Poster Prize Winners

P-01

DISORGANIZATION OF NEUROFILAMENTS IN NEURONS RESULTS IN NEUROPATHOLOGICAL CHANGES IN YOUNG MICE WITH DISRUPTION OF EXPRESSION OF NEUROFILAMENT LIGHT SUBUNIT

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It is still not fully understand what pathological changes would be in earlier pathogenesis of neurodegenerative diseases when there is no obvious clinical sign of neural dysfunction. We have reported that in early age of mice with interruption of expression of neurofilament light subunit (NFL−/−), neurofilament high subunit (NFH) were moved away from the axon, NFH and NFM were accumulated in cytoplasm of motor neurons and at the same time microglial functions were suppressed. In the current study, we have investigated morphological alterations in axons of descending motor neurons in the white matter of the NFL−/− spinal cord when neurofilament organization has been disrupted. The diameters of axons became smaller and twisted. There were partial demyelination and remarkable increase in thickness of the myelin sheath in NFL−/− mice, especially in smaller axons ($\Phi < 2\,\mu m$). The length of paranodes from axonal diameter ranging from 0.5 to 1 μm in 6 month old NFL−/− mice was longer than that of controls. Immunohistochemical staining suggested disorganization of the Na^+ channels at the node of Ranvier and K^+ channels in the juxtaparanode in the white matter of the spinal cord after deletion of the NFL gene. Western blot has shown that there was no significant difference in the amount of MAG and CNPase between knockout and wild-type mice at 2 and 6 months. The results showed that profound pathological changes can be detected even before onset of neurological signs, suggesting that the earlier diagnosis and treatment are of importance in therapeutic strategy.

P-02

DEVELOPMENT OF AN ARTIFICIAL NEURONAL NETWORK WITH POST-MITOTIC RAT FETAL HIPPOCAMPAL CELLS BY POLYETHYLENIMINE

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The selection of appropriate surface materials that promote cellular adhesion and growth is an important consideration when designing a simplified neuronal network *in vitro*. In the past, extracellular matrix proteins such as laminin (LN) or positively charged substances such as poly-l-lysine (PLL) have been used. In this study, we examined the ability of another positively charged polymer, polyethyleneimine (PEI), to promote neuronal adhesion, growth and the formation of a functional neuronal network *in vitro*. PEI, PLL and LN were used to produce grid-shape patterns on glass coverslips by micro-contact printing. Post-mitotic neurons from the rat fetal hippocampus were cultured on the different polymers and the viability and morphology of these neurons under serum-free culture conditions were observed using fluorescent microscopy and atomic force microscopy (AFM). We show that neurons cultured on the PEI- and PLL-coated surfaces adhered to and extended neurites along the grid-shape patterns, whereas neurons cultured on the LN-coated coverslips clustered into clumps of cells. In addition, we found that the neurons on the PEI and PLL-coated grids survived for more than two weeks in serum-free conditions, whereas most neurons cultured on the LN-coated grids died after one week. Using AFM, we observed some neurosynapse-like structures near the neuronal soma on PEI-coated coverslips. These findings indicate that PEI is a suitable surface for establishing a functional neuronal network *in vitro*.

P-03

SRC SUPPRESSED C KINASE SUBSTRATE REGULATES THE LIPOPOLYSACCHARIDE-INDUCED TNF-α BIOSYNTHESIS IN RAT ASTROCYTES

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The protein kinase C (PKC) is known to be a critical component in the signaling cascades that lead to astrocyte-activation. To further understand the mechanism of PKC signaling in astrocyte-activation, we investigated the effect of SSeCKS, a PKC substrate, on LPS-induced cytokine expression in astrocytes by RT-PCR and enzyme- linked immunosorbent assay. Exposure of the cells to LPS induced rapid translocation of SSeCKS to the perinuclear sides, ERK activation and pronounced TNF-α production, which can be inhibited by the PKC inhibitor Gö6983. By using siRNA knockdown of SSeCKS expression, LPS-induced signaling events were partly inhibited, including ERK activation, inducible TNF-αbiosynthesis and secre- tion. These results suggest that SSeCKS is involved in the LPS-induced TNF-alpha expression in astrocytes mediated by PKC.

P-04

EFFECT OF NOURISHING ''YIN'' REMOVING ''FIRE'' CHINESE HERB MIXTURE ON THE HYPOTHALAMIC KISSPEPTIN EXPRESSION IN PRECOCIOUS RATS

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Nourishing ''Yin'' -removing ''Fire'' Chinese herb mixture, a Chinese herb-based formulation, has been successfully used for the treatment of idiopathic precocious puberty (IPP) for more than thirty years. Precocious model rats induced by danazol were used to investigate the effect of the herb mixture on the hypothalamic kisspeptin expression, which is the ''central processor of GnRH''. Female Sprague-Dawley rats were divided into intact normal (N), IPP model (M), vehicle with no IPP (V), IPP model exposed to herb mixture (HM) and IPP model exposed to saline (S) groups. On postnatal day 5 (P5), a single subcutaneous injection of 300 μg danazol was given to establish precocious model rats. From P15, rats in HM were continuously fed with the mixture 2 ml, until 2 consecutive regular estrous cycles were established. The hypothalamic Kiss-1 expression was detected by RT-PCR and immunohistochemistry. The day of vaginal opening and establishment of two regular estrous cycles were delayed in HM compared with M and S ($P<0.05$, respectively). Hypothalamic Kiss-1 mRNA, kisspeptin-immunoreactive (kisspeptin-ir) cells in arcuate nucleus (ARC), preoptic area (POA) and periventricular nucleus (PeN), the both were decreased significantly in HM than those in M and S ($P<0.01$, respectively) on the day of onset-puberty. The nourishing ''Yin'' -removing ''Fire'' Chinese herb mixture could significantly delayed the sexual development of the precocious rats, and down-regulate the expression of hypothalamic Kiss-1 mRNA and kisspeptin-ir in ARC, POA and PeN on the day of onset-puberty. Based on above, kisspeptin signaling pathway might involve in the effective treatment of herb mixture on IPP.

P-05

ALTERED GENE EXPRESSION PROFILES IN APPV717I-CT100 TRANSGENIC MICE

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Many carboxy-terminal (C-terminal) fragments of the human amyloid precursor protein (APP) are neurotoxic, including β amyloid (Aβ) and APP-CT100. We used a transgenic mouse model expressing the familial Alzheimer's disease (AD) ''London mutation'' with the V717I (valine to isoleucine) substitution within APP-CT100, which includes the Aβ sequence. β amyloid, in the form of diffuse non-congophilic extracellular plaques, deposits in the brain of transgenic mice expressing APPV717I-CT100 from 6 months of age. To elucidate the neurodegenerative mechanisms of AD, we evaluated several genes that might be involved in APP pathology in 11-month-old APPV717I-CT100 transgenic mice using microarray analysis. We compared the profiles of the altered genes in the hippocampal region of transgenic mice with that of age-matched wild-type transgenic mice. We selected several genes that might have essential roles in AD pathology and their altered expression in the cortical and hippocampal brain tissues used in the microarray anaysis was confirmed with RT-PCR and Western blot analysis. In addition, the expression pattern of the selected genes was examined in human AD brain by immunohistochemistry and Western blot, and their pathogenic mechanisms were studied in an APP intracellular domain -overexpressing cell culture system. These findings suggest that APPV717I-CT100 overexpression selectively alters the levels of specific transcripts in the brains of transgenic mice. This study provides valuable information about several AD-related genes and their pathogenic roles.

P-06

A DIRECT EVIDENCE FOR THE RECIPROCAL REGULATION OF ASTROCYTES AND FOS POSITIVE NEURONS IN THE NEUROPATHIC PAIN STATE

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Emerging research implicates the participation of astrocytes in the neuropathic pain induced by nerve injury. However, the underlying relationship between neurons and astrocytes is not so clear. Using a L5 spinal nerve ligation pain model, we provide here a direct evidence for the reciprocal regulation of astrocytes and neurons after nerve injury-induced neuropathic pain. Glial fibrillary acidic protein (GFAP) was used as the astrocytic specific marker and Fos, protein of the protooncogene c-*fos* was used as a marker of activated neurons. Spinal nerve liagation induced a significant mechanical allodynia which can be attenuated by intrathecal administration of c-*fos* antisense oligodeoxynucleotides or astroglial toxin L-α-aminoadipate. Double immunofluorescent histochemistry indicated that most of the Fos positive cells were nuclei of activated neurons. However, intrathecal administration of c-*fos* antisense oligodeoxynucleotides significantly suppressed the astrocytic activation, as well as the up-regulation of Fos protein expression induced by nerve inury. And astroglial toxin shortened the duration of Fos expression after SNL. These results indicate that neuronal and astrocytic activation are closely related with each other in neuropathic pain state. Our study offered a direct evidence for the neuronal-astrocytic crosstalk under neuropathic pain states. It emphasizes that we should consider neuronal and non-neuronal elements integrally in nociceptive transmission. And breaking this interaction may offer some novel pain treatment strategy.

POSTERS

Neurodegeneration

P-07

CHARACTERIZATION OF A NOVEL *P*-TYPE ATPASE CHOLESTEROL TRANSPORTER IN BRAIN

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Brain contains the highest levels of cholesterol. Synthesized locally, cholesterol is transported from glial cells to neurons. However, little is known of how cholesterol transport is achieved from cell organelles to plasma membrane and regulated in brain. Our studies in membrane trafficking over the last ten year led to an identification of a brain-specific ATPase. Molecular cloning of the gene showed a *P*-type ATPase transporter with multiple transmembrane domains. Following development of monoclonal antibodies, we found that the novel ATPase is localized predominantly in the substantia nigra as well as cerebral cortext on plasma membrane. In addition, the ATPase is also localized in the endosome, lysosome and Golgi vesicular transport systems. ATPase activity analysis and molecular interaction studies revealed that the novel ATPase bound to cholesterol. Binding to cholesterol regulated the ATPase activity, whereas binding to adenine nucleotides also regulated cholesterol binding. Moreover, we found that the *P*-type ATPase transporter interacted with Niemann Pick C2 (NPC2) that also binds cholesterol and is mutated in Niemann Pick C2 disease. Thus, our work suggests that the novel *P*-type ATPase is a cholesterol transporter that collaborates with NPC2 in cholesterol homeostasis in brain, and is involved in neurodegenerative diseases.

P-08

CLONING, EXPRESSION AND PURIFICATION OF MOUSE ESTROGEN RECEPTOR (ER) β AND ITS INTERACTION WITH NUCLEAR AND MITOCHONDRIAL PROTEINS OF MOUSE BRAIN

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Estrogen exerts multiple effects in brain through two well characterized estrogen receptors (ER) α and ERβ. These have three functional domains – *N terminal transactivation domain* (*TAD*) containing activation function (AF)-1, *DNA binding domain* (*DBD*) and *C terminal ligand binding domain* (*LBD*) containing AF-2. ERα and β act through LBD in response to estrogen, its agonist or antagonist by recruiting coregulators (coactivators or corepressors) in nuclear gene regulation. Recently, ERβ ERβ has been located in mitochondria and hypothesized its possible role in mitochondrial gene regulation. Here, we studied the interaction of LBD with the nuclear and mitochondrial proteins of mouse brain. For that mouse ERβ LBD was subcloned in a prokaryotic expression vector pRSETA. Then, pRSETALBD was transformed into BL21 (DE3) competent cells, and expressed as a recombinant protein by induction with isopropyl -β-D thiogalactoside (IPTG). Subsequently, it was detected by western blot analysis and purified by nickel affinity column chromatography as 22 kDa and its dimer. Furthermore, we used purified mERβ LBD for pull down assay to study its interaction with the nuclear and mitochondrial proteins from male and female mouse brain. We found six proteins in the range of 45–160 kDa interacting with nuclear and mitochondrial proteins in both sexes. The present study will lead to the identification of coregulators and elucidation of their function in the mouse brain with advancing age, and to understand their role in estrogen action during neurological disorders and dementia in old age.

P-09

MEMANTINE SUPPRESSES THE CHANGES IN THE LEVELS OF GLIAL CELLS, NEUROPEPTIDES AND THEIR METABOLIZING ENZYMES IN Aβ-INDUCED AD MODEL RAT BRAIN REGIONS

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Soluble forms of amyloid β (Aβ) have recently been considered to be responsible for cognitive dysfunction prior senile plaque formation in Alzheimer's disease (AD) brain, although their mechanism is not well understood. We therefore examined the effects of repeated intracerebroventricular infusion of soluble $A\beta_{25\text{-}35}$ on peptidergic system and glial cells as well as their possible cross talk in the pathogenesis of AD. The present study further aimed to investigate the protective effects of memantine on $A\beta_{25\text{-}35}$-induced changes in peptidergic and glial systems. Infusion of $A\beta_{25\text{-}35}$ decreases the level of somatostatin (SS) and substance P (SP) in the hippocampus prior to neuronal loss. Immunohistochemical studies with GFAP and CD11b, markers of astroglia and microglia respectively, and biochemical experiment with prolyloliopeptidase (POP) and endopeptidase 24.15 (EP 24.15), peptide-degrading enzymes, demonstrate a concomitant increase in the hippocampus. Double immunostaining experiments of EP 24.15 and GFAP/CD11b antibodies clearly demonstrated the co-localization of neuropeptidases with astrocytes and microglia. Treatment with memantine significantly attenuated $A\beta_{25\text{-}35}$-induced changes of neuropeptides, their metabolizing enzymes and glial marker proteins. Taken together, the present data suggest that

memantine exerts its protective effects by modulating the neuropeptide system as a consequence of suppressing the glial cells in AD model rat brain regions.

P-10
POSTMORTEM AUTOPSY ADULT HUMAN BRAIN MATERIAL USED TO ESTABLISH MICROGLIAL AND OLIGODENDROCYTES CELL CULTURES

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The present study describes the simultaneous establishment of highly enriched human adult microglial and oligodendrocyte cell cultures. Brain tissue specimens were collected at rapid autopsy with short postmortem delay (3–6 hours) from various regions of the CNS of Alzheimer's disease, Pick's disease and non-demented control cases. Cultures were derived from the subcortical white matter, corpus callosum, and frontal, temporal, and occipital cortex. The adherent microglial cells were immunoreactive for CD68, CD45, CD11c, and major histocompatibility complex (MHC) class II markers, and were not immunoreactive for astrocyte or oligodendrocyte markers. In addition, some functional characteristics of the isolated microglial cells were also studied. Upon stimulation with lipopolysaccharide (LPS), microglial cells secreted pro- and antiinflammatory mediators, i.e., interleukin- (IL)-6, prostaglandin E2 (PGE2), and IL-10, indicating the functional capacity of cultured microglia. We developed a method to isolate viable glial cells from human adult brain tissue as surgical material was not available. Non-adherent cells isolated from the same brain tissue samples expressed oligodendrocyte-specific markers. The current described culture system may provide a valuable tool in studying human CNS biology and disease. Cell cultures are dynamic, highly accessible and permit direct experimental manipulations where cause-effect relations can be more definitely assayed.

P-11
THE EFFECT OF LOW DOSE OF EXTRACELLULAR BETA-AMYLOID (Aβ) IN INDUCING NEW Aβ PRODUCTION IN CULTURED HIPPOCAMPAL NEURONS OF ADULT RAT

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Introduction: Alzheimer's disease (AD) progression involves beta-amyloid peptide (βAP) aggregation as plaques. These depositions in extracellular space of neurons have been linked to extensive neuronal degeneration in AD brains. βAP is normally produced as a nontoxic soluble peptide but it is cleared from neuronal tissues and its remaining will result in βAP aggregation. The exact mechanisms underlie extracellular βAP elevation which result in plaque producing is unknown. The present study investigated whether fibrilar βAP affected the neurons to produce more βAP in hippocampal neuronal cultures of adult rats.
Methods: We used fibrilar beta-amyloid $(A\beta)_{42}$ as the most toxic part of βAP plaques in low nontoxic levels at the concentrations of of 2×10^{-6}, 2×10^{-5} and 2×10^{-4} μM and then we measured extracellular Aβ1-40. Our findings show that even very. The adult neurons were exposed to $(A\beta)_{42}$ for 6 days. Beta-amyloid $(A\beta)_{40}$ measurement has been performed as the most prevalent part secreted βAP in plaques by $(A\beta)_{40}$ assessment in extracellular medium through ELISA technique.
Results: Our findings show that βAP can induce its own production in a dose dependent manner. It can be as one of the basic mechanism for extracellular βAP elevation in AD.
Conclusion: It could be suggested that remaining even low levels of fibrillar Aβ in extracellular space could possibly trigger a lethal positive feedback procedure that leads to extracellular Aβ elevation.

P-12
DJ-1 AND α-SYNUCLEIN INFLUENCE CELLULAR SENSITIVITY TO OXIDATIVE STRESS BY ERK KINASE SIGNALING PATHWAY

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DJ-1 and α-synuclein are associated with oxidative stress and mutations in them are involved in the onset of Parkinson's disease (PD). The ERK kinase signaling pathway plays important roles in neuroprotection during oxidative stress. Whether there are correlation between DJ-1and α-synuclein with ERK is unknown. To answer this question we overexpressed DJ-1 and α-synuclein respectively in two cell lines and evaluated the level of ERK phosphorylation. We first found DJ-1 significantly increased the phosphorylation of ERK while α-synuclein decreased the level of ERK phosphorylation. We then found the change of ERK induced by DJ-1 and synuclein were dependent on MEK and always accompanied with the change of protein phosphatase 2A (PP2A), the main inhibitor of ERK signaling pathway dephosphorylating and inactivating MEK and ERK family kinases. Moreover, inhibition of ERK pathway with ERK1/2 inhibitor was able to eliminate the change of sensitivity to oxidative stress caused by DJ-1 and α-synuclein. Based on these results, we concluded that DJ-1 and synuclein could affect the activation of ERK and further influenced the sensitivity of cells to oxidative stress.

P-13
THE LOSS OF NEOCORTICAL $P2Y_2$ NUCLEOTIDE RECEPTORS IS ASSOCIATED WITH ALZHEIMER'S DISEASE NEUROPATHOLOGY

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The uridine nucleotide-activated $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors are widely expressed in the brain and are involved

in many CNS processes, including those which malfunction in Alzheimer's disease (AD). However, the status of these receptors in the AD neocortex, as well as their putative roles in the pathogenesis of neuritic plaques and neurofibrillary tangles, remain unclear. In this study, we used immunoblotting methods to measure $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors in the postmortem neocortex of a cohort of neuropathologically assessed AD patients and aged controls. $P2Y_2$ immunoreactivity was found to be selectively reduced in the AD parietal cortex, while $P2Y_4$ and $P2Y_6$ levels were unchanged. In contrast, all three receptors were preserved in the occipital cortex, which is known to be minimally affected by AD neuropathology. Furthermore, the loss of parietal $P2Y_2$ immunoreactivity correlated with both neuritic plaque and neurofibrillary tangle scores, but not with dementia severity. These results provides a basis for adding $P2Y_2$ receptor loss to the list of neurochemical abnormalities which may contribute to AD neuropathology, and point towards uridine nucleotide-activated P2Y receptors as novel targets for disease-modifying AD pharmacotherapeutic strategies.

P-14
ALPHA-SYNUCLEIN INCREASES CELL ADHESION MOLECULE, CD44 AND MT1-MMP

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One of the pathological features of Parkinson's disease (PD) is loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). It has been known that α-synuclein, is one of the major causative agents of PD. During the dopaminergic neuronal loss in SNpc, activated microglia were centered in SNpc. Existing reports indicate microglial activation by aggregated α-synuclein hence, microglial recruitment into the SNpc is important in the pathogenesis. We hypothesize that α-synuclein may play a role in microglial activation and to perform the neuronal cytotoxicity. We demonstrate that α-synuclein induces the CD44 expression on microglia which participates in cell adhesion with surrounding extracellular matrix (ECM). To induce the cell migration, proteolytic processing of CD44 is required. We found that α-synuclein also enhanced MT1-MMP (membrane-type 1 matrix metalloproteinase) to shed off CD44 at the cell surface and degrades surrounding ECM to open the migratory way. A53 T mutant α-synuclein showed greater level of CD44 shedding and cell migration. Extracellular treated α-synuclein also increased CD44 and MT1-MMP expressions dose-dependently as in the transfected cells. Among the multiple signaling pathways, ERK pathway was involved in α-synuclein induced cell migration. These induced cell migration were also confirmed in human PD patients. These findings shed a new light on the functional link between α-synuclein and microglial migration in the pathogenesis of Parkinson's disease.

P-15
DIFFERENT CONCENTRATIONS OF FIBRILLAR Aβ 1–42 PROMOTE HIPPOCAMPAL ADULT NEURONS INTO THE DIFFERENT PHASES OF CELL CYCLE

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Introducton: The expression of cell cycle proteins in terminally differentiated neurons precedes cell death in Alzheimer's disease (AD). This attempt by neurons to re-enter mitosis is a response to mitogenic stimuli like beta amyloid (Aβ). It was a question that in many areas of AD brains, cell cycle markers appeared without obvious plaque formation. In the other hand, cell cycling re-entry is not an immediate cause of cell death AD. Instead the affected neurons live for many months before death with the Alzhimerous morphology such as cell cycle markers expression and tau pathology. Because abnormalities in mitotic mechanisms are early events in AD, we examined the possibility that picomolar levels of Aβ 1–42 could trigger the cell cycle re-entry and the effect of different concentrations of Aβ in promoting neurons to different cell cycle phases.
Methods: Adult neuronal cultures were treated with Aβ 1–42. Cyclin D1 and B1 (G1 and G2 phase markers) were assessed by immunocytochemistry and apoptosis by TUNEL.
Results: Treatment with toxic concentrations of Aβ resulted in extensive apoptosis but to lower levels of Aβ promotes the neurons into G1 and G2 phase relative to the treated doses, without noticeable apoptosis.
Conclusion: It suggests that very low doses of Aβ induce neurons to re-enter cell cycle and its different concentrations have variable progressing strength for promoting neurons to enter into different phases of cell cycle, or causing their death. It could explain why some neurons in AD would be degenerated while the others just show elevated cell cycle markers and Alzheimerous pathology.

P-16
INTRACELLULAR DOMAINS OF AMYLOID PRECURSOR-LIKE PROTEIN 2 INTERACT WITH CP2 TRANSCRIPTION FACTOR IN THE NUCLEUS AND INDUCE GLYCOGEN SYNTHASE KINASE-3BETA EXPRESSION

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Amyloid precursor protein (APP) is a member of a gene family that includes two APP-like proteins, APLP1 and 2. Recently, it has been reported that APLP1 and 2 undergo presenilin-dependent gamma-secretase cleavage, as does APP, resulting in the release of an approximately 6 kDa intracellular C-terminal domain (ICD), which can translocate into the nucleus. In this study, we demonstrate that the APLP2-ICDs interact with CP2/LSF/LBP1 (CP2) transcription factor in the nucleus and induce the expression of glycogen synthase kinase 3beta (GSK-

3beta), which has broad-ranged substrates such as tau- and beta-catenin. The significance of this finding is substantiated by the *in vivo* evidence of the increase in the immunoreactivities for the nuclear C-terminal fragments of APLP2, and for GSK-3beta in the AD patients' brain. Taken together, these results suggest that APLP2-ICDs contribute to the AD pathogenesis, by inducing GSK-3beta expression through the interaction with CP2 transcription factor in the nucleus.

P-17
GEPHYRIN IN ALZHEIMER'S DISEASE

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"Gephyrin," derived from the Greek word meaning "bridge", binds inhibitory glycine and $GABA_A$ receptors to the subsynaptic cytoskeletal elements, serving as an important receptor-microtubule linker for the assembly and stabilisation of inhibitory post-synaptic terminals in the central nervous system. Excitotoxicity, or neuronal loss due to excessive excitatory transmission, depends on the balance of excitatory and inhibitory factors, and has been postulated by several research groups as a crucial factor contributing to synapse loss in Alzheimer's disease. Thus aberrant gephyrin levels in AD might contribute to disease pathology by altering the normal inhibitory modulation of excitation impulses. This is the first study of gephyrin in relation to AD. Gephyrin protein levels were investigated in two AD susceptible areas and a spared area in *post mortem* brain tissues from normal ($n = 15$) and AD ($n = 15$) patients. Quantification of the protein levels was achieved by interpolation from known concentrations of a recombinant truncated gephyrin protein containing the immunogenic epitope. A significant reduction ($P < 0.01$) in gephyrin was seen in the disease condition. Analysis, using indices of pathological severity, suggested that gephyrin levels decline until a moderate pathological condition is reached, but rise thereafter. This may indicate a compensatory mechanism for the excessive excitatory damage in the final stages of the disease. A second immunoreactive band was detected, presumed to be a splice variant of gephyrin, in all the cases in all three brain areas which requires further characterisation. This study contributes to the understanding of excitotoxicity in AD.

P-18
α-SYNUCLEIN FRAGMENTS INVOLVE IN MITOCHONDRIAL OF MN9D CELLS

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder, however, there still existed controversial ideas on the mechanisms, and the α-synuclein-centric theory and mitochondrial dysfunction theory are related to PD closely. Most of PD patients were found to have the phenomenon of mitochondrial dysfunction. α-synuclein can act with the membrane of mitochondrial through it's special domains which can influence the mitochondrial structures by binding to mitochondrial membranes. Previously, our group found α-synuclein can co-localize with mitochondrial, but how does it form this function and which part of α-synuclein can really act with mitochondrial? To make those clear we cloned the α-synuclein fragments which were named as N-terminal (from 1–65aa of α-synuclein), NAC terminal (from 60–95aa of α-synuclein) and C-terminal (from 96–140aa) and insert them into retroviral vector then made stable expressing cell models in packing cell lines. We use MN9D as target cells which were infected by virus to detect various indexes. We found that α-synuclein/N seems to have colocalization with mitochondrial while others don't. Over-expressing NAC demonstrated that some kind of aggregations appeared in nuclear, and a cluster of different cells like apoptosis which can induce extremely lower mitochondrial membrane potential qualified by JC-1 staining which were tested by flow-cytomertic assay. Over-expressing N-terminals may be toxic to target cells, over-expressing α-synuclein/N results the lower mitochondrial membrane potential, however, after a period time this phenomenon can be tolerated. This research can make clearly the exact function of α-synuclein and enhance our understanding of the role of mitochondrial dysfunction in Parkinson's disease.

P-19
DSCR1 OVEREXPRESSION INDUCES CASPASE-3 DEPENDENT NEURONAL APOPTOSIS

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Individuals with Down syndrome (DS) will inevitably develop Alzheimer's Disease (AD) neuropathology including neuritic plaques, neurofibrillary tangles and neuronal loss in various brain regions after middle age. The mechanism underlying neurodegeneration in AD and DS remains elusive. The Down Syndrome Critical Region 1 (DSCR1) gene is located on Chromosome 21 and has been implicated in the pathogenesis of DS. Our data show that DSCR1 protein expression is elevated in the cortex of DS and AD patients. To investigate the mechanism of DSCR1 upregulation in AD, we characterized the molecular transcription of *DSCR1* isoform 1. *DSCR1* isoform 1 expression can be activated by the stress hormone dexamethasone. A functional glucocorticoid response element

(GRE) was identified in *DSCR1* isoform 1 promoter region. Our study provides a novel mechanism by which DSCR1 is upregulated by overactivity of HPA axis in AD brain. Furthermore, we show that overexpression of DSCR1 in primary neurons activates caspase-9 and caspase-3 and subsequently induces neuronal apoptosis. Furthermore, we found that the neurotoxicity of DSCR1 is inhibited by knockout of caspase-3 in caspase-$3^{-/-}$ neurons. Our study provides a novel mechanism by which DSCR1 function as a mediator of stress and Aβ-induced neuronal death. Our studies demonstrate that overexpression of DSCR1, due to extra copy of DSCR1 gene on chromosome 21 or HPA overactivity in AD, leads to neurodegeneration and contributes to AD pathogenesis.

P-20

HSP22 IS INVOLVED IN SELECTIVE VULNERABILITY OF NIGRAL DOPAMINERGIC NEURONS EXPOSED TO 6-OHDA

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Selective vulnerability of neuronal populations in neurodegenerative diseases is exemplified by Parkinson's disease (PD), in which there is relative vulnerability among neighboring midbrain populations of neurons releasing the same neurotransmitter, dopamine (DA): A9 and A10 group. A9 DA neurons are preferentially affected in PD, whereas A10 DA neurons are relatively spared. Similar patterns of degeneration appear in rodents and primate models of PD and even postmortem of human PD brains, indicating that the physiological differences between A9 and A10 DA neurons may be conserved between species. Certain proteins differentially expressed in A9 and A10 DA neurons may play critical roles in susceptibility to or protection against neurodegenerative processes in PD. In the present study, expressions of several proteins were validated in A9 and A10 DA neurons. *In situ* hybridization showed higher expression of HSP22 in A9, and immunohistochemistry indicated HSP22 positive cells by *in situ* hybridization were almost TH positive. Real-time PCR also indicated the different expression of HSP22 between A9 and A10 DA neurons. Knocking down HSP22 by transfecting siRNA increased survival of MES23.5, the DA cell line, against 6-OHDA. On the contrary overexpression of HSP22 in MES23.5 decreased its survival significantly. In the primary VM cultures overexpressed HSP22 increased the susceptibility to 6-OHDA. Results suggested that HSP22 is predominantly expressed in A9 dopaminergic neurons, and HSP22 appeared to increase the susceptibility of MES23.5 and primary VM cultures to 6-OHDA.

P-21

THE ROLE OF PINK1 IN AUTOPHAGY OF MN9D

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Mutations in PINK1 cause autosomal recessive Parkinson's disease (PD). PINK1 encodes a putative serine/threonine kinase with an N-terminal mitochondrial targeting sequence and PINK1 protein is mainly located inside mitochondria. There is evidence that PINK1 is one of the component of Lewy's bodies. In this study, We have generated stable dopaminergic cell lines MN9D knocked down PINK1 using small interfering RNA (siRNA). Lines knocked down PINK1 show: autophagic cell death, disruption of the mitochondrial cristae, loss of mitochondrial membrane potential, and subsequent entrapment of disorganized mitochondria within autophagosomes or autophagolysosomes along with degradation of mitochondrial proteins were noted, showing that PINK1 is involved in autophagy in which mitochondria serve as the main target. These findings support the hypothesis that PINK1 participates in the protection of dopaminergic neurons through autophagic passway.

P-22

ASSOCIATION PINK1 OF WITH α-SYNUCLEIN

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Parkinson's disease (PD) is one of the most frequent neurodegenerative disorders. The pathological hallmarks are the massive loss of dopaminergic neurons in the pars compacta of the substantia nigra and presence of Lewy bodies (LBs) α-synuclein and pink1 have been found to be the major components of LBs. They both effect mitochondrial function and cellar apoptosis. Here we try to find the possible mechanism – the relation between α-synuclein and pink1 in PD pathogenesis. A GST-α-synuclein fusion was created by inserting α-synuclein into pGEX4T. The protein was expressed in Escherichia coli and purified on glutathione-Sepharose beads. An approximately equimolar amount of GST or GST-α-synuclein was mixed with same amount of MN9D cells lysates. Pre-equilibrated glutathione-Sepharose beads were added and washed after further incubation. The bound proteins were eluted by buffer and were detected by SDS–PAGE followed by autoradiography. We detected that only pink-1 was retained by GST-α-synuclein. For co-immunoprecipitation (IP), following the precipitation of MN9D cells lysates with precleared protein A/G-agarose beads and anti-α-synuclein antibodies, the immune complexes were released

from the beads and subjected to 10% SDS–PAGE. The precipitates were then analyzed by WB using rabbit anti-pink-1 antibodies. IP revealed that α-synuclein specifically interacted with pink-1. In summary, here we report a specific interaction between α-synuclein and pink-1 that was confirmed *in vitro* by GST pull-down and *in vivo* by IP.

P-23

ALPHA-SYNUCLEIN LOCATED IN MITOCHONDRIA AND INVOLVED IN MITOCHONDRIAL PATHOLOGY IN RAT

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The pathological features of Parkinson's disease are the selective loss of dopaminergic neurons in the substantia nigra (SN), and the presence of Lewy bodies (LB) which contain alpha-synuclein (αSNY) in the surviving SN dopaminergic neurons. Triplication of the wild-type (WT) locus has been linked to autosomal dominated Parkinson's. Adeno-associated viral (AAV) vector-mediated overexpression of αSNY has been shown to cause neurodegeneration of the nigrostriatal dopaminergic pathway in rodents and primates. However, the physiological function of this protein, which might help explain its involvement in pathological processes, remains poorly understood. In this work, the AAV carrying αSNY cDNA (rAAV-αSNY) were unilaterally injected into the SN and leaded to decrease of tyrosine hydroxylase positive neuron in SN compared with the injection of AAV alone. The silver intensified gold staining was employed to describe the ultrastructural distribution of alpha-synuclein and demonstrates that the protein is also found associated with mitochondria in rat SNpc neurons. Mitochondrial abnormalities, perinuclear cytoplasmic inclusions contain αSNY and neuron apoptosis were also found in the SN of rats overexpressing αSNY. These data suggest that αSNY has a direct interaction with mitochondria. Furthermore, overexpression of αSNY lead to mitochondrial abnormalities which may form Lewy bodies (LB).

P-24

GSK3β AND ENDOPLASMIC RETICULUM STRESS MEDIATES ROTENONE-INDUCED DEATH OF SK-N-MC CELLS

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Rotenone, an environmental toxin that inhibits mitochondrial complex I, has been used to induce experimental Parkinsonism in animals and cell cultures. We investigated the mechanism underlying rotenone-induced death of SK-N-MC neuroblastoma cells. Rotenone-induced cell death preceded intracellular accumulation of reactive oxygen species and antioxidants failed to protect cells, indicating that oxidative stress was minimally involved in rotenone-induced death of SK-N-MC cells. Glycogen synthase kinase 3β (GSK3β), a multifunctional serine/threonine kinase, has been implicated in the pathogenesis of neurodegeneration. We showed that rotenone activated GSK3β by enhancing its phosphorylation at tyrosine 216 while inhibiting phosphorylation at serine 9. Inhibitors of GSK3β and dominant negative (kinase deficient) GSK3β partially protected SK-N-MC against rotenone cytotoxicity, indicating that GSK3β activation was involved in rotenone cytotoxicity. Rotenone also induced endoplasmic reticulum (ER) stress which was evident by an increase in phosphorylation of PERK, PKR, eIF2α and the expression of GRP78. Rotenone had a modest effect on the expression of CHOP. An eIF2α siRNA significantly reduced rotenone cytotoxicity. ER stress was experimentally induced by tunicamycin and thapsigargin; but tunicamycin/thapsigargin did not activate GSK3β in SK-N-MC cells. Down-regulation of eIF2α also offered a partial protection against tunicamycin/thapsigargin cytotoxicity. Combined treatment of GSK3β inhibitors and eIF2α siRNA provided much greater protection than either treatment alone. Taken together, the results suggest that GSK3β activation and ER stress contribute separately to rotenone cytotoxity.

P-25

EXPRESSION OF PHOSPHORYLATED α-SYNUCLEIN IS INCREASED IN THE ROTENONE-INDUCED RAT MODEL OF PARKINSON'S DISEASE

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The deposition of the α-synuclein as fibrillary aggregates in the dopaminergic neurons of the substantia nigra (SN) is a hallmark lesion in Parkinson's disease (PD). The predominant modification of α-synuclein in Lewy bodies is a single phosphorylation at Ser 129. In the present study, phosphorylated α-synuclein expression is increased and the retrograde degenerative changes in the dopaminergic neurons of SN in rotenone-induced rat model of PD. Twenty-four male SD rats

were selected and divided into rotenone group and control group. rotenone group were stereotactically injected with rotenone to the left SN and Medial forebrain bundle (MFB), respectively. Praxiology change was observed at days 7, 14, 21, 28 after surgery. Mean speed and 30-minute movement distance were calculated with Ethovison software. Twenty Eight days after injection,animals were deeply anesthetized and killed. Tyrosine hydroxylase(TH)-positive neurons and phosphorylated α-synuclein expression of left SN were demonstrated with immunohistochemical staining. The number of TH-positive neurons and phosphorylation of α-synuclein average gray value in left SN were measured. The mean speed and 30-minute movement distance in the rotenone group were lower than those in the control group ($P<0.05$). TH-positive neurons counts in left SN in the rotenone group was lower than that in the control group ($P<0.01$). Mean gray values of α-synuclein phosphorylation in lesioned substantia nigra of the rotenone group was lower than the control group ($P<0.01$). The lower gray value, the expression is stronger. our results indicate that phosphorylated α-synuclein expression is increased by sterotaxic injection of rotenone, suggesting rotenone-induced rat model can reproduce neuropathological features of PD.

P-26

MATRIX METALLOPROTEINASE-3 PLAYS A CRITICAL INTRACELLULAR ROLE DURING APOPTOSIS OF DOPAMINERGIC CELLS

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We have previously demonstrated that the active form of matrix metalloproteinase-3(actMMP-3) is released from dopamine(DA)rgic neurons undergoing apoptosis. Herein, whether actMMP-3 might be generated intracellularly, and if so, whether it is involved in apoptosis of DArgic neurons itself was investigated in primary cultured DA neurons of wild type and MMP-3 knockout animals and CATH.a cells. During apoptosis, gene expression of MMP-3 is induced, specifically among the various classes of MMPs, generating the proform (55kD), which is subsequently cleaved to the catalytically active actMMP-3 (48kD) involving a serine protease. Intracellular actMMP-3 activity is directly linked to apoptotic signaling in DArgic cells: 1) Pharmacologic inhibition of enzymatic activity, repression of gene expression by siRNA, and gene deficiency all lead to protection; 2) Pharmacologic inhibition causes attenuation of DNA fragmentation and caspase-3 activation, the indices of apoptosis; and 3) Inhibition of the proapoptotic enzyme JNK leads to repression of MMP-3 induction. Under the cell stress condition MMP-3 is released as actMMP-3 rather than proMMP-3, and catalytically active MMP-3 added to the medium does not cause cell death. Thus, actMMP-3 seems to have a novel intracellular role in apoptotic DArgic cells and this finding provides an insight into the pathogenesis of Parkinson's disease.

P-27

GELSOLIN, AN ACTIN-BINDING PROTEIN, REGULATES AMYLOID BETA-PROTEIN FIBRILLOGENESIS IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by the deposition of amyloid plaques extracellularly and of neurofibrillary tangles intracellularly. Amyloid beta-protein (Aβ) is a major component of amyloid plaques. Under normal conditions, Aβ is present in soluble form. However, Aβ gets fibrillized and deposits as amyloid plaques in the brains of AD patients. The identification of compounds that can inhibit the fibrillization of Aβ or dissociate the Aβ fibrils are considered to be of importance for the therapeutic intervention in AD. We have reported previously that extracellular (plasma) gelsolin binds to Aβ and inhibits its fibrillization. Gelsolin exists as cytoplasmic and extracellular forms. Compared with cytoplasmic gelsolin, the plasma form has an extension of 25 amino acids residues at its amino-terminus. In addition, five Cys residues in cytoplasmic gelsolin are free thiols, while three Cys residues in plasma gelsolin are free thiols and other two are disulfide-linked. We report here that Aβ also forms complex with cytosolic gelsolin in PC 12 cells. These results suggest that structural differences between plasma and cytoplasmic gelsolin do not play a key role in their complex formation with Aβ. It also suggests that intracellular Aβ fibrillization may also be regulated by cytoplasmic gelsolin.

Stroke/Hypoxia

P-28

DOPAMINE D_2 RECEPTOR FUNCTIONAL REGULATION IN THE CEREBRAL CORTEX OF HYPOXIA INDUCED NEONATAL RATS: EFFECT OF GLUCOSE, OXYGEN AND EPINEPHRINE TREATMENT

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Brain is sensitive to reductions in oxygen supply. Hypoxia leads to neuronal functional failure and neuro-developmental delay with molecular changes with permanent or transitory neurological sequelae. The initial resuscitation of newborns asphyxiated at birth was practiced with administration of 100% oxygen, epinephrine and 10% glucose. The neonatal resuscitation were not analysed at the molecular level for brain function. We investigated the dopamine D_2 gene expression in the cerebral cortex of hypoxia induced neonatal rats and role of oxygen, epinephrine and glucose, in regulating the receptor function due to hypoxia. Wistar 4-days neonatal rats were used for experiments and grouped as follows: Control (C); Hypoxia (Hx); Hypoxic rats injected with 10% dextrose (500 mg/Kg body wt) (i.p.)(Hx + G); Hypoxic rats injected with 10% dextrose and treated with 100% oxygen (Hx + G + O); Hypoxic rats, 10% dextrose and epinephrine (0.1 μg/Kg body wt) i.p. and then treated with 100% oxygen (Hx + G + E + O). DA and HVA contents significantly decreased in Hx compared to C. Glucose treatment to Hx-Hx + G and Hx + G + O reversed the DA and HVA contents. Gene expression studies using Real-time PCR for dopamine D_2 receptor showed a significant increase in hypoxia which was reversed to near control in Hx + G and Hx + G + O + E. Dopamine modulates cGMP mediated Ca^{+2} release in the brain. We observed a decrease in cGMP content in hypoxic groups whereas in Glucose treatment to Hx- Hx + G and Hx + G + O reversed to control. Our study suggests that glucose supplementation to hypoxic neonatal rats play an immense role in reversing the altered dopamine D_2 in the developing brain. This has clinical significance in neonatal care with intellectual developmental life.

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P-29

TRANSCRIPTION FACTOR EARLY GROWTH RESPONSE-1 INDUCTION MEDIATES INFLAMMATORY GENE EXPRESSION AND BRAIN DAMAGE FOLLOWING TRANSIENT FOCAL ISCHEMIA

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Early growth response-1 (Egr1) is a sequence-specific transcription factor (TF) which is induced under hypoxic conditions. We presently report that transient middle cerebral artery occlusion (MCAO) leads to increased expression of Egr1 in the brains of adult mice and rats between 2 hour to 5 days of reperfusion with a peak increase of 8 to 12 fold at 1 day. When subjected to transient MCAO and 3 days of reperfusion, $Egr1^{-/-}$ mice showed significantly smaller infarcts (by $44.9 \pm 8.4\%$, $P<0.05$) and improved neurological function than $Egr1^{+/+}$ littermates. Following transient MCAO, brains of $Egr1^{-/-}$ mice showed less water accumulation and decreased neutrophil infiltration (by $42 \pm 8\%$, $P<0.05$) compared to $Egr1^{+/+}$ mice. The number of activated microglia/macrophages were also significantly lower ($OX42^+$ cells by $53 \pm 9\%$, $P<0.05$ and $ED1^+$ cells by $59 \pm 11\%$) in the post-ischemic cortex of $Egr1^{-/-}$ mice compared to $Egr1^{+/+}$ mice. In addition, post-ischemic inflammatory gene expression was less pronounced in the brains of $Egr1^{-/-}$ mice compared to $Egr1^{+/+}$ mice. Preventing cerebral Egr1 protein induction with small interference RNAs that target Egr1 decreased inflammatory gene expression and led to smaller infarcts (by $40.2 \pm 6.9\%$, $P<0.05$) and reduced neurological deficits in rats subjected to transient MCAO. Conversely, transient MCAO following adenoviral-mediated Egr1 overexpression exacerbated the infarct volume (by $29 \pm 5.3\%$, $P<0.05$) and worsened the neurological deficits in rats. These studies indicate Egr1 as a significant contributor of inflammation and neuronal damage after stroke.

Acknowledgment: Supported by US NIH.

P-30

PHYSOSTIGMINE IMPROVES SPATIAL MEMORY IMPAIRMENT IN RATS FOLLOWING HYPOBARIC HYPOXIA

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Hypobaric hypoxia (HBH), a predisposing environmental condition at high altitude (HA) faced by mountaineers, sojourners has deleterious effect on cognitive functions. We hypothesized that disturbances of cholinergic system may be involved in HA induced memory impairment. The present study was aimed to investigate the involvement of cholinergic system in HBH induced memory impairment and the effects of Physostigmine (PHY) in its prevention. Rats were exposed to HBH 6100 m for 7 days and memory functions were evaluated in Morris Water Maze. Neuronal damage and apoptotic cell death were studied by morphological changes and TUNEL staining. Cholinergic markers like acetylcholine (ACh), acetylcholinestrase (AChE), cholineacetyltransferease (ChAT), α7nicotinic acetylcholine receptor (α7nAChR) and M1 muscarinic acetylcholine receptor (M1 mAChR) were investigated. There was impairment of memory function after exposure to HBH as indicated by increased latency and path length and rats supplemented with PHY showed improvement in memory functions. Cellular damage including DNA fragmentation was observed after HBH exposure and treatment with PHY improved these changes. Decreased ACh level and increased AChE activity were also observed in HBH exposed animals with significant down regulation of M1mAChR, ChAT and α7nAChR. PHY treatment resulted in significant increase in ACh level, decrease in AChE activity and upregulation of M1 mAChR, ChAT and α7 nAChR levels. Our data suggested that HBH induced memory impairment may be due to disturbances in cholinergic system and decrease in ACh level may be due to the increased activity of AChE or decreased synthesis of ChAT activity where as PHY ameliorated HBH induced memory impairment by improving cholinergic system.

P-31

TRANSCRIPTION FACTOR EARLY GROWTH RESPONSE-1 MEDIATES INFLAMMATORY GENE EXPRESSION AND BRAIN DAMAGE FOLLOWING STROKE

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Early growth response-1 (Egr1) is a sequence-specific transcription factor which is induced under hypoxic conditions. We presently report that transient middle cerebral artery occlusion (MCAO) leads to increased expression of Egr1 in the brains of adult mice and rats between 2 hours to 5 days of reperfusion with a peak increase of 8 to 12 fold at 1 day. When subjected to transient MCAO, $Egr1^{-/-}$ mice showed significantly smaller infarcts (by $44.9 \pm 8.4\%$, $P<0.05$) and improved neurological function than $Egr1^{+/+}$ littermates. Brains of $Egr1^{-/-}$ mice also showed less water accumulation, decreased neutrophil infiltration (by $42 \pm 8\%$, $P<0.05$), and lower numbers of OX42 and ED1 positive activated microglia/macrophages (by $57 \pm 11\%$; $P<0.05$) compared to $Egr1^{+/+}$ mice. In addition, post-ischemic inflammatory gene expression was less pronounced in the brains of $Egr1^{-/-}$ mice compared to $Egr1^{+/+}$ mice. Preventing cerebral Egr1 protein induction with siRNAs that target Egr1 decreased inflammatory gene expression and led to smaller infarcts (by $40.2 \pm 6.9\%$, $P<0.05$) and reduced neurological deficits in rats subjected to transient MCAO. Conversely, transient MCAO following adenoviral-mediated Egr1 overexpression exacerbated the infarct volume (by $29 \pm 5.3\%$, $P<0.05$) and worsened the neurological deficits in rats. These studies indicate Egr1 as a contributor of inflammation and neuronal damage after stroke.

Acknowledgment: Supported by US NIH.

P-32

POTENTIAL CARDIAC CAUSES OF ISCHEMIC STROKE IN YOUNG ADULTS: A STUDY FROM THE INSTITUTE OF NEUROLOGY, SRI LANKA

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Ischemic stroke in young adults (<45 years) is proportionately more common in India 19–32% and Sri Lanka 10.- 34% in contrast to 5–9% in the West. The objectives was to study the potential cardiac causes of ischemic stroke in young adults (< 45 years). Sociodemographic data, potential cardiac causes and laboratory investigations were recorded in 41[21 male and 20 female (3 patients below the age of 15 years)] patients with first-ever ischemic stroke occurring on or less than 45 years of age. 11 (27%) [8 male and 3 female between 15–45 years and 1 female less than 15 years] had potential cardiac causes. 5 (12%) patients had valvular heart diseases, 4 (10%) [3 male and 1 female] with mitral valve prolapse (MVP): (2 with mitral regurgitation (MR) and 1 with mitral stenosis and MR) and 1 male had tricuspid regurgitation, 2 females had atrial septal defect, one had patent foramen ovale (PFO) with right to left shunt, 1 male with cardiomyopathy and irregular rhythm, 1 male with irregular rhythm, one male with myocardial infarction, 1 female had a history of rheumatic fewer. All patients were Sinhalese except one Tamil male with MVP with MR and MS. Potential cardiac causes are more common among male compared to females, 3 of the 4 patients with MVP had MR being the most common potential cause of ischemic stroke in young adults.

P-33

ALTERATIONS OF GLUCOSE METABOLISM AND ATP LEVELS IN THE BRAIN OF MOUSE REPEATEDLY EXPOSED TO HYPOXIA

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Upon hypoxia, newborn mammals are usually more effective in down-regulation of their metabolic rates than their adults. Except depression of the metabolism, hypoxia stimulates ATP production by anaerobic glycolysis in order to match the reduced energy demand under the status of hypoxic hypometabolism. We have developed an animal model in which a mouse, when repeatedly exposed to hypoxia, increases its hypoxic tolerance by several folds and represent a hypometabolic state, which is featured by dramatic reduction in oxygen consumption, body temperature, and heart and respiration rates. In this study, several rate-limiting proteins in the mouse brain were investigated in order to know the possible change in the activity of the anaerobic glycolysis pathway in the hypoxia-exposed mouse. During repeatedly hypoxic exposures (HE), the levels of GLUT1, GLUT3, PFK and LDH mRNAs in the brain increased during the first three HE but fell down to control values after the fifth HE. Quite similar to the mRNA expressions, the protein levels of GLUT1, GLUT3, PFK and LDH also up-regulated during the first three HE but reduced to control values after the fifth HE. Very interestingly, the brain ATP levels decreased during the first two HE but recovered afterwards. No morphological changes were observed in the brain of the mouse. The above results suggest that the activity of the anaerobic glycolysis and ATP levels in the mouse brain undergo a regular change during repeated hypoxic exposures, which may be an adaptable regulation for the brain to increase its hypoxic tolerance.

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Drugs of Abuse

P-34
RATS PRENATALLY EXPOSED TO HEROIN–NEUROTRANSMITTER CHANGES DURING WEANLING AND ADULTHOOD

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Previous studies in our lab showed prenatal heroin exposure in rats caused postnatal (PN) increase in locomotor activity and rearing plus a decrease in habituation rate at PN3weeks. These rats showed behavioral recovery of behavioral to normal level in adulthood (PN3months) reflecting strong developmental plasticity. Pair-fed animals on the other hand showed a decrease in locomotor activity in adulthood suggestive of developmental effects of prenatal malnutrition.

Purpose: To examine whether neurotransmitter changes in different brain regions at weanling and adulthood reflect the behavioral changes observed. Sprague-Dawley rats ($n = 9$ each comparison group) that were exposed to heroin (10 mg/kg/day, s.c.) prenatally from gestational day 8 to 20 were studied. Offspring borne to dams from pair-fed and free food and water groups were compared to the heroin-exposed group.

Results: Heroin-exposed rats showed an increased dopamine (DA) turnover at PN3wks in the nucleus accumbens (increased DOPAC and HVA) and hypothalamus (increased DOPAC). At PN3 months, there is a decrease in D1 and D2 receptor binding in the frontal cortex in the females. DA and/or DOPAC and HVA are significantly lower in the NAc, striatum and midbrain of the pair-fed group compared to controls.

Conclusion: Neurotransmitter changes are in concordance to increase locomotor activities observed in PN3weeks and the decrease in locomotor activities observed in pair-fed rats.

P-35
ROLE OF ASCORBATE ON PREVENTION OF MORPHINE ADDICTION IN RATS

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Today, addiction is one of the most important social problems. Morphine is an addictive drug which causes several alterations in human body. Both acute and chronic administration of morphine increase releasing of dopamine which leads to dependence and tolerance to it. One of the most important factors that prevent addicted people from abandonment is painful symptoms of withdrawal syndrome. So finding a method to decrease withdrawal symptoms can be a good protocol to defeat this challenge. Since vitamin C which is released from glutaminergic neurons is a modulator of central dopaminergic and glutaminergic transmissions, we decided to study role of it on prevention and decreasing physical dependence of morphine addiction in rats. In this study we evaluated withdrawal symptoms (e.g. jumping, wet dog shaking) after naloxane injection in sham (Normal saline IP injection), control (Fixed doses of morphine IP injection) and test (500 mg/kg vitamin C IP injection before morphine daily) groups. Our data showed that both the tendency and most of the withdrawal Sings (jamping, standing and wet dog shaking) in the test animals were significantly less than the control animals ($P < 0.05$). Our findings support the use of vitamin C as a potent agent in treatment of addicts.

P-36
THE ROLE OF THE CANNABINOID CB1 RECEPTOR AND DOWN-STREAM cAMP/DARPP-32/PP-2B SIGNAL IN THE NUCLEUS ACCUMBENS OF METHAMPHETAMINE-SENSITIZED RATS

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In this study, we intended to investigate the significance of brain CB_1 receptors on the development of behavioral sensitization to methamphetamine. Male Sprague-Dawley rats treated with chronic methamphetamine (4 mg/kg, i.p.) for either 7 or 14 days developed behavioral sensitization to methamphetamine at withdrawal day 7. A progressive decrease in numbers of CB_1 receptor (both *Bmax* and mRNA) but increase in binding affinity (*Kd*) was noticed during withdrawal days 3 to 7. Microinjection of CB_1 antagonist SR 147778 into the nucleus accumbens (NAc) at withdrawal day 7, significantly suppressed the methamphetamine sensitization. In NAc slices, acute incubation with CB_1 agonist CP 55940 dose-dependently enhanced cyclic AMP accumulation in sensitized rats; no change was noticed in controls. Consequently, treatment of CP 55940 induced a dose-dependent (10 nM–10 μM) phosphorylation on down-stream DARPP-32/Thr34 in sensitized rats, barely enhance the phosphoDARPP-32/T34 in control groups. Alternatively, both basal activity of PP-2B and CP 55940-induced changes in the amount of PP-2B in the NAc were both decreased in sensitized rats, but not in controls. Overall, we demonstrated that brain CB_1 receptor and its down-stream cAMP/DARPP-32/T34/PP-2B signaling are profoundly altered in methamphetamine-sensitized animals. The up-stream transcription regulator (s) involved in the CB1 expression and CB_1-D_2 dopamine receptor interplay are currently under investigation in construct expressed HEK-293 cells.

P-37

EFFECT OF MELATONIN ON AMPHETAMINE-INDUCED STRUCTURAL CHANGE OF NIGROSTRIATAL PATHWAY IN EARLY POSTNATAL RATS

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Chronic amphetamine (AMPH) abuse creates temporary or permanent disturbances in dopaminergic systems of the brain that may predisposed individual to parkinsonism. The formation of dopamine-related reactive oxygen species (ROS) such as superoxide and hydroxyl radicals appears to play an important role in amphetamine-induced neurotoxicity. Melatonin, the main secretory product of pineal gland, is well known for its protective effects that are currently attributed mainly to its radical scavenging and antioxidant properties. The present study has been performed to investigate the effect of melatonin on amphetamine (AMPH)-induced neurotoxicity in nigrostriatal pathway of the postnatal rats. Wistar rats at postnatal age 4 (P4) were injected with either D-AMPH alone or D-AMPH with 30 min-pretreated with melatonin daily for 7 days and sacrified on P10. The substantia nigra and caudate were, then, stained by an immunohistochemical technique using antibodies raised against tyrosine hydroxylase. There was a loss of dopaminergic neurons and nerve fibers in AMPH-treated substantia nigra. In addition, most of these neurons had morphological changes. The number of nerve fibers and terminals were also decresed in the caudate. Pretreatment with melatonin prevents AMPH-induced loss of these neurons and fibers in substantia nigra and caudate. Interestingly, there was an increasing of fibers in the melatonin-treated group much more than those in the control. The results of this study suggest that melatonin not only protects the AMPH-induced neurotoxicity, it also possibly promotes the neurite outgrowth.

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P-38

MELATONIN ATTENUATES EFFECT OF METHAMPHETAMINE ON MAMMALIAN TARGET OF RAPAMYCIN (MTOR) SIGNALING PROTEIN

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Methamphetamine (METH) is a well known drug abuses which damage nerve terminal by inducing dopamine (DA) release, causing formation of many reactive oxygen species (ROS), apoptosis, and finally neuronal damage. Autophagy is a type of program cell death independent of apoptosis, though whether autophagy is involved in METH-induced neurotoxicity is not yet clarified. Autophagy is negatively regulated by mammalian target of rapamycin (mTOR) signaling pathway. Therefore, in the present study we investigated the effect of METH on autophagy and its upstream regulator, mTOR signaling pathway. We found that METH dose dependently induced autophagy in SK-N-SH cells as detected by immunoblot analysis of LC3-II, a protein associated on autophagosome membrane. Moreover METH inhibited mTOR and its down stream target 4EBP1 activities as observed by their phosphorylation status. However upon mTOR activation, METH failed to induce autophagy, indicating that METH inhibited mTOR to induce autophagy in SK-N-SH cells. Melatonin, a hormone releases from pineal gland, has a protective effect against METH-induced neurotoxicity by inhibiting ROS production. We identified whether melatonin has an effect on METH-induced mTOR deactivation and METH-induced autophagy. We found that melatonin pretreatment promoted mTOR activity but has no effect on autophagy in cell expose to METH. In conclusion, we demonstrated that METH induced autophagy by reducing mTOR activity in SK-N-SH cells. Moreover, we identified a novel role of melatonin in promoting mTOR activity in METH treated cells, which might help explaining a neuroprotective effect of melatonin against METH induced toxicity.

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P-39

THE CIRCADIAN RHYTHM OF *PER1* GENE EXPRESSION IN THE RAT STRIATUM

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The circadian rhythm is generally regulated by the suprachiasmatic nucleus (SCN), the master clock. However, some circadian patterns of expression are controlled from outside of the SCN. The endogenous rhythmicity of the SCN is driven by negative and positive transcriptional-translational feedback loops considering the periodical expression of clock genes. Among clock genes, *Per1* is important for maintenance of circadian rhythmicity and entrainment to light cues. Outside of the SCN, there are at least two types of oscillations; a food-associated oscillation produced by daily restricted feeding and a methamphetamine associated oscillation produces by daily injection of methamphetamine. However, the mechanism underlying these SCN-independent circadian rhythms are unknown. It is interesting to determine whether a circadian rhythm of *Per1* exists in the striatum. In this experiment, adult (8 weeks old) rats were housed under LD 12:12 with lights on at 7 am and rats were sacrificed at different time of daily cycle. The striatum was dissected, mRNA expression of *Per1* gene was detected by real-time PCR. The result showed that *Per1* clearly expressed a circadian rhythm. It gradually increased at 2 pm and reached to the highest level at 10 pm. The level of highest expression (at 10 pm) is 230% of the lowest expression (at 10 am). The present result suggests clearly that the molecular rhythms exist in the striatum this may play some roles related to methamphetamine addiction.

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P-40

REDUCTION OF DEATH SIGNALING IN METHAMPHETAMINE-TREATED HUMAN NEUROBLASTOMA SH-SY5Y CULTURED CELLS BY MELATONIN

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Methamphetamine can increase oxidative stress, which regulates many pathways to induce neuronal cell death. Melatonin has property to exceed direct radical scavenging, and include up- and down-regulations of antioxidant and pro-oxidant enzymes, respectively. Several studies have demonstrated that melatonin can protect dopaminergic cell death caused by amphetamine. The objective of this study was to investigate the neuroprotective properties of melatonin on methamphetamine-induced induction of death signaling and neuronal cell degeneration in human neuroblastoma SH-SY5Y cultured cells. The results of the present study demonstrated that methamphetamine significantly decreased cell viability in SH-SY5Y cultured cells and melatonin reversed the toxic effects of methamphetamine. In addition, induction of Bax/Bcl-2 proteins was observed in SH-SY5Y cultured cells treated with methamphetamine and this effect was diminished by melatonin. These finding might demonstrate some protective roles of melatonin in methamphetamine-treated neuronal cells.

P-41

THE CHANGES OF SPATIAL LEARNING ABILITY AND NEUROGENESIS AFTER ACUTE CIGARETTE SMOKING EXPOSURE

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To investigate the effects of cigarette smoking on spatial learning ability and neurogenesis of immature mice, we gained 30 postnatal mice finally after filtrating the differences of physical and spatial learning ability through the tests of Rota rob and square water maze. Then treated them three times per day during 8 days with no cigarette (control), 2 cigarettes (high intensity) 30 min, and 1 cigarette (low intensity) 10 min separately. The training of Morris Water Maze started at the fourth day. After five days training, the animals were sacrificed and the brain slices were labeled with BrdU antibody and BSI-B4 separately. The results shows that the animals of CSE groups spent much more times than control before they reach the hidden plate ($P<0.05$) which reflect the impaired spatial learning ability. Meanwhile, the $BrdU^+$ and $BSI\text{-}B4^+$ cells in dentate gyruse decreased significantly ($P<0.05$, $P<0.001$). From the results above, we supposed that the impaired spatial learning ability quite possibly due to the inhibited neurogenesis and microglia. The parallel changes of spatial learning ability, neurogenesis and microglia revealed that there are some kinds of relationships among them.

P-42

IMPLICATION OF PARAFASCICULAR THALAMIC NUCLEUS IN THE DEVELOPMENT OF MORPHINE DEPENDENCE AND WITHDRAWAL OF RATS

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Parafascicular thalamic nucleus is a critical relay of ascending system that mediates motor control in central nervous system

(CNS). Yet, little was known about nPf whether or not it involves in the development of morphine dependence and withdrawal. In the present study, Wistar rats were used to destruct the nPf chemically by KA, and then animal models of morphine dependence and withdrawal were established. Morphine withdrawal symptoms were scored in each group. Electrophysiological method was invited to decide the changes of spontaneous discharge of nPf neurons. μ-opioid receptor (MOR) mRNA level in nPf was detected using RT-PCR. The ultrastructural alterations were examined by transmission electronic microscope. Results showed that the bilateral lesion of nPf had remarkable influence on the withdrawal syndrome of morphinomania rats ($P<0.05$) The average of frequency and the sum of nPf neurons which exhibited spontaneous discharging were increased in morphine withdrawal group in contrast with the control group ($P<0.05$, respectively). MOR mRNA in nPf was decreased in morphine dependence group in comparison with saline control group (1.45 ± 0.38 vs. 5.37 ± 0.94, $P<0.01$). In morphine withdrawal group which underwent 40 h morphine withdrawal, the significance was still obvious (2.97 ± 0.73 vs. 1.45 ± 0.38, $P<0.05$). Remarkable ultrastructural injuries of nPf neurons were detected including nucleus, organelles and neuropil. Our study indicated that nPf played an important role in the development of morphine dependence and withdrawal, which suggested that nPf might become one of the therapeutic targets when treating with the morphine withdrawal syndrome.

P-43
LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN BLOOD OF AMPHETAMINE ABUSERS

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Amphetamine (AMPH) is a currently drugs of abuse in throughout the world. It has been shown to be a potential brain neurotoxic effect by the production of free radicals. Reactive oxygen species indirectly generated by this drug have been indicated as an important factor in the appearance of neuronal damage. The data from human studies are limited. Therefore the purpose of this study was to examine the change in blood of amphetamine abusers (age between 17–48 years) used AMPH 180–360 mg/day in the duration of drug abused 1–10 year to compare the control group (age between 17–30 years) by measuring the parameters of oxidative stress, such as lipid peroxidation and antioxidant enzymes (i.e., glutathione peroxidase and catalase). Malondialdehyde, an end product of lipid peroxidation, content was significantly increased in amphetamine's users ($P<0.05$). But the activity levels of antioxidant enzymes were significantly decreased in amphetamine's users ($P<0.1$, $P<0.05$, respectively). Increased lipid peroxidation and decreased antioxidant enzymes activity in the blood of amphetamine's users indicate oxidative stress. These results indicated the important role of oxidative stress in amphetamine addiction.

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P-44
NUCLEUS ACCUMBENS CORE ACETYLCHOLINE IS SELECTIVELY ACTIVATED DURING DRUG VERSUS FOOD REINFORCEMENT ACQUISITION

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Acquisition of drug reinforcement is accompanied by a systematic increase of release of the neurotransmitter acetylcholine rather than dopamine, the expected prime reward neurotransmitter candidate, in the nucleus accumbens core (AcbC) (Crespo et al. 2006, J. Neurosci. 26, 6004–6010), with activation of both muscarinic and nicotinic acetylcholine receptors in the AcbC by ACh volume transmission being necessary for the drug conditioning. The present findings demonstrate that activation of the AcbC ACh system is selective for drug reinforcers, because (1) acquisition of food reinforcement was not paralleled by activation of ACh release in the AcbC whereas acquisition of morphine reinforcement (like that of cocaine or remifentanil, tested previously) was, and because (2) local intra-AcbC administration of muscarinic or nicotinic ACh receptor antagonists (atropine and mecamylamine, respectively) did not block the acquisition of food reinforcement whereas acquisition of drug reinforcement had been blocked. Interestingly, the speed with which a drug of abuse distributed into the AcbC and was eliminated from the AcbC determined the size of the AcbC ACh signal, with the temporally better defined drug stimulus producing a more pronounced AcbC ACh signal. In conclusion, activation of muscarinic or nicotinic ACh receptors in the AcbC during reward conditioning does not seem to represent a general learning phenomenon but seems to be selective for drugs of abuse.

Neuroprotection

P-45

NEUROPROTECTIVE EFFECT OF CURCUMIN AGAINST INHIBITION OF MITOCHONDRIAL COMPLEX I *IN VITRO* AND *IN VIVO*: IMPLICATIONS FOR PARKINSON'S DISEASE EXPLAINED VIA *IN SILICO* STUDIES

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Parkinson's disease (PD) is a progressive, age-associated neurodegenerative disease characterized by loss of dopaminergic neurons of the substantia nigra (SN). Oxidative/nitrosative stress mediated mitochondrial dysfunction within SN neurons is the central event during PD. Mitochondrial complex I is the central target for oxidative/nitrosative damage during mitochondrial dysfunction in these neurons. Peroxynitrite (PN), the chief reactive nitrogen species inhibits brain complex I probably by 3-nitrotyrosine and nitrosothiol formation. Our data indicate that pretreatment with the dietary polyphenol curcumin protects brain mitochondria against PN *in vitro* by direct detoxification and prevention of 3-nitrotyrosine formation and *in vivo* via elevation of cellular glutathione (GSH) level. We also show that curcumin treatment protects against GSH depletion mediated oxidative stress, protein oxidation and mitochondrial dysfunction. Using systems biology, we have built a dynamic *in silico* model linking curcumin, PN, GSH metabolism and mitochondrial physiology. "What if" analyses of the dynamic model corroborate the major findings of our experimental work. These data suggest that curcumin has potential therapeutic value for neurodegenerative diseases involving nitrosative stress and GSH depletion mediated oxidative stress.

P-46

POSSIBLE NITRIC OXIDE MODULATION IN THE PROTECTIVE EFFECT OF TRAZODONE, ATYPICAL ANTIDEPRESSANT AGAINST ANIMAL MODEL OF CHRONIC FATIGUE SYNDROME-INDUCED BEHAVIOURAL ALTERATIONS AND OXIDATIVE DAMAGE

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Present study was designed with an aim to elucidate the possible nitric oxide mechanism in the neuroprotective effect of trazodone by using mice model of chronic fatigue syndrome. Male albino laca mice ($n = 6$ in each group) were forced to swim for each 6 min session for 7 days and immobility period was measured on every alternate day. Trazodone (5 mg/kg, 10 mg/kg), was administered each day 30 min before the forced swimming test. However, L-arginine (100 mg/kg) and L-NAME (5 mg/kg) was administered 15 min before administration of Trazodone (5 mg/kg) treatment. Different behavior tests such as locomotor (actophotometer), anxiety (mirror chamber and plus maze test) followed by biochemical parameters (lipid peroxidation, reduced glutathione, catalase and nitrite) were assessed from animal brains. Present study showed that forced swimming for seven days caused a chronic fatigue like condition, anxiety like behavior, impairment in locomotor activity and oxidative stress (increased lipid peroxidation and nitrite levels and depleted reduced glutathione and catalase activity) in animals. L-NAME (5 mg/kg) pretreatment with trazodone enhanced neuroprotective effect of trazodone (5 mg/kg). However, L-arginine (100 mg/kg) pretreatment with trazodone (5 mg/kg) reversed the neuroprotective effect of trazodone ($P < 0.05$). Present study suggests the neuroprotective effect of trazodone possibly by L-arginine-nitric oxide-cyclic guanosine monophoshate signaling pathway.

P-47

INVOLVEMENT OF RAS PROTEINS IN HYDROGEN PEROXIDE AND MELATONIN-TREATED SH-SY5Y CULTURED CELLS

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It has been reported that overproduction of reactive oxygen species occurs after brain injury and mediates neuronal cell death. In addition, melatonin has been reported to have an efficient antioxidant capacity. In the present study, activation of cell death signaling by hydrogen peroxide and the protective mechanism against cell death by melatonin was investigated in dopaminergic neuroblastoma SH-SY5Y cells. An inhibitor of the enzyme that catalyzes the farnesylation of Ras proteins, FTI-277 and a high dose of guanosine 5′-O-(2-thiodiphosphate) or GDP-β-S, an inhibitor of G-protein activation significantly decreased hydrogen peroxide-induced reduction in cell viability in SH-SY5Y cultured cells. Melatonin is able to reverse the toxic effects of hydrogen peroxide on reduction in cell viability while low not high doses of GDP-β-S, diminished the protective effect of melatonin. The results of this study might indicate that a Ras-dependent signaling pathway plays a role in the death of cells and melatonin can protect and restore the function of hydrogen peroxide-treated SH-SY5Y cells.

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PROTECTION OF CELL DEATH AND SUSTAINED TYROSINE HYDROXYLASE PHOSPHORYLATION IN HYDROGEN PEROXIDE TREATED HUMAN NEUROBLASTOMA SH-SY5Y CULTURED CELLS BY MELATONIN

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The neuroprotective effect of melatonin against oxidative stress-induced neuronal cell degeneration in human SH-SY5Y neuroblastoma cells was investigated in the present study. The results of this study demonstrated that H_2O_2 significantly decreased cell viability in SH-SY5Y cultured cells. Desipramine, a monoamine uptake blocker was able to abolish the toxic effects of MPP^+ but not H_2O_2 on reduction of cell viability. Contradictory, melatonin was able to reverse the toxic effects of H_2O_2 on cell viability reduction. In addition, reduction of phosphorylation of tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis and phosphorylation of cyclic AMP responsive element-binding protein by H_2O_2 was also diminished by melatonin. These results demonstrate some effective roles of melatonin on neuroprotection and its action on the modulation of tyrosine hydroxylase phosphorylation.

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P-49

ENDOCANNABINOID 2-AG PROTECTS NEURONS BY LIMITING COX-2 ELEVATION

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Endocannabinoids are involved in synaptic signaling and neuronal protection, however, our understanding of the mechanisms by which endocannabinoids protect neurons from harmful insults remains elusive. 2-Arachidonoylglycerol (2-AG), the most abundant endogenous cannabinoid and a full agonist for cannabinoid receptors (CBRs), is a substrate for cyclooxygenase-2 (COX-2), and can be metabolized by COX-2. Here we show, however, that 2-AG is also capable of suppressing elevation of hippocampal COX-2 expression in response to proinflammatory and excitotoxic stimuli in mice. 2-AG prevents neurodegeneration from toxic assaults that elevate COX-2 expression, and inhibits the COX-2 elevation-enhanced excitatory glutamatergic synaptic transmission. The action of 2-AG on suppression of COX-2 expression appears to be mediated via the PTX-sensitive G protein-coupled CB1 cannabinoid receptor (CB1) and MAPK/NF-κB signaling pathways. Our results reveal that 2-AG functions as an endogenous COX-2 inhibitor protecting neurons from harmful insults by preventing excessive expression of COX-2, which provides a mechanistic basis for opening up new therapeutic approaches of protecting neurons from inflammation- and excitotoxicity-induced neurodegeneration.

P-50

GALANIN PROTECTS AGAINST INTRACELLULAR AMYLOID TOXICITY IN HUMAN PRIMARY NEURONS

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Galanin and galanin receptors are upregulated in the brain regions associated with Alzheimer's disease (AD). However, the consequence of this overexpression is still unknown, particularly in human neurons. Here, we investigate the time and dose effects of galanin to intracellular amyloid β $(Aβ)_{1–42}$ toxicity, as well as other insults including staurosporine, etoposide, hydrogen peroxide and serum depletion in cultured human primary neurons. The results show that galanin is protective against intracellular Aβ cytotoxicity and all of the above insults at sub-nanomolar physiological concentrations. The galanin protection is mediated by galanin receptors and downregulation of Bax level. The data from the present study provide a potential drug target for therapy or prevention of neurodegenerative diseases, including AD.

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PROTECTIVE EFFECT OF *CENTELLA ASIATICA* EXTRACT ON 1-METHYL-4-PHENYL-1,3,4,6-TETRAHYDROPYRIDINE (MPTP) INDUCED MITOCHONDRIAL OXIDATIVE STRESS IN THE DISCRETE BRAIN REGIONS RELATED TO PARKINSONISM IN AGED ALBINO RATS

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Reactive oxygen species (ROS) have been hypothesized to play an important role in ageing and age related neurodegenerative diseases such as parkinsonism. There exists an imbalance between reactive oxygen species (ROS) generation and antioxidant defense-repair mechanisms in the discrete regions of brain related to parkinsonism, which may lead to cell death. Biochemical evidence suggests PD involves oxidative neurodegeneration and that L-dopamine (L-DOPA) therapy further adds to the oxidative burden. Our study was designed to determine whether *Centella asiatica* extract (CAE) enriched with bioflavonoid and triterpenes antioxidants, when administered orally (300 mg/kg body weight/day, started 3 days prior to MPTP administration) for 21 days would prevent age-

related mitochondrial oxidative stress itself as well as the neurotoxin MPTP induced changes in antioxidant defense system and the markers of oxidative stress such as lipidperoxidation (LPo), protein carbonyl content (PCC), xanthine oxidase (XO) activity and DNA content in the mitochondria of aged rat brain regions such as striatum and hippocampus. Aged control and Aged rats challenged with MPTP elicited a significant decline ($P<0.05$) in GSH, MN-SOD and DNA and a significant increase ($P<0.05$) in LPo, PCC and XO activity, with an enormous change in MPTP-treated group of animals. Administration of CAE was found to be effective in augmentation of mitochondrial antioxidants in ageing and amelioration of MPTP-toxicity and thereby reduction of oxidative stress and sparing of DNA in the brain regions such as striatum and hippocampus and proved to be effective in the treatment of ageing related neurodegenerative disorders such as stroke and Parkinsonism.

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MICROGLIAL ACTIVATION CONTRIBUTES TO HEPATIC ENCEPHALOPATHY AND BRAIN EDEMA IN EXPERIMENTAL ACUTE LIVER FAILURE: BENEFICIAL EFFECT OF MINOCYCLINE.

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Purpose: Encephalopathy and brain edema are serious complications of acute liver failure (ALF). The precise pathophysiologic mechanisms responsible have not been fully elucidated, but involvement of brain-derived proinflammatory cytokines were reported. Therefore, the effects of the anti-inflammatory drug minocycline were investigated on the progression of hepatic encephalopathy and brain edema in rats with ALF resulting from hepatic devascularization.

Methods: ALF rats ($n = 6$ per group) were administered saline or minocycline (22.5 mg/kg) on days −2, −1 and day 0 of surgery. ALF rats were sacrificed at precoma (loss of righting reflex) and coma (loss of corneal reflex) stages of encephalopathy along with their appropriate sham-operated controls. Minocycline-treated animals were sacrificed in parallel with saline-treated comatose rats. IL-1β in serum and brain was measured by ELISA. IL-1 β mRNA in the brain was assessed by real-time PCR. Microglial activation was assessed by immunohistochemistry using anti-OX42 antibody.

Results: Minocycline delayed the onset of coma and significantly reduced brain water content in ALF rats. Minocycline treatment also significantly attenuated the increase of IL-1β protein in serum, together with IL-1 β mRNA expression and IL-1 β protein in cerebral cortex of ALF animals. Furthermore, induction of OX-42 immunoreactivity observed in the frontal cortex of ALF rats was suppressed by minocycline treatment.

Conclusion: These results further support a role for brain-derived cytokines in the pathogenesis of encephalopathy and brain edema in ALF and suggest that minocycline may be beneficial in the prevention of these neuropsychiatric complications in ALF patients.

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ROLE OF THE METABOTROPIC P2Y RECEPTOR IN PERIPHERAL NERVE REGENERATION

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Using the model of rat sciatic nerve crush we studied influence of P2Y-receptor antagonist (Reactive blue 2) and probable agonist (pyrimidine derivative Xymedon) on number of myelinated and unmyelinated axons. Xymedon significantly increased the number of regenerating myelinated fibers by 33.5%. Reactive blue 2 decreased the number of unmyelinated fibers by 73.8%. When co-injected, the number of fibers was unchanged. These results suggest the involvement of P2Y receptors in the peripheral nerve regeneration.

P-54

ANTIPARKINSONIAN PROPERTIES OF AN *AYURVEDIC* PREPARATION: ROLE OF NOVEL INGREDIENTS

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A potential therapy for Parkinson's disease (PD) is described in the classical Indian textbook, "Charakasamhitha". A prospective clinical study suggested beneficial effects of such treatment in patients, and analyses of the medicine showed significantly high levels of the dopamine precursor, 3,4-dihydroxyphenylalanine (L-DOPA) in *Mucuna pruriens* (MP), one of the four herbs used in preparing the drug. Recent studies pointed to better effectiveness of the *Ayurveda* medicine as compared to L-DOPA alone treatment. In the present study we investigated the extracts of each of the plants in 6-hydroxydopamine (6-OHDA) and/or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD. The aqueous alcoholic extracts of seeds of two herbs viz. MP and for the first time in literature, *Hyoscyamus reticulatus* (HR) showed significant neuroprotection in terms of behavior and striatal dopamine recovery in these animal models of PD. HR extract exhibited significant inhibition of mitochondrial monoamine oxidase B (MAO-B) activity, suggesting that the neuroprotection may be deriving from the enzyme inhibitory action. Neuroprotective action of MP was visible even when L-DOPA was removed employing column chromatographic techniques. MP has been reported to contain biologically isoquinoline alkaloids, based on which four molecules have been synthesized. Of these one naturally occurring and two novel synthetic molecules showed significant neuroprotective effects in MPTP model of PD, and suggest that the plant extract contains neurologically active entities other than L-DOPA, and studies on active principles in plants are extremely important for the discovery of novel drug molecules active against Parkinson's disease.

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GENISTEIN ATTENUATES HYPERPHOSPHORELATION OF TAU PROTEIN INDUCED BY DIARRHETIC SHELL-FISH TOXIN, OKADAIC ACID, IN SH-SY5Y CELLS *IN VITRO*

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Environmental toxins made by marine and freshwater micro-organisms represent a significant health problem. Okadaic acid, produced by marine dinoflagellates, is an enterotoxin involved in diarrhetic shellfish poisoning in humans. These poisons are inhibitors of protein phosphatases 1 and 2A (PP-1 and 2A), and exert their toxicity through a general overphosphorylation of cellular proteins. Tau protein is a microtubule-associated protein (MAP) that normally interacts with tubulin to stabilize microtubules and promote tubulin assembly into microtubules. Hyperphosphorylation of tau protein in neuronal cells is a major component of neurofibrillary tangles (NFTs) seen in Alzheimer's disease (AD). The tangle-containing neurons eventually die. However, the exact causes of tau hyperphosphorylation remain unclear. The imbalance of enzyme kinase and phosphatase activity is interesting as the underlying mechanism of AD. Glycogen synthase kinase-3β (GSK-3β) and PP-1 and 2A play a significant role in the regulation of phosphorylation and dephosphorylation of tau protein, respectively. In this study, we aim to investigate the possibility of using genistein as an alternative estrogenic agent for the neuroprotection by examining the effects of genistein on tau phosphorylation and inactivation of GSK-3β in SH-SY5Y cells induced by diarrhetic shellfish toxin, okadaic acid (OA). The results show that genistein is able to prevent toxin-induced protein phosphorylation. Genistein also increases the inactive form of GSK-3β. In addition, genistein inactivating GSK-3β activity was mediated by estrogen receptor activation. This study demonstrates that genistein is neuroprotective via estrogen receptor in OA-induced death of SH-SY5Y cells.

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MELATONIN AND SALICYLIC ACID PROTECT AGAINST STRIATAL GENERATION OF 6-OHDA IN MICE TREATED WITH L-DOPA AND MPTP

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Parkinson's disease (PD) is a progressive neurodegenerative motor disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compacta resulting in reduction of dopamine in the striatum. Dopamine replacement therapy by L-3, 4-dihydroxyphenylalanine (L-DOPA) is the most effective medication till date for the treatment of PD. We report here that L-DOPA treatment in normal mice leads to increased formation of 6-hydroxydopamine (6-OHDA) in the striatum, which can be detected by a highly sensitive electrochemical detector after separation by high performance liquid chromatography. We found a dose and time dependent increase of 6-OHDA in the striatum after 7 days of L-DOPA treatment. Further, a single dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on the 7th day in L-DOPA treated animals significantly increased the level of 6-OHDA in the striatum as compared with that in animals treated with L-DOPA alone. We also confirmed the generation of 6-OHDA from dopamine *in vitro* using a ferrous ascorbate hydroxyl radical generating system. This led us to investigate whether melatonin, salicylic acid and L-deprenyl, which are known to scavenge hydroxyl radicals, could reduce the levels of 6-OHDA *in vitro* as well as *in vivo*. Melatonin and salicylic acid treatment significantly decreased the generation of 6-OHDA in the striatum. However, L-deprenyl did not prevent the generation of 6-OHDA, rather it increased the L-DOPA induced generation of 6-OHDA in the striatum. Both L-deprenyl and melatonin protected against the generation of 6-OHDA *in vitro*. The present study demonstrates the possible neurotoxic effect of L-DOPA administration in PD and suggests the concomitant use of an antioxidant such as melatonin or salicylate in order to increase the therapeutic potential.

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VITAMIN D3 SCAVENGES HYDROXYL RADICALS, AND PROTECTS AGAINST MPP^+-INDUCED DOPAMINERGIC NEURODEGENERATION IN A NARROW THERAPEUTIC WINDOW

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We investigated the effect of 1,25-dihydroxyvitamin D3 (D3) or cholecalciferol, an antioxidant neuroprotector, in 1-methyl-4-phenyl pyridinium ion (MPP^+)-induced neurotoxicity in Sprague-Dawley rats. Rats were injected vehicle (peanut oil), or D3 (1, 2.5, 5 and 10 μg/kg, i.p.) for seven days, and thereafter infused MPP^+ (16 nmol in 1 μl) unilaterally into the substantia nigra (SN), and followed with seven more injections of D3 in neuroprotective studies. MPP^+ alone treatment caused about 60% striatal dopamine depletion by 7 days, but no apoptosis in the SN was discernable. Significant nuclear DNA breaks in the SN that received the non-apoptotic dose of MPP^+ were visible for animals treated with 1 and 2.5 μg/k D3. While the lowest and the highest doses of D3 treatment caused exacerbation of the MPP^+ effect, the medium doses studied (2.5 and 5 μg/kg, i.p.) either did not change or significantly attenuated the MPP^+-induced striatal DA depletion. Interestingly, 5 μg/kg dose of D3 failed to produce chromosomal DNA breaks following MPP^+ administration. MPP^+-induced hydroxyl radical generation in SN was significantly reduced dose-dependently by D3 in rats. The above findings suggest potent free radical scavenging action of D3 *in vivo*. These results also indicate significant pro-apoptotic action of D3 when used arbitrarily. The overall data suggest that D3 provides an effective, but a narrow therapeutic window in MPP^+-induced PD in rats, and caution that D3 may not be used indiscriminately.