypercholesterolemia.
Methods: The animal model of hypercholesterolemia was induced by feeding high cholesterol forage containing 1% cholesterol and 0.3% cholic acid for 10 weeks. TSG was given orally at doses of 30, 60 and 120 mg/kg body weight/day also for 10 weeks. The ability of learning and memory was tested by Morris Water Maze after giving high cholesterol forage for 8 weeks, the content of β-amyloid was measured by immunohistochemistry and radioimmunoassay methods, the serum cholesterol and low density lipoprotein (LDL-C) were measured by automatic biochemical analytical methods and some indexes of hemorheology were also observed after giving high cholesterol forage for 8 weeks.
Results: The learning and memory ability was damaged after feeding high cholesterol forage for 8 weeks and TSG could improve it. The content of Aβ in the hippocampus of rat model was increased, and the serum cholesterol and low-density lipoprotein level were obviously elevated after 10 weeks. TSG could reduce the content of Aβ in the hippocampus, the serum cholesterol and low-density lipoprotein level obviously. The indexes of hemorheology showed that blood viscosity (ηb, high and low shear rate) and RBC adhesion index (AI) were increased in rats. TSG could decrease blood viscosity and lower RBC adhesion index (AI) in the rat model.
Conclusion: Hypercholesterolemia, by increasing A(β production, leads to more Aβ deposition in the brain and caused damage in the ability of learning and memory. TSG reduced the content of Aβ in hippocampus, decreased serum cholesterol and low-density lipoprotein, promoted blood circulation and removed blood stasis. These actions may be related to the therapeutic mechanisms of AD.

PS3C-03

Cystatin F induction in demyelinating disease of CNS

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Demyelination and remyelination coincide with numerous gene expression changes. We try to discover the novel gene, which is dependent to demyelination and remyelination process. We performed cDNA microarray against spontaneous chronic late-onset demyelination disease animal model, proteolipid protein transgenic mice (plp^{tg}/-). We successfully extract the induction of Cystatin F, one of the endogenouse papain-like cathepsin inhibitors, in plp^{tg}/- mice. According to histological analysis, we showed that Cystatin F mRNA expression dominates in demyelinating lesion and its cellular localization is in microglia. This induction was not specific to the case of this model mouse and was also observed in cuprizone-treated demyelinating mouse. However, the upregulation of Cystatin F mRNA was not observed in dys- or hypomyelinating mice, jimpy and shiverer mutant. Furthermore, the expression level was lower or disappeared in the advanced stage of plp^{tg}/- mice or lasting cuprizone treatment. In both cases, demyelination continues and remyelination ceases. These *in vivo* data suggest that cystatin F induction in microglia coincides with the presence of degraded myelin. To test this possibility, we isolated primary microglia and treated them with myelin directly. This *in vitro* experiment showed that the expression level of cystatin F mRNA was increased in

microglia taken up myelin. In conclusion, during myelin degenerating period, cystatin F mRNA was increased in microglia in white matter and its expression was dependent to the phagocytosis of myelin.

PS3C-04

Non-targeted profiling of lipids during kainate-induced neuronal injury

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Kainate is a glutamate analog that has been widely used in pharmacological studies of neuronal injury related to ischemic conditions and epilepsy. While altered lipid metabolism has been implicated in kainate action no study has yet investigated the associated changes in lipid metabolites on a systems level scale. Here we describe a mass spectrometry based approach for profiling of lipid mixtures in a non-targeted fashion. Combined with tandem mass spectrometry this method aims at identifying lipids that are altered between two conditions, the kainate-treated and the control hippocampal tissues. In addition to reductions in major phospholipids with mainly polyunsaturated fatty acyl chains we find elevated levels of ions that correspond to acylated forms of phosphatidylethanolamines and ceramides. Acylated phosphatidylethanolamines are neuroprotective lipids and precursors for anandamide that signals via cannabinoid receptors. Quantitative analysis of ceramides shows that many molecular species with different acyl compositions are increased during kainate treatment. This increase is mainly restricted to neurons rather than other brain cells in the hippocampus as revealed by immunohistochemistry of brain slices.

PS3C-05

PTPα acts as a modulator of notch signaling during CNS myelination

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Axoglial interactions between neurons and oligodendrocytes regulate myelination in the central nervous system. F3/contactin promotes oligodendrocyte maturation via the Notch/Deltex1 pathway and also acts as a co-receptor of protein tyrosine phosphatase α (PTPα). We report that PTPα was expressed in oligodendrocytes and neurons. PTPα was required for the Notch/CSL/Hes1 signaling pathway while suppressed the interaction between Notch and Deltex1. PTPα deficiency did not affect oligodendrocyte maturation promoted by F3-triggered Notch/Deltex1 signaling pathway. Overexpression of PTPα prevented F3/contactin clustering at the cell surface *in vitro*, whereas PTPα deficiency facilitated early formation of axonal transverse bands that are mainly constituted by F3 and Caspr and advanced myelination *in vivo*. Moreover, hypermyelination, a pronounced increment in the thickness of myelin sheaths and in the number of non-landed oligodendrocyte loops at paranodes, was observed in PTPα knockout mice. Thus, PTPα acts as a dual-role modulator of Notch signaling during CNS myelination via positively regulating the myelination-inhibiting Notch/Hes1 cascade and negatively mediating the myelination-promoting F3/Notch/Deltex1 pathway.

PS3C-06

Lovastatin modulates increased cholesterol and oxysterol levels and has a neuroprotective effect on rat hippocampal neurons after kainate injury

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The present study was carried out to elucidate the effect of a brain permeable statin (lovastatin) on cholesterol and oxysterol levels of the hippocampus after neuronal injury induced by the excitotoxin, kainic acid. Increased immunolabeling to cholesterol and the oxysterol biosynthetic enzyme, cholesterol 24-hydroxylase, was observed in the rat hippocampus after kainate lesions. This was accompanied by increased levels of cholesterol, 24-hydroxycholesterol (product of cholesterol 24-hydroxylase enzymatic activity) and 7-ketocholesterol in homogenates of the degenerating hippocampus, as detected by gas chromatography / mass spectrometry (GC/MS). Hippocampi from rats or organotypic slices that had been treated with kainate plus lovastatin showed significantly lower levels of cholesterol, 24-hydroxycholesterol, and 7-ketocholesterol, compared to those that had been treated with kainate only. Lovastatin also modulated hippocampal neuronal loss after kainate treatment, *in vivo* and *in vitro*. The level of 24-hydroxycholesterol detected *in vivo* after kainate treatment (>50 μM) was found to be neurotoxic in hippocampal slice cultures. The above results suggest that brain permeable statins such as lovastatin could have a neuroprotective effect by limiting the levels of oxysterols in brain areas undergoing neurodegeneration.

PS3C-07

Double assurance of Hes5 on inhibiting myelin gene expression: repression and sequestration

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The myelin gene expression is tightly regulated during the differentiation of oligodendrocytes both *in vitro* and *in vivo*. Although a lot of factors have been found to play crucial roles in the regulation of myelin gene expression, the precise mechanism is still largely unknown. We show here that the myelin gene

expression is negatively regulated by Hes5. Although Hes5 can directly bind to MBP promoter, this direct binding has no significant repression effect on MBP promoter activity *in vitro*. Instead, Hes5 regulates myelin gene expression through inhibiting the expression and function of transcription factors favoring myelin gene expression. Initially Hes5 works as a transcription inhibitor repressing the expression of Sox10 and Mash1. Secondly, Hes5 sequester the expressed Mash1 and Sox10.

Poster Session 3D
Parkinson's disease

PS3D-01

Susceptibility genes and genotype combinations for pathogenesis of Parkinson's disease

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Parkinson's disease is considered to be a multifactorial disease. Seventy to ninety percent of cases are estimated to be sporadic, while ten to thirty percent are familial. Multiple genetic and environmental factors are thought to cause idiopathic Parkinson's disease (PD). To search for the susceptibility genes and the possible genotype combinations as one of the predisposing factors for Parkinson's disease, a case-control association study was carried out by studying single nucleotide polymorphisms (SNPs) in the candidate genes involved in dopamine metabolism and regulation such as dopamine receptor-D2 (DRD2), dopamine transporter (DAT), monoamine oxidase-B (MAO-B) and those involved in toxication-detoxication mechanisms such as cytochrome P450 2D6 (CYP2D6) and glutathione S-transferase (GSTs). Genomic DNA was isolated from the blood samples, drawn from 60 patients suffering from Parkinson's disease and equal number of age and gender matched healthy controls. PCR based RFLP and allele-specific PCR methods were used to detect the polymorphisms in above said genes. Our data in 60 cases showed increased frequency of CYP2D6*4 (G1934A), GST-T1 (null), DAT (A1215G) and MAO-B (intron-13A/G) polymorphisms in patients as compared to controls; while no significant changes in the frequency of wild type and variant alleles of DRD2Taq1A and 1B were observed in forty patients studied so far. Our data also revealed that combined genotype of CYP2D6 and MAO-B were found to be slightly increased in PD cases as compared to controls. Further analysis is in process to identify the causative genes as well as genotype combinations for pathogenesis of PD.

PS3D-02

The neuroprotective effect of melatonin against 1-methyl, 4-phenyl, pyridinium ion-induced common oxidative stress and cell death in cultured SK-N-SH cells

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Neurodegenerative diseases such as Parkinson's disease are illnesses associated with high morbidity and mortality with few, or no effective, options available for their treatment. In addition, the direct cause of selective dopaminergic cell loss in Parkinson's disease has not been clearly understood. Recent evidence has shown that oxidative stress induced c-Jun-N-terminal kinase (JNK) pathways may have some role in the death of neuronal cells. Taken together, several studies have documented that melatonin has a neuroprotective effect both *in vivo* and *in vitro*. Accordingly, the effects of melatonin on neurotoxin, 1-methyl, 4-phenyl, pyridinium ion (MPP^+)-treated cultured human neuroblastoma SK-N-SH cell lines were investigated in the present study. The results showed that MPP^+ significantly decreased cell viability as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. By contrast, an induction of reactive oxygen species (ROS) formation, phosphorylation of c-Jun and cleavage of DNA fragmentation factors 45 (DFF45) was observed in MPP^+-treated cells. The protective effect of melatonin on the viability of MPP^+-treated cells was determined. The results showed that melatonin was able to diminish the toxic effect of MPP^+ on cell viability. These results demonstrate the cellular mechanisms of neuronal cell degeneration induced via oxidative stress, and the potential role of melatonin on protection of neuronal cell death induced by neurotoxin.

Acknowledgment: This work was supported by a research grant from the Thailand Research Fund (RSA4780018).

PS3D-03

Alpha-synuclein overexpression in human neuroblastoma SH-SY5Y cells prevents mitochondrial deficit and oxidative stress

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Parkinson's disease (PD) is characterized pathologically by the selective loss of dopaminergic neurons and the presence of Lewy bodies in the substantia nigra. alpha-synuclein, a major component of Lewy bodies, is believed to play an important role in the pathogenesis of PD. However, the function of alpha-synuclein remained unclear. The present study was designed to investigate the function of alpha-synuclein in mitochondria of human neuroblastoma SH-SY5Y cells treated by rotenone and to find out its exact role in the pathogenesis of PD. Human neuroblastoma SH-SY5Y cells were transfected with alpha-synuclein. Cells were selected in G418 after transfection. RT-PCR and Western-blot was carried out to test the expression of alpha-synuclein in SH-SY5Y cells. SH-SY5Y cells with alpha-synuclein overexpression (SH-SY5Y-α-syn) and control cells (SH-SY5Y-Ctr) were both treated by 2 nmol rotenone

for 1, 2, 4 weeks. Then cell viability, the activity of mitochondrial complex I, the degree of mitochondria membrane swelling and the content of superoxide anion in mitochondria were measured. Results of RT-PCR and Western-blot showed that SH-SY5Y cells did overexpress alpha-synuclein. When tested at 1 and 2 weeks post rotenone treated, cell viability of SH-SY5Y-α-syn were significantly higher than SH-SY5Y-Ctr ($P < 0.01$). On the contrary, cell viability of SH-SY5Y-α-syn were lower than SH-SY5Y-Ctr ($P < 0.01$) 4 weeks after rotenone treated. The activity of complex I, the degree of mitochondrial membrane swelling and the content of superoxide anion showed similar results. The present study showed that alpha-synuclein may partially prevent mitochondrial deficit and oxidative stress induced by rotenone at early stage, however, with long term rotenone treatment, alpha-synuclein may act as an accomplice of neurotoxin.

PS3D-04

α-synuclein expression in SH-SY5Y cells resists oxidative stress induced by rotenone

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α-Synuclein is the major component of Lewy bodies and mutations in the α-synuclein gene are responsible for a few early onset familial PD indicating that α-synuclein may have a critical role in the pathogenesis of both familial and sporadic PD. Although the aetiology of PD is unknown, a variety of evidence has supported that environmental factors shared with the common mechanisms of resulting in α-synuclein aggregation by inhibiting complex I of mitochondria and leading to oxidative stress. To investigate the relationship between α-synuclein and oxidative stress, we used human dopaminergic SH-SY5Y cells transfected with α-synuclein-GFP. α-Synuclein gene expression in cells was determined by GFP fluorescence, immunocytochemistry, and RT-PCR. Cells were then treated with different concentrations of rotenone for different times. The viability and oxidative stress were detected by MTT assay and DCF assay. SOD activity was assessed with xanthine peroxidase method. The results showed that α-synuclein was constantly expressed in transfected cells. Rotenone significantly reduced cell viability and simultaneously induced mitochondrial swelling and oxidative stress in a dose-dependent manner. During rotenone exposure, the cell viability, mitochondrial membrane potential and complex I activity of SY5Y/Syn cells were better than those of SY5Y cells (*$P < 0.01$). In addition, a quantitative correlation between rotenone-induced cell death and rotenone induced ROS production was also observed. Antioxidant capacities in SY5Y/Syn cells were higher than that of SY5Y cells ($P < 0.01$). Moreover, The JNK signaling pathway was inhibited by the α-synuclein expression in SH-SY5Y cells. Thus it seemed that α-synuclein expression had a tendency to resist oxidative stress induced by rotenone and protected cells from rotenone insult. These results suggest that there is a complex interplay between α-synuclein and environmental factors and oxidative stress may play an important role in the cause of dopamine neuron death.

PS3D-05

Synergistic effects of zinc-metallothionein against methamphetamine induced mitochondrial damage in SK-N-SH cells

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Methamphetamine (METH) is a drug of abuse and also a neurotoxin that induces Parkinsonian-like pathology after chronic usage by targeting the nigrostraital dopaminergic pathway. The autopsied brains of chronic METH users showed similar patterns of brain pathology to that seen in Parkinson's disease. Now it is believed that the damage to the dopamine system from long-term METH use may also lead to symptoms of Parkinson's disease. However, the precise mechanism by which METH induces neuronal damage is poorly understood. Elucidation of the intracellular mechanisms that underlie METH-induced dopaminergic neuron toxicity may help in understanding the pathogenesis of Parkinson's disease. In the present study, we investigated the mechanisms involved in METH-induced death of dopaminergic neuronal cells and further assessed the neuroprotective ability of metallothionein against METH-induced toxicity in culture. In our previous study, we demonstrated that metallothionein isoforms can scavenge different reactive oxygen species i.e., superoxide and hydroxyl radicals. Our current data demonstrates that METH increases the production of reactive oxygen species (ROS), decreases intracellular ATP levels and finally reduces the cell viability. Pretreatment with zinc markedly prevented the loss of cell viability caused by METH treatment via up-regulating the metallothionein expression and reducing the generation of reactive oxygen species and ATP depletion. Furthermore, our results suggest that generation of reactive oxygen species by METH is an early event that precedes cell death and metallothionein protects these cells from METH-induced toxicity via scavenging the reactive oxygen species and thus preventing the ATP depletion.

Acknowledgment: The authors greatly acknowledge Royal Golden Jubilee Award from the Thailand research fund that supports AA.

PS3D-06

New evidence for DNA nuclease activity of alpha-synuclein: a molecular mechanism in understanding Parkinson's disease

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The pathological hallmark of Parkinson's disease is the formation of insoluble protein aggregates known as *Lewy bodies*. The major constituent of these fibrillar structures is α-synuclein, a 140 amino acid protein with a basic amino terminal and an acidic carboxy terminal. Little information is available about the neurobiology of α-synuclein under normal and neurodegenerative conditions. Recent observations have shown that α-synuclein is localized in the

chromatin region of nuclei in the brain. Moreover, the presence of the majority of the lysine residues in the N-terminal region of α-synuclein suggests a possible DNA binding role for α-synuclein. We demonstrated in the present study, two novel properties of α-synuclein. Firstly, α-synuclein binds to DNA and alters the conformation of DNA. Secondly, α-synuclein has DNA nicking activity and it behaves like a nuclease enzyme. These are new evidences on α-synuclein binding to DNA. It was also observed that the nicking activity involves the formation of only single strand breaks. The altered DNA topology is studied using Electron Microscopy, Gel studies etc. Further, the ability of known nuclease inhibitors, Aurintricarboxylic acid (ATA) and Diethyl pyrocarbonate (DEPC) to abolish DNA nicking activity of α-synuclein is evidenced. It has also been shown that conformational change or oligomerisation of α-synuclein would enhance the nicking activity. This indicates that the oligomers of α-synuclein are more toxic in terms of DNA nicking than monomers and aggregates. The potential implications of the above *in vitro* findings to neurodegenerative changes associated with PD will be discussed.

PS3D-07

Redistribution of cytochrome c and apoptosis inducing factor in dopamine neurons after exposure to MPP^+ and rotenone

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Oxidative stress and mitochondrial dysfunction contribute to neuronal injury in Parkinson's disease (PD), where dopamine (DA) neurons of the ventral mesencephalon (VM) are sensitive to the redox milieu. The neurotoxins, MPTP and rotenone, which produce experimental models of PD, are inhibitors of mitochondrial complex I. The release of apoptogenic factors (e.g. cytochrome c and apoptosis inducing factor (AIF)) from mitochondria to activate death cascades may also be a consequence of oxidative stress. Here the roles of cytochrome c and AIF in the apoptosis of DA neurons were investigated in primary VM cultures after exposure to MPP^+ and rotenone. Cultures were established from embryonic rat VM and maintained under serum-free conditions (96 well plates and glass coverslips) in NBM/B27. At d6 cultures were exposed (48 h) to MPP^+ (300 μM) and rotenone (30 μM). Cellular injury was assessed by confocal microscopy and MTT assay. Cells were fixed for immunocytochemistry (AIF, cytochrome c, tyrosine hydroxylase (TH)) and labeled with propidium iodide (PI) for confocal microscopy. MPP^+ and rotenone produced injury that was slow in time-course, but extensive at 48 h: injury was attenuated by Z-DVED-fmk (40 μM). Neurite blebbing and cell body shrinkage were minimal at 24 h, but widespread at 48 h when 30% and 40–50% of TH-positive and -negative neurons, respectively, displayed apoptotic nuclei. At shorter timepoints PI labeled few apoptotic profiles, and cytochrome c was generally not found in the cytosol, but release occurred at longer time intervals. AIF was redistributed earlier than cytochrome c in TH-positive neurons with both insults, however the reverse was true in MPP^+-treated TH-negative neurons with cyt c being released first. These data provide new insights into the role of AIF and apoptotic injury in DA neurons, which involve caspase-sensitive and -insensitive mechanisms, and mitochondrial activation with the differential redistribution of apoptogenic proteins.

PS3D-08

Nitric oxide, a mediator in rotenone-induced Parkinsonism in rats

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A specific and potent mitochondrial complex-I inhibitor, rotenone has been shown to cause parkinsonian like symptoms in rats but the etiology of it is still not known. We examined the role of nitric oxide (NO), if any, in mediating dopaminergic neurotoxicity exerted by unilateral intranigral infusion of rotenone in Sprague-Dawley rats. For these, we studied the level of inducible nitric oxide synthase (iNOS) mRNA and NADPH-diaphorase (NADPH-d) activity in the substantia nigra pars compacta (SNpc) region after rotenone treatment. Our results revealed an up-regulation of iNOS 96 hours after rotenone administration in the nigral region and intense NADPH-d staining in the SNpc on the 5th day following the administration of rotenone. We also found significant decrease in striatal complex I activity and dopamine (DA) levels, and decreased tyrosine hydroxylase (TH) immunoreactivity in rotenone treated SNpc. Interestingly, a relatively specific neuronal NOS inhibitor, 7-nitroindazole, and a non-specific NOS inhibitor, N-ω-nitro-L-argenine methyl ester significantly attenuated rotenone-induced (i) complex I inhibition, (ii) increased NADPH-d enzyme activity in SNpc, (iii) loss of TH immunoreactivity in both SNpc and the striatum, (iv) DA depletion in the striatum, as well as (v) apomorphine and amphetamine-induced circling behavior. Upregulation of the synthesizing machinery, increase in the rate limiting enzyme activity and reversal by NOS inhibitors of the neurotoxic parameters included in the present study provide convincing evidences for the involvement of NO in rotenone-induced dopaminergic neurodegeneration.

PS3D-09

Mitochondrial involvement in Parkinson's disease: evidence from animal models, human brains and PD cybrids

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Investigations in our laboratory employing animal models of Parkinson's disease (PD) have generated data that indicated involvement of mitochondria. This prompted us to investigate intrinsic cell signaling pathways that led to apoptosis in neurons of the brains of rats intracranially administered neurotoxins such as MPP^+, homocysteine and rotenone. We employed HPLC-electrochemistry, patch clamp recording, fluorimetry, immunoblot and RT-PCR respectively for measuring free radicals, membrane currents, many of the capsases activity, the proteins and mRNA levels of mitochondria-linked signaling in the brain. For the first time, we report here a retrograde mode of neuronal death operating in rats treated with MPP^+. We also report here the mitochondrial intrinsic pathway operating in the dopaminergic neuronal death caused by MPP^+ and rotenone. Using a number of antioxidants such as quercetin, melatonin and keeping L-deprenyl as a positive control we have observed that the former drug molecules act via interfering the signaling events in the neurons. Coenzyme Q_{10} and vitamin D_3 were also tested for neuroprotective or therapeutic effects, and

report here that these molecules also interfere with neuronal signaling. We also demonstrate here the loss of complex-I and –IV activities as well as changes in some of the protein subunits in the postmortem brains of PD patients. Establishment of PD- and age-matched cybrids helped us to verify the loss of mitochondrial complex-I and -IV activities, as well as changes in a number of protein subunits of the electron transport chain enzymes. The work carried out in the various animal models, human brains and cybrids provided significant data in support for a direct involvement of mitochondrial electron transport chain dysfunction as a major causative factor of PD.

PS3D-10

Oxidative stress and MAPK activation are implicated in tetrahydrobiopterin-induced dopaminergic cell death in SH-SY5Y cells

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Oxidative stress has been proposed as a potential mechanism responsible for degeneration of dopaminergic neurons in substantia nigra (SN) seen in Parkinson's disease (PD). Evidence has shown that endogenous molecules present in dopaminergic neurons may play a role in creating such damaging oxidative environment. Tetrahydrobiopterin (BH4) is an obligatory cofactor for tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine (DA) synthesis. Exogenously applied BH4 (100–400 μM) to human SH-SY5Y neuroblastoma cells resulted in cell death in a dose dependent manner as observed by cytotoxicity assays. Intracellular reactive oxygen species (ROS) were found to be elevated along with the increasing dose of BH4 as determined by measurement of dichlorofluorescein (DCF) fluorescent intensity, indicating the importance of oxidative stress. Western blot analysis showed that mitogen-activated protein kinase (MAPK), p38 and extracellular signal-regulated kinase (ERK), were activated within 15 min upon BH4 exposure to SH-SY5Y cells. Pretreatment with SB203580 (10 μM) or PD98059 (20 μM), specific inhibitors of p38 and ERK, respectively, could attenuate BH4-induced cell death. Our data suggest that extracellular BH4 are toxic to dopaminergic neurons partly through its ability to generate ROS and activate signaling molecules such as p38 and ERK, which eventually lead to cell death. This increase in ROS production may be a result of BH4 autoxidation and may involve the oxidation of DA, rendering the selective vulnerability of dopaminergic neurons. However, this link between BH4 and DA oxidation needs to be further elucidated.

PS3D-11

Behavioral alterations and dopaminergic deficit following acute intranigral homocysteine administration in rodents: relevance to Parkinson's disease

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Homocysteine is implicated in a number of geriatric multisystem disorders including coronary artery diseases, stroke, dementia and cognitive abnormalities, depression, cerebral atrophy and psychosis. A number of neurodegenerative disorders, viz., Parkinson's disease (PD), Alzheimer's disease and Huntington's disease are suggested to be associated with hyperhomocysteinemia. Elevated plasma levels of this molecule in PD patients receiving long-term L-DOPA therapy prompted us to investigate whether homocysteine is neurotoxic to the nigrostriatal dopaminergic system in Sprague-Dawley rats. Animals infused unilaterally in substantia nigra pars compacta (SNpc) with different doses of homocysteine (250–1000 nmol in 1 μl) caused dose dependent dopamine loss in the ipsilateral striatum. Animals received the highest dose of the neurotoxin exhibited significant abnormalities in stride-length and spontaneous and dopaminergic drug-induced circling behavior. Amphetamine administration on the 14th day in the animals receiving the highest dose of homocysteine caused ipsilateral, while apomorphine treatment on the 16th day elicited contralateral turning behavior. In these animals a clear loss of neurons was visible in SNpc, which are shown to be dopaminergic in nature by tyrosine hydroxylase immunoreactivity. While 3,4-dihydroxyphenylacetic acid level in the striatum showed a dose-dependent decrease, homovanillic acid was found to be inhibited only for the highest dose. Neurotransmitter levels in the serotonergic perikarya or terminals were unaltered 19 days following intra-raphe infusion of homocysteine, which suggested the specificity of its action to dopaminergic neurons. The results of the present study provide evidence for the neurotoxic nature of homocysteine to dopaminergic neurons, and suggest that L-DOPA induced chronic hyperhomocysteinemia in treated PD patients may enhance the progression of the disease.

PS3D-12

Amantadine, but not trihexyphenidyl, adjunct therapy with levodopa reduces oxidative stress

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Oxidative stress is incriminated to play a central role in Parkinson's disease (PD) and increasing evidence shows reactive oxygen species induced alterations outside the brain as well, particularly in blood cells. Previous studies prompted concern about the possible cytotoxic nature of antiparkinsonian medications and the purpose of this study was to evaluate and correlate the changes in the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in erythrocytes of PD patients taking amantadine or trihexyphenidyl as an adjunct therapy with levodopa. Activities of antioxidant enzymes were determined by established assays in 45 PD patients: levodopa monotherapy $n = 13$, levodopa with amantadine $n = 12$ and levodopa with trihexyphenidyl $n = 20$. Patients treated with levodopa in combination with amantadine showed higher CAT activity ($P = 0.024$) when compared with levodopa monotherapy. Though elevated levels of SOD and GPx were also witnessed in this group, they were not significant. No significant changes were found in levodopa with trihexyphenidyl group when compared with levodopa monotherapy. Our results show that amantadine adjunct therapy with levodopa reduces oxidative stress to an extent by improving CAT activity while trihexyphenidyl does not show this property.

PS3D-13

Leucine-rich repeat kinase 2 (LRRK2) mRNA expression in the mouse brain: effects of dopamine depletion

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Recently mutations in the gene encoding LRRK2 (Leucine-rich repeat kinase 2) have been identified as the cause of familial parkinsonism at the PARK-8 locus. In order to learn more about the possible function of LRRK2 in the CNS we used in situ hybridisation to investigate the distribution of LRRK2 mRNA in the mouse brain and also to look for possible changes in expression of this transcript in two mouse models of parkinsonism, namely the MPTP treated mouse and a VMAT2 hypomorph developed in this laboratory. (Mooslehner *et al.* 2001). Radioactive 'in situ' hybridisation using mouse LRRK2 specific antisense oligonucleotides end labelled with ^{35}S-dATP showed high levels of expression of LRRK2 transcripts in neurones in the cerebral cortex, caudate-piutamen, nucleus acumbens, olfactory tubercle and cerebellum. Moderate levels of expression were detected in neurons in the hippocampal CA regions (CA1-CA3) and the dentate gyrus. Interestingly only very low levels of LRRK2 transcript were detected in the dopamine cells of the substantia nigra. Given the strong LRRK2 signal in the striatum of the mouse we used quantitative 'in situ' hybridisation and grain counting to monitor changes in expression of LRRK2 in the VMAT2 hypomorph and MPTP treated mice (30 mg/kg). MPTP treated mice were killed at 7 and 14 days after a single MPTP injection when dopamine levels were substantially reduced (≈ 90% depletion). There was no detectable change in caudate-putamen levels of expression of LRRK2 transcript in the VMAT2 mice despite a striatal dopamine depletion of around 85–90% of normal (Mooslehner *et al.* 2001). There was however a decrease in LRRK2 mRNA in the MPTP treated mice at 7 days post injection but by 14 days striatal expression had returned to normal paralleling recovery of dopamine cell tyrosine hydroxylase content. The focus of LRRK2 expression in the basal ganglia, at least in the mouse is the caudate – putamen suggesting that LRRK2 kinase activity will be important in signalling in striatal output neurons. How this possible role in striatal signalling relates to the ability of mutations in this kinase in man to cause parkinsonism remains to be established and development of transgenic or knock-out mice for this gene may clarify this issue.

PS3D-14

Association of MAO-B intron 13 polymorphism with smoking and risk for Parkinson's disease

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Monoamine oxidase B (MAO-B), a mitochondrial enzyme, plays an important role in metabolism of dopamine and neurotoxin e.g. MPTP, and therefore, has been suggested to be involved in human behavior e.g. smoking and Parkinson's disease (PD), which have been shown to be inversely associated with each other by many previous studies. MAO-B polymorphism A to G substitution in the intron 13 has been found to affect its enzyme activity and be associated with smoking behavior and risk for Parkinson's disease but little information is available in Chinese. It is also not known whether the relationship between MAO-B and smoking modifies the inverse association between smoking and risk for PD. In order to further investigate the relationship among MAO-B polymorphism, smoking and risk for PD, we examined the distributions of MAO-B intron 13 polymorphism in both Chinese PD and controls. Total 176 PD subjects and 354 community normal controls were recruited and detailed information on smoking habit was collected. DHPLC and sequencing methods were applied to detect the intron 13 A/G substitutions. MAO-B G allele was significantly associated with smoking behavior in men ($P = 0.025$, OR = 7.02, 95% CI 0.92–147.07), while it was not associated with smoking in women ($P = 0.486$). Smoking was found to provide a dose and duration dependent protective effect for PD ($P = 0.000$, OR = 0.06, 95% CI 0.02–0.17). MAO-B genotype was not associated with increased risk for PD ($P = 0.122$), and did not modify the inverse association between smoking and risk for PD when PD patients were stratified according to MAO-B genotype ($P = 0.000$,OR = 0.06 in A allele, $P = 0.017$,OR = 0.07 in G allele). In conclusion, our study supports that MAO-B polymorphism is involved in smoking behavior but does not affect the role of smoking on PD, suggesting that smoking is an independent protective factor for PD development.

PS3D-15

Melatonin protects D-amphetamine-induced cell death in SK-N-SH dopamine cell

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Several hypotheses regarding the mechanism underlying amphetamine-induced neurotoxicity have been proposed. One of them is based on the observation of free radical formation and oxidative stress produced by auto-oxidation of dopamine. The formation of dopamine-related reactive oxygen species such as superoxide and hydroxyl radicals appears to play an important role in amphetamine-induced neurotoxicity. Melatonin, a secretory product of the pineal gland, plays a key role in a variety of important physiological response including free radical scavenging. The objective of this study was to investigate the effect of melatonin on D-amphetamine-induced neurotoxicity in cultured SK-N-SH cells. The present results showed that D-amphetamine significantly decreased cell viability as determined by the 3-(4, 5-dimethyl thiazol-2-yl)-2, 5 di phenyl-tetrazolium bromide (MTT) assay, in a concentration-dependent manner, and this effect was abolished by melatonin. In addition, D-amphetamine induced the production of reactive oxygen species (ROS) and decreased intracellular ATP levels; these effects were significantly prevented by the presence of melatonin. Understanding the antioxidative stress effect of melatonin may lead to the understanding of the mechanism by which D-amphetamine induces neuronal degeneration.

Acknowledgment: This work was supported by a Mahidol University Research Grant (PG) and a Royal Golden Jubilee Fellowship (AA, SK).